A Diffusion-Based Model of Kinesin Recycling in Neurons

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Introduction

The axon terminal of neuronal cells has complex signaling and metabolic demands. Transmitting signals to neighboring neurons through synapses involves macromolecules, presynaptic vesicles, and organelles (such as mitochondria), many of which are synthesized in or near the cell body [1]. Diffusion of large molecular complexes over long distances is slow, and neuronal axons can be meters long, so supplying the synapse with cargo synthesized in the soma through diffusion would be inefficient. There are motor proteins that actively transport cargo.

One motor protein is kinesin, which consumes energy to walk down the microtubule (MT) tracks of the axon, pulling cargo along and thus supplying the needed proteins to the terminus [1]. But what happens to kinesin after transport is completed? Recent experimental work and mathematical modeling have pointed away from degradation or recycling via dynein, a retrograde motor, and instead suggest that kinesin may be recycled via a diffusion-based mechanism, at least in cells with short neurites/axons [2]. But will such a mechanism be viable in long axons, given the slowness of diffusion over long distances?

Continuing this line of exploration, we propose a "bucket-brigade" mechanism, in which cargoes "change hands" during active transport and diffusion of unbound kinesin motors is restricted by cargo jams and therefore no longer a limiting step in kinesin recycling. The cargo jams occur when active transport is disrupted by spatial inhomogeneities due to MT discontinuities or occlusion of MTs by other microtubule associated proteins (MAPs).

Objective

Investigate, by formulating and solving a mathematical model of axonal transport in the presence of spatial inhomogeneities, whether the bucket-brigade mechanism of kinesin recycling results in cargo transport that is efficient over long distances.

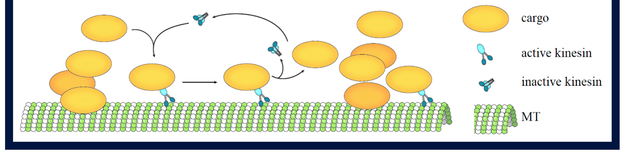
Bucket Brigade Model

Mass-action kinetics:

- kinesin is conserved over the length of the axon
- kinesin exists in two states, active and inactive [3]

Other assumptions:

1. Kinesin performs active transport when bound to cargo and diffuses when unbound
2. When unbound kinesin meets cargo, it binds cargo & MT simultaneously and begins active transport
3. Accumulated cargo impedes diffusion of free kinesin (excluded volume effect)
4. Spatial inhomogeneities disrupt active transport and binding of kinesin to cargo/MT



Methods

Partial Differential Equations (PDEs)

	Variables	Equations	Boundary Cond.
Free kinesin (diffusion)	$P(x,t)$	$\frac{\partial P}{\partial t} = \frac{\partial}{\partial x}\left(D_{eff} \cdot \frac{\partial P}{\partial x}\right) + R$	zero flux
Kinesin bound to cargo (advection)	$Q(x,t)$	$\frac{\partial Q}{\partial t} = -\frac{\partial}{\partial x}(Q \cdot v_{eff}) - R$	zero flux
Free cargo (diffusion)	$C(x,t)$	$\frac{\partial C}{\partial t} = D_{cargo} \cdot \frac{\partial^2 C}{\partial x^2} + R$	$C\|_{x=0} = C_0$ right: zero flux

R is the rate of change of concentrations due to binding and unbinding of kinesin and cargo:

$$R = -k_{on_{eff}} \cdot P \cdot C + k_{off} \cdot Q$$

We used VCell [4], a biological modelling software, to compute and run simulations.

Parameters

D_{eff}	effective kinesin dif. rate
$k_{on_{eff}}$	effective assoc. rate
k_{off}	dissociation rate
l	segment length
n	number of segments
$L = n \cdot l$	axon length
v_{eff}	effective adv. velocity

Mathematical Interpretations of Assumptions

Excluded Volume Effect

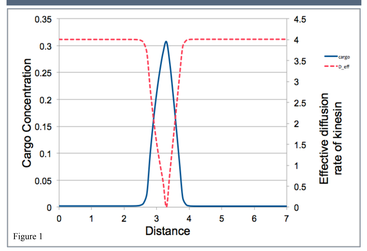


Figure 1

It has been shown that particles in the presence of obstacles have a slower diffusion rate. This is the excluded volume effect.

This decrease is given by the power law below (see, e.g., [5,6] and references therein):

$$D_{eff} = D_{kinesin}\left(1 - \frac{C_{tot}}{C_p}\right)^{\alpha}$$

where $C_{tot} = C + Q$

and C_p is the percolation limit: as total cargo approaches this limit, D_{eff} goes to 0, and α is a constant related to the shape of the obstructions.

- MAPs, e.g. tau, can cause slowdowns in kinesin advection and decrease binding [3]
- MTs are discontinuous

This is modeled by periodic decreases in the velocity and binding rate.

h defines the width of the intervals.

$$\text{step} = \left\lfloor \frac{x}{l} \right\rfloor l + \frac{l}{2}$$

$$\text{gaps}_v = \left(x \geq \text{step} - \frac{h}{2}\right)\left(x \leq \text{step} + \frac{h}{2}\right)$$

$$v_{eff} = v(\text{length} - \text{gaps}_v)$$

$$\text{gaps}_{kon} = \left(x \geq \text{step} - \frac{3h}{2}\right)\left(x \leq \text{step} + \frac{h}{2}\right)$$

$$k_{on_{eff}} = k_{on_0}(\text{length} - \text{gaps}_{kon})$$

Spatial Inhomogeneities

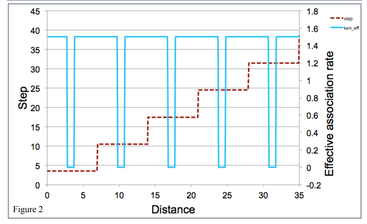


Figure 2

Results

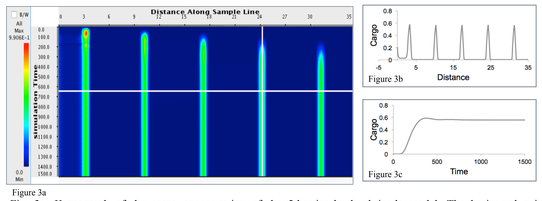


Figure 3a

Fig. 3a: Kymograph of the cargo concentration of the 5-barrier bucket-brigade model. The horizontal axis corresponds to the length of the axon, while the vertical axis is time. The cross hairs through the fourth pile are shown in Fig. 3b and Fig. 3c, the horizontal and vertical, respectively.

Figure 3b

Figure 3c

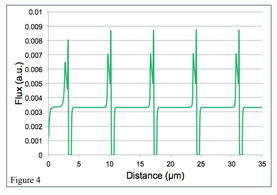


Figure 4

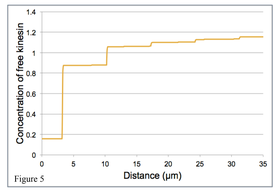


Figure 5

Fig. 4 & 5: Variables Q (Fig. 4) and P (Fig. 5) at steady state of the 5-barrier model

Fig. 6: Steady state cargo concentration (left axis) and effective diffusion (right axis) as a function of distance along the axon for the 5-barrier bucket-brigade model. The figure illustrates restricted diffusion of free kinesin.

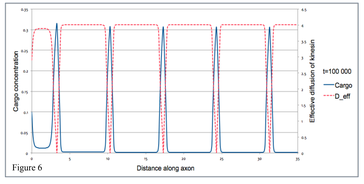


Figure 6

Fig. 7 & 8: Active flux as a function of time for the bucket-bridge model with 32 barriers (Fig. 7) and for the model with no barriers of the same length (Fig. 8). They illustrate the time it takes for the models to reach steady states as well as the differences in flux values in the two models.

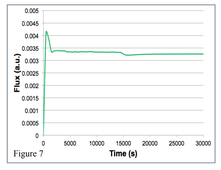


Figure 7

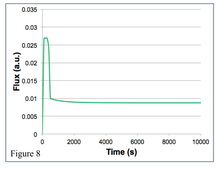


Figure 8

Fig. 9: Active steady-state flux as a function of axonal length for the bucket-brigade and no-barrier models. The flux of the bucket-brigade mechanism appears to become more efficient over longer distances as it is virtually independent of the axonal length. The latter makes the bucket-brigade mechanism a competitive diffusion-based alternative to the recycling of kinesin by retrograde motors.

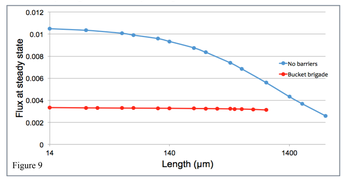


Figure 9

Discussion

To test competitiveness of the bucket-brigade mechanism of kinesin recycling, we compared active steady-state fluxes of the bucket-brigade model and the model with no barriers with the same model parameter values. In doing this, we confirmed that the unrestricted kinesin diffusion is inefficient for long axons (Fig. 9), but the bucket brigade maintains a virtually constant flux, even though it is initially low. This, preliminarily, confirms our hypothesis that the bucket brigade is more efficient in longer neurons (Fig. 9). For a definitive answer, we must run long-length simulations. But reaching a steady state by solving the time-dependent PDEs becomes computationally expensive, because for all lengths, we must use a small spatial step to avoid errors. Since these simulations are computationally expensive, our current numerical solver has yet to complete the simulations needed to confirm our hypothesis.

The bucket brigade model takes longer time to reach a steady state (Fig. 7). This is because the flux in this model is limited by the rate with which the cargo is passed over the cargo jam; the latter relies on cargo diffusion which is slow. This rate can be increased by increasing the diffusion of cargo within the jam, but this will lower the diffusion barriers for kinesin, and the model becomes more like the no-barrier model. We can also reconsider the definition of "steady state" for the bucket-brigade model. After an initial peak, the flux stays steady, but later decreases to a true, mathematical steady state (Fig.7). The initial steady value corresponds to fluctuating concentrations in the cargo jams, which is similar to the environment in real neurons. Considering this to be an actual steady-state flux would make the comparison of the models less challenging.

Future Directions

We would like to complete the currently-running long-length simulations to see if our hypothesis holds. This computational problem might be solved more efficiently by a time-independent PDE solver, however implementation of a stable and fast-converging time-independent solver is a challenge in and of itself.

As described above, emergence of cargo jams and movements of cargo in the vicinity of the cargo jams is a limiting factor. To model these processes more realistically, one could construct a multi-scale hybrid deterministic-stochastic model, in which the kinesin advection and diffusion over areas with no barriers are described continuously, as we do now, but the processes in the vicinity of the cargo piles, and the piles themselves, are modeled stochastically. Resolving the piles stochastically on a small spatial scale would more accurately depict the collisions of molecules and excluded volume effect. By allowing the piles to stochastically appear, dissipate, and reappear in different places, the model would better describe the dynamics of the axon and the cellular environment. We hope to use the results of our model to make testable hypotheses for our experimental collaborators.

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