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The Axon as a Metabolic Compartment: Protein Degradation, Transport, and Maximum Length of an Axon

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We present a model that predicts the maximum axonal length from the apparent velocity of slow axonal transport and cytoskeletal protein half-life. The model assumes that in mature axons the apparent velocity of slow transport varies with position, but that the density of cytoskeletal proteins and protein degradation are uniform. The model predicts that the apparent transport velocity of cytoskeletal proteins is highest near the cell body and decreases linearly along the axon, and that when axons branch the apparent velocity of transport decreases across the branch point. The predictions of this model are shown to be consistent with experiments. These results explain the variation in these fundamental metabolic parameters in different axons and species.

Introduction

Neurons typically have a single axon that can extend over long distances, and they synthesize the majority of their proteins in the cell body (Craig & Banker, 1994) [but for review of protein synthesis in the axon see Van Minnen, (1994)]. It is widely accepted that after synthesis, the proteins move down the axon by both fast and slow transport mechanisms (Grafstein & Forman, 1980; Vallee & Bloom, 1991). Fast transport involves the movement of membrane bound organelles at an average rate of approximately 400 mm day^{-1} along cytoskeletal elements such as actin filaments and microtubules by the action of molecular motors (Morris & Hollenbeck, 1995; Cyr & Brady, 1992). Less is known about slow axonal transport, but it is well established that the cytoskeletal proteins tubulin, actin, and the neurofilament proteins make up 75 to 85% of the total protein that is moved at the slow transport rate of approximately 0.1 to 2 mm day^{-1} (Grafstein & Forman, 1980; Lasek, 1988).

Once a neuron has matured, the role of axonal transport is to carry newly synthesized substances into the axon to replace those that have been removed or degraded (Nixon, 1980; Koenig *et al*., 1985; Hollenbeck & Bray, 1987; Nixon & Cataldo, 1995; Hollenbeck, 1993). While it is well established that proteinases can degrade proteins in minutes (Hasilik, 1992; Seglen & Bohley, 1992), the half-lives of proteins transported in the axon in many different species have been estimated to be much longer, on the order of months (Karlsson & Sjostrand, 1971; Neale *et al*., 1974; Cancalon, 1979). If degradation happens at a constant rate, then the density of the protein along the axon would decline exponentially with time unless these damaged proteins are replaced.

We present a simple, unified model that relates protein transport, protein degradation, and axonal length. The model predicts that the mechanism responsible for slow axonal transport must be systematically regulated along the length of the axon, and we also predict that the velocity of slow axonal transport changes dramatically across axonal branch

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points. We review experiments that have measured these parameters, and derive theoretical limits for the lengths of axons based on the slow transport of cytoskeletal proteins, with an understanding that the transport of other substances consumed in the normal activity of the neuron may place even further constraints on the axon length.

An Analogy for Axonal Transport

One analogy that can be made for protein transport and degradation within the axon is that of several lines of people where each person attempts to maintain a uniform distance from his neighbors. The people in this analogy represent any given transported protein, and the distance between the people is an abstract representation of protein density. While the people in this analogy are in several lines this is not meant to imply that they represent polymeric cytoskeletal elements or that there are channels in the axon through which protein can be transported.

Over time, people randomly leave the line and others behind them move forward or from the sides to fill the gap. The people leaving the line represent protein degradation. The forward motion of the people represents slow transport. The net effect of this is that a person's rate of forward movement depends on that person's position in the line. At the back of the line, people move forward quickly to replace the large number of people in front of them that are dropping out of the line. Conversely, the people near the front of the line move forward slowly because there are fewer people ahead of them dropping out.

This analogy is not meant to imply that slow axonal transport is necessarily regulated by protein density in the axon. An equally plausible mechanism would be that the transported protein moves forward at a uniformly decreasing apparent velocity that is in balance with protein degradation. Nevertheless, the point of the analogy is to illustrate that in order to maintain protein density along the axon in the face of protein degradation the flux of protein must vary with position.

Before we proceed to a discussion of slow axonal transport, we must carefully define "velocity". First, it is widely accepted that at any given moment at least part of the cytoskeleton is stationary, but over time all of it is transported [Miller & Joshi, 1996; Terada *et al*., 1996; Funakoshi *et al*., 1996; Lasek, 1986; but also see Nixon & Logvinenko (1986)]. Based on this, it is apparent that the velocity of a pulse of labeled protein as measured over days or months does not reflect the velocity of an individual protein that is actually in motion in the axon. To distinguish between these two velocities, we will define the velocity averaged over all the transportable proteins as the apparent velocity, V_app. We choose this name because this is the velocity that would be measured with a radioactive tracer, since the measured velocity here is averaged over all tagged protein. We will call the velocity of any individual protein that is actually undergoing transport the instantaneous velocity, V_inst. Therefore $V\_app =$ (fraction of protein in motion at a given time point, defined as $F\_m) * V\_inst$. Finally, we will define the velocity of an individual transported protein averaged over a long time (i.e. containing many stationary and moving episodes) as the net velocity, V_net. These three velocities may be very different, and it is important that this distinction be made when discussing a "transport velocity".

These definitions lead us into a consideration of flux and the mechanisms whereby the rate of protein transport in the axon may be modulated. The true flux in the case of axonal transport will be considered to be the number of molecules in motion multiplied by the instantaneous velocity V_inst. But, since it is agreed that at least part of the cytoskeleton is always temporarily stationary or paused we take this into consideration with the following equation.

$$\begin{aligned} Flux\_app &= V\_inst * P\_tot * F\_m \\ &= V\_app * P\_tot \end{aligned} \tag{1}$$

The apparent flux ($Flux\_app$) may be changed by either changing the total concentration of protein (P_tot), the velocity of proteins in motion V_inst, or the fraction of protein in motion F_m. We simplify our discussion by assuming that protein concentration is uniform along the axon. This allows us to remove the term P_tot by dividing the $Flux\_app$ by the total concentration of protein, which gives the apparent velocity, V_app, defined above. This can now be related back to our original question of how axons balance slow axonal transport and protein degradation in the axon.

The exponential decay of cytoskeletal proteins in the axon has been reported in several pulse-chase experiments (Karlsson & Sjostrand, 1971; Neale *et al*., 1974; Cancalon, 1979; Nixon & Logvinenko, 1986). This decay can be described by the equation

$$\mathrm{d}P/\mathrm{d}t = -P/\tau \tag{2}$$

where P is the density of a given cytoskeletal protein and τ is the degradation time constant of that protein. The degradation time-scale τ is related to the more

familiar half-life $T_{1/2}$ (the time necessary for half the proteins to decay) by the equation $T_{1/2} = \ln(2) * \tau$.

At each point X along the axon we consider two processes which can change the local cytoskeletal protein density and which balance to zero at a steady state so that the protein density remains constant in time. These processes are the flux [eqn (1)] through the point X and the degradation of protein [eqn (2)]. Together, these give the differential equation

$$0 = (-\mathrm{d}(V\_app * P\_tot)/\mathrm{d}X) - (P\_tot/\tau) \quad (3)$$

where the first term represents the change in the amount of cytoskeletal protein P_tot that is moving through X at V_app, and the second term represents the decrease in the density of protein due to degradation which is assumed to be uniform at all points along the axon.

If we were to assume a uniform apparent velocity of transport along the axon, then according to eqn (3) the density of protein must decrease with distance from the cell body. But considerable evidence shows that the density of polymerized cytoskeletal protein along the mature axon does not decrease in density (Bray & Bunge, 1981; Goslin *et al*., 1990; Black *et al*., 1994). To maintain a uniform density, eqn (3) requires a gradient in the apparent transport velocity along the axon.

$$\mathrm{d}V\_app/\mathrm{d}X = -1/\tau \quad (4)$$

The resulting transport velocity profile $V\_app(X)$ is

$$V\_app(X) = V\_app_0 - X/\tau \quad (5)$$

where V_app_0 is the apparent velocity at the proximal end of the axon. The transport velocity is highest at the proximal end of the axon and drops linearly. At some finite length the apparent transport velocity is zero. Note that since $V\_app = V\_inst * F\_m$ a gradient in V_app does not require a gradient in V_inst. The spatial variation in V_app may be due to a variation in the fraction in motion while V_inst remains uniform.

Equation (5) defines a natural maximum length for the axon. The axon must be supplied with new protein from the soma to replace the degraded cytoskeletal protein. The length at which the apparent transport velocity goes to zero is the maximum distance that new cytoskeletal protein can be transported from the soma. From eqn (5) this maximum length L_{max} is

$$L_{max} = V\_app_0 * \tau = (2/\ln(2)) V\_app_{avg} * T_{1/2} \quad (6)$$

where $V\_app_{avg} = (1/2) * V\_app_0$ is the average velocity taken over the maximum length of the axon. We introduce V_app_{avg} here in order to facilitate comparisons with experiments which only measure an average velocity.

The results derived here are for the simple case where the cytoskeletal protein density and the protein decay rate are uniform across the axon. Naturally, there are variations in these values in a real axon, such as the nodes of Ranvier and in the distribution of protein degradation (Hollenbeck, 1993). These nonuniform effects can be incorporated into this model by making τ and P_tot functions of position instead of constants. Until these variations are better known, a uniform distribution is a good approximation that does not conflict with available data.

Experiments and Implications of the Model

There is a wide range of axon lengths present in animals. Only a few strategies are possible that increase the maximum length of an axon: (a) increase the average speed of transport V_app_{avg}, (b) stabilize the cytoskeleton protein and increase the half-life $T_{1/2}$, or (c) allow the density of cytoskeleton protein P_tot to decrease toward the distal end of the axon instead of the uniform profile that we have assumed in our analysis. Strategy (c) seems unlikely because axonal arborization actually increases the net amount of cytoskeletal protein P_tot with distance from the cell body (summed over the axon branches). Strategies (a) and (b) are viable, indeed higher velocities of slow axonal transport have been reported in neurons with longer axons (Murray, 1974; Komiya & Kurokawa, 1978; Mori *et al*., 1979).

Table 1 lists the results of several experiments which have measured the rate of slow axonal transport and the half-life of the transported proteins in various species. The lengths of the axons involved in these experiments cover a wide range from 10 mm in the goldfish optic nerve to 280 mm in the garfish olfactory nerve. Both the measured average apparent transport velocity and the protein half-life vary in these experiments with longer axons tending to have both a faster apparent transport velocity and longer half-lives, though there are exceptions to both these trends. The calculated $V\_app_{avg} * T_{1/2}$ from this data is graphed in Fig. 1. The axon lengths from all the species fall below the line $L_{max} = (2/\ln(2)) * V\_app_{avg} * T_{1/2}$ in good agreement with this theory. While the axon lengths in Table 1 vary by a factor of 30, the apparent velocities and half-lives both vary by approximately a factor of 5. The burden of increasing the product $V\_app_{avg} * T_{1/2}$ seems to be shared fairly evenly between V_app_{avg} and $T_{1/2}$. The longer axons are supported by a combi-

TABLE 1
Data from slow axonal transport experiments compared with values calculated from theory

Animal	Nerve	V_{avg} (mm day^{-1})	$T_{1/2}$ (days)	Length (mm)	L_{max} (mm)	L/L_{max}
Garfish	Olfactory	2.38	75	280	515	0.54
Rat	Sciatic	1.2	51	140	176	0.80
Rabbit	Optic	2	14	25	81	0.31
Mouse	Optic	0.6	20	10	35	0.29
Goldfish	Optic	0.4	67	10	77	0.13

The transport velocity V_{avg}, *protein half-life* $T_{1/2}$, and the axon length L were measured in these experiments. The axon lengths vs V_{avg} $T_{1/2}$ are plotted in Fig. 1. The data are taken from: garfish (Cancalon, 1979), rat (Hoffman & Lasek, 1975), rabbit (Sjostrand & Karlsson, 1969), mouse (Karlsson & Sjostrand, 1971), goldfish (Neale *et al*., 1974; Grafstein, 1967). The protein half-life in the rat sciatic nerve was determined from the data in Hoffman & Lasek (1975) by calculating the total radioactive signal and fitting an exponential decay to those values. All other half-lives were given by the original authors.

nation of increased apparent transport velocity and the stabilization of cytoskeletal proteins.

These data have several areas where uncertainty could arise. The first is the estimation of the axon lengths. For example in the visual system, the lengths of the nerves vary a great deal because the axons project to several different parts of the brain (Cuenod & Schonbach, 1971, Grafstein, 1967). In addition, the measured average velocities are a rough estimate of the actual average velocity. Since it is impractical to measure the average velocity over the entire length of the axon, these values represent averages taken over only a section of the axons, typically leaving out the most distal ends of the axon. If our theory is correct the reported average velocities are upper limits on the actual values of V_app_{avg}. Finally, the estimation of the half-lives of the proteins is imprecise for two reasons. The first is that recycling of labeled amino acids from degraded proteins will lead to an overestimation of the protein half-lives. The second is that in the cases where nerve branching occurs and only one branch of the nerve was followed the half-lives of the proteins will be underestimated (Neale *et al*., 1974). For consistency we have limited this table to experiments that used the same type of radioactive labeled amino acid (H3-leucine) and have used the authors reported half-lives, and velocities of transport where available (see Table 1).

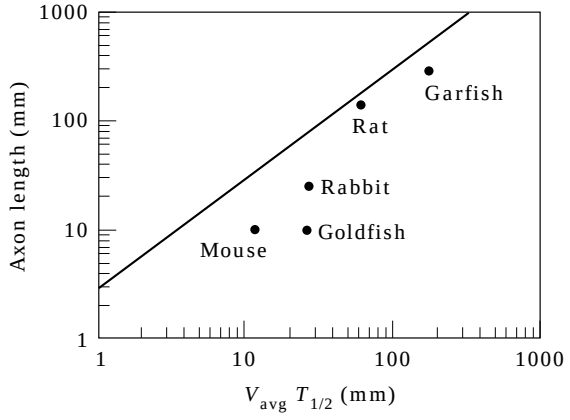

FIG. 1. Comparison of theory to data. The length of axons is plotted for various species where the velocity of slow transport and the rate of protein degradation have been measured. The solid line is the theoretical maximum length $L_{max} = (2/\ln(2))\ V_{avg}\ T_{1/2}$. The data and references are given in Table 1.

While the evidence that the velocity decreases for cytoskeletal protein transport along the axon is limited, currently good evidence suggests that at the very least it can occur (Lasek, 1968; Hoffman *et al*., 1983, 1985; Watson *et al*., 1989). In addition, there appears to be evidence for the slowing of slow axonal transport in papers that did not directly address this topic (Hoffman & Lasek, 1975) (see Fig. 2). In Hoffman & Lasek (1975) the position of a labeled pulse of protein was measured as it was transported down the sciatic nerve. In Fig. 2 we have plotted the measured position of the peaks of labeled protein vs time. Using our predicted linear profile in the apparent transport velocity [eqn (5)] we can derive the position of the peak of labeled protein as it moves down the axon. The equation for the peak position, X_p, is

$$X\_p = (V\_app_0 * \tau) + (X\_0 - V\_app_0 * \tau)\exp(-t/\tau) \qquad (7)$$

where X_0 is the position of the peak at time $t = 0$. Using the value of $T_{1/2} = 51$ days (therefore $\tau = 74$ days) from Table 1, we can make a linear fit of eqn (7) to the data of Hoffman & Lasek (1975). This fit gives the parameters $V\_app_0 = 1.66 \pm 0.05$ mm dat^{-1} (quite close to the value of 1.2 mm day^{-1} that

Hoffman & Lasek derive as the velocity of transport) and $X\_0 = -11 \pm 12$ mm. This fit is shown as the solid curve in Fig. 2. For comparison we also show the peak position assuming the reported constant velocity of 1.2 mm day^{-1} (dashed line) (Hoffman & Lasek, 1975). The agreement of this model with the data is superior to that of the constant velocity model.

The slope of the predicted curve in Fig. 2 appears nearly linear for much of the length of the axon. This is because the slow but constant decrease in velocity has little effect on the large velocity in the part of the axon close to the cell body. It is only further along the axon, when the predicted transport velocity becomes much lower, that the slowing of the peak becomes clearly visible. Given that the majority of the measurements of slow axonal transport have been taken along the proximal end of the axon, it is easy to see how a slowing in the transport velocity could go undetected.

While it is not explicitly stated in Hoffman & Lasek (1975) that the apparent velocity of transport is uniform along the axon, this has been generally assumed. Therefore, it was surprising that when the Hoffman & Lasek data were plotted in Fig. 2 there was a decreasing trend in the velocity which was consistent with the predictions of our theory. It is clear that this overlooked aspect of slow axonal transport will require further careful investigation. For example, this slowing of the peak of transported protein may also be responsible, in part, for the tightness of the peak as it moves down the axon, in a manner analogous to a band moving down a SDS-PAGE gel with a gradient of polyacrylamide density.

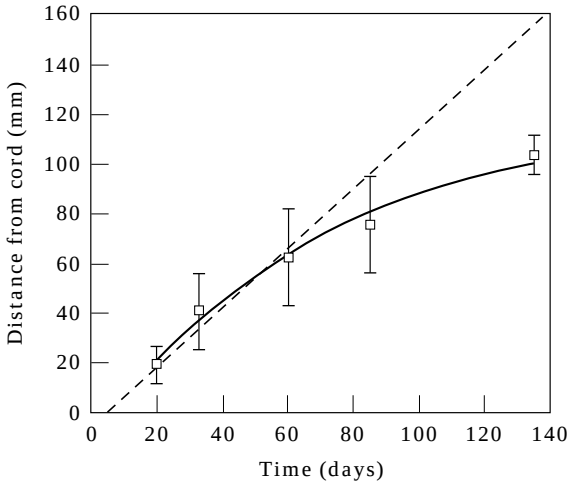

FIG. 2. Comparison of the predicted position of the peak of the labeled protein distribution and the data of Hoffman & Lasek (1975). The solid line shows the predicted peak position from eqn (7) with the value of τ taken from Table 1 and the values for V_app_0 and X_0 set by a least squares fit to the data. The apparent velocity in this fit varies from 1.66 mm day^{-1} at $t = 0$ days to 0.25 mm day^{-1} at 135 days. For comparison, the dashed line shows the peak position for constant velocity of 1.2 mm day^{-1}. The peak positions plotted as squares were taken directly from Fig. 1 of Hoffman & Lasek (1975). The error bars are the width of the peak at 75% of the maximum peak height. Key: — constant velocity = 1.2 mm day^{-1}; —— model prediction [eqn(7)]; □ Hoffman & Lasek (1975).

There currently exists no direct evidence to support the assumption that the density of soluble protein is uniform along the length of the axon. Nevertheless, the simplest theory is that the density of soluble protein will in some way reflect the density of the polymeric protein. Regardless, this may not be the case. Microtubule associated proteins or protein phosphorylation may alter the ratios between the polymeric and the soluble phases of the protein dramatically. In fact gradients in the distribution of microtubule associated proteins along the axon (Black *et al*., 1996) and/or gradients in protein phosphorylation may be altering the ratios of the polymeric and the soluble phases. If we consider for a moment that the soluble phase is being moved (Miller & Joshi, 1996; Terada *et al*., 1996; Funakoshi *et al*., 1996), one way of decreasing the flux of a particular protein along the axon would be to decrease the concentration of soluble protein along the length of the axon. While this would not directly affect the instantaneous velocity of what is being transported, it would have the effect of slowing the apparent velocity of transport. Alternatively, even if the ratios of the soluble and the polymeric protein are uniform along the axon, it is possible that while the concentration of soluble is uniform, the fraction of the total soluble protein that is being transported is decreasing.

Axon Branch Points

When axons branch the microtubule mass in the daughter branches is greater than the microtubule mass in the parent branch (Watson *et al*., 1989). The assumption of a uniform cytoskeletal protein density along the axon together with the conservation of transported protein flux through an axon branch point requires a change in the apparent transport velocity at the branch point. Assuming that the axon diameter remains uniform through the branch point, conservation of protein flux gives

$$(V * P)_{main} = (V * P)_{b1} + (V * P)_{b2} \qquad (8)$$

where the subscript "main" refers to the axon before the branch and the subscripts "b1" and "b2" refer to the two branches. If the protein density P

remains uniform across the axon branch then flux conservation requires that the transport velocity V drops across the branch point.

$$V_{main} = V_{b1} + V_{b2}. \quad (9)$$

If the protein flux into the two branches is equal, the velocity of slow transport must decrease by a factor of two across a branch point of the axon. Note that this drop in the velocity is not caused by protein degradation but rather by the geometry of the branch point. This could be experimentally tested in a system with well-defined axonal branches where slow axonal transport could be measured before and after the branch point. One difficulty with this test is that the velocity of transport is predicted to decrease with distance from the cell body [eqn (5)] and this could produce false positive evidence supporting this hypothesis unless the velocity measurements are made close to the branch point.

Summary

We present a model of axonal maintenance based on slow transport and protein degradation. The conclusion of this model is simple: an axon of length L must have an average slow transport rate V_app_{avg} and a cytoskeletal protein degradation half-life $T_{1/2}$ such that the ratio $(\ln(2)/2)\ L/V\_app_{avg} * T_{1/2}$ is below one. This conclusion agrees well with experiments carried out on many different species (Table 1 and Fig. 1). But more importantly, this model provides us with an understanding of the relationship between protein transport, protein degradation, and the maximum length of an axon. The variations of the measured slow transport velocities and the protein half-lives are systematic, with longer axons tending to have both a faster transport velocity and a longer protein half-life. This points out the danger of taking these values from experiments on small animals and applying them to larger animals, particularly humans. We cannot safely predict exactly how these values will change for longer axons. Either V_app_{avg} or $T_{1/2}$ may eventually reach a maximum value, and any further increase in the product $V\_app_{avg}T_{1/2}$ would have to come solely from the parameter that can vary. Nevertheless, the values will be different for longer axons. The slow axonal transport velocity and the protein half-life are not constants, but are dependent on neuron morphology.

The primary testable prediction of this theory is that in non-growing axons, where the diameter of the axon is not changing and net protein degradation is occurring in the axon, the apparent velocity of slow axonal transport must decrease along the axon to maintain a uniform density of the cytoskeletal proteins. There is some evidence for this in observations that the apparent transport velocity is highest at the proximal end of the axon (Lasek, 1968; see Fig. 2 and Hoffman & Lasek, 1975; Hoffman *et al*., 1983, 1985; Watson *et al*., 1989). The dependence of the average slow axonal transport velocity and protein half-life on the axon length is also a testable prediction of this theory. The observation of this could be complicated by heterogeneity in the distribution of protein degradation machinery along the axon. The number of experiments that have measured both protein half-life and velocity is small and further experiments should be done. It would be interesting to see if this variation in the average velocity of transport and the half-life of the transported proteins holds for axons of different lengths within the same animal, as well as between species. We would like to point out that while we have written the predicted maximum axon length in eqn (6) as a function of the average apparent transport velocity and the protein half-life, this is not meant to imply that V_app_{avg} and $T_{1/2}$ are the more fundamental parameters that determine the actual axon length. The actual processes that the cell uses to satisfy this relation are unknown and in principle any two of these parameters may be taken to determine the third. Furthermore, it should be noted that the apparent velocity of transport could be changed by either a change in the instantaneous velocity of what is being transported, or a change in the fraction that is being moved, or some combination of both. This must be taken into consideration in the experiments that measure the velocity of slow axonal transport in time spans that are shorter than the rate of exchange between the moving and the temporarily stationary or paused pools of protein.

Unlike fast axonal transport, the mechanism of slow transport remains unknown and has been a matter of some debate for many years. We may find vital clues to this transport mechanism in the transport velocity changes along the axon and across axon branches that we have derived here simply from the observed properties of cytoskeletal proteins in axons.

This paper proposes for the first time that there is a relationship between the protein degradation, protein transport, and the length of the axon. A profound implication is that instead of generating a large amount of protein that will be wasted during transport, neurons work within the confines of protein degradation and transport to economically distribute protein along the axon.

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