

## Synaptic Democracy and Vesicular Transport in Axons

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Synaptic democracy concerns the general problem of how regions of an axon or dendrite far from the cell body (soma) of a neuron can play an effective role in neuronal function. For example, stimulated synapses far from the soma are unlikely to influence the firing of a neuron unless some sort of active dendritic processing occurs. Analogously, the motor-driven transport of newly synthesized proteins from the soma to presynaptic targets along the axon tends to favor the delivery of resources to proximal synapses. Both of these phenomena reflect fundamental limitations of transport processes based on a localized source. In this Letter, we show that a more democratic distribution of proteins along an axon can be achieved by making the transport process less efficient. This involves two components: bidirectional or “stop-and-go” motor transport (which can be modeled in terms of advection-diffusion), and reversible interactions between motor-cargo complexes and synaptic targets. Both of these features have recently been observed experimentally. Our model suggests that, just as in human societies, there needs to be a balance between “efficiency” and “equality”.

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A fundamental problem in cell biology is how newly synthesized proteins are delivered to subcellular targets located within the cell membrane. This is particularly acute for neurons with their extensively branched dendrites that receive information from other neurons, and a single long axon that delivers information over long distances to other neurons or muscle cells. Both the axon and dendrites contain highly regulated, protein-rich subcellular compartments that constitute synaptic contacts between neurons. Some synaptic junctions appear partway along an axon—*en passant* synapses—while others appear as terminals at the ends of axonal branches. In order to generate new synaptic contacts during synaptogenesis or to maintain and modify existing synapses in response to synaptic activity from other neurons, it is necessary to synthesize new protein products and localize them at the appropriate synaptic site [1]. The long distances between the soma and distal synapses on the axon and dendrites necessitates active motor-driven transport of vesicles along microtubular filament tracks. Microtubules are directionally polarized polymeric filaments with biophysically distinct (+) and (−) ends, and this polarity determines the preferred direction in which an individual molecular motor moves. For example, kinesin moves towards the (+) end, whereas dynein moves towards the (−) end [2]. Motor driven transport along microtubules is responsible for the distribution of many components necessary for the proper function of a neuron [3], and the breakdown of such transport has been implicated in many neurological diseases such as Alzheimer’s and Parkinson’s disease [4].

One major issue regarding neuronal function is how vesicles containing newly synthesized proteins are distributed evenly across the *en passant* synapses of an axon (or

the postsynaptic sites of a dendrite). Since vesicles are injected from the soma (anterograde transport), one might expect that synapses proximal to the soma would be preferentially supplied with resources. This problem persists even when the stochastic nature of motor transport and delivery of cargo at synaptic targets is taken into account—the probability of delivery would still be higher for proximal synapses. In principle, the challenge of so-called synaptic democracy could be solved by routing cargo to specific synaptic targets, but there is no known form of molecular address system that could support such a mechanism, particularly in light of the dynamically changing distribution of synapses. Note that the notion of synaptic democracy has previously arisen within the context of equalizing synaptic efficacies, that is, ensuring that synapses have the same potential for affecting the postsynaptic response regardless of their locations along the dendritic tree [5,6]. From a mathematical perspective, the issue of synaptic democracy reflects a fundamental property shared by the one-dimensional advection-diffusion equation used to model active transport and the cable equation used to model ionic current flow [7], namely, they generate an exponentially decaying steady-state solution in response to a localized source of active particles or current.

A number of recent experimental studies of axons in *C. elegans* and *Drosophila* have shown that (i) motor-driven vesicular cargo exhibits “stop and go” behavior, in which periods of ballistic anterograde or retrograde transport are interspersed by long pauses at presynaptic sites, and (ii) the capture of vesicles by synapses during the pauses is reversible in the sense that the aggregation of vesicles can be inhibited by signaling molecules resulting in dissociation from the target [8,9]. Thus, it has been

hypothesized that the combination of inefficient capture at presynaptic sites and the back-and-forth motion of motor-cargo complexes between proximal and distal ends of the axon facilitates a more uniform distribution of resources [10]. In this Letter, we construct a biophysical model of bidirectional vesicular transport in axons and establish that the hypothesized mechanism can support a form of synaptic democracy.

Let us begin by considering a simple three-state model of a single motor-cargo complex moving on a semi-infinite 1D track and carrying a single synaptic vesicle precursor (SVP), as shown in Fig. 1. The particle is taken to be in one of three states labeled by  $n = 0, \pm$ : unbound from the track and stationary or slowly diffusing ( $n = 0$ ), bound to the track and moving to the right (anterograde) with speed  $v_+$  ( $n = +$ ), or bound to the track and moving to the left (retrograde) with speed  $-v_-$  ( $n = -$ ). Transitions between the three states are governed by a discrete Markov process. Let  $p_n(x, t)$  denote the probability that the particle is at position  $x$ ,  $x \in (0, \infty)$ , and in state  $n$  at time  $t$  given some fixed initial condition. The evolution of the probability is described by the following system of partial differential equations [11,12]:

$$\frac{\partial p_{\pm}}{\partial t} = \mp v_{\pm} \frac{\partial p_{\pm}}{\partial x} - \beta p_{\pm} + \alpha p_0, \quad (1a)$$

$$\frac{\partial p_0}{\partial t} = D_0 \frac{\partial^2 p_0}{\partial x^2} + \beta p_+ + \beta p_- - 2\alpha p_0 - k\chi_A(x)p_0. \quad (1b)$$

For the moment, we take the end  $x = 0$  to be reflecting so that  $v_+p_+(0, t) = v_-p_-(0, t)$ . (In our population model, we will assume a constant, nonzero flux at  $x = 0$ .) Here,  $\alpha, \beta$  are the transition rates between the stationary and mobile states, and  $D_0$  is the diffusivity in the unbound state  $n = 0$ . We are also assuming that there is a uniform, continuous distribution of presynaptic targets along a region  $A$  of the axon, and that the motor complex can irreversibly deliver its SVP to a presynaptic target at a uniform rate  $\kappa$ . Thus,  $\chi_A(x)$  denotes an index function with

$\chi_A(x) = 1$  if  $x \in A$  and  $\chi_A(x) = 0$  if  $x \notin A$ . For future reference, we note that, in the above model, we are really keeping track of the SVP bound to the motor complex so that the irreversible delivery of the SVP to a presynaptic target is treated as an absorption event.

For intracellular transport, one finds that the transition rates are fast compared to  $v_{\pm}/l$ , where  $l$  is a fundamental microscopic length scale such as the size of a synaptic target ( $l \sim 1 \mu\text{m}$ ). One can then use a quasi-steady-state (QSS) diffusion approximation to obtain the following advection-diffusion equation for the total probability density  $p(x, t) = \sum_n p_n(x, t)$  [11,12]:

$$\frac{\partial p}{\partial t} = -V \frac{\partial p}{\partial x} + D \frac{\partial^2 p}{\partial x^2} - k\chi_A(x)p, \quad (2)$$

with mean velocity  $V = (v_+ - v_-)\rho_+$ , effective diffusivity  $D$  given by

$$D = D_0\rho_0 + \frac{\alpha}{\beta(2\alpha + \beta)} [(v_+ - V)^2 + (v_- + V)^2],$$

and effective delivery rate  $k = \kappa\rho_0$ . Here  $\rho_0 = \beta/(2\alpha + \beta)$  and  $\rho_{\pm} = \alpha/(2\alpha + \beta)$  are the stationary probabilities of the discrete Markov process for the states  $n = 0$  and  $n = \pm$ , respectively. The basic idea of the QSS reduction is to fix units so that  $v_{\pm} = O(1)$  and  $\alpha, \beta = O(1/\epsilon)$  with  $0 < \epsilon \ll 1$ . In this regime, there are typically a large number of transitions between different motor-complex states  $n$  while the position  $x$  hardly changes at all. This suggests that the system rapidly converges to the (quasi) steady state  $\rho_n$ , which is then perturbed as  $x$  slowly evolves. This motivates decomposing the probability densities as  $p_n(x, t) = p(x, t)\rho_n + \epsilon w_n(x, t)$  with  $\sum_n w_n(x, t) = 0$ . Substituting such a solution into Eqs. (1) and performing an asymptotic expansion in  $w_n$  then leads to Eq. (2) to leading order in  $\epsilon$ . In particular,  $D - D_0\rho_0 = O(\epsilon)$ .

Now, suppose that we have a population of motor complexes injected at one end of the axon at a fixed rate  $J_1$ , each of which carries a single SVP. As a further

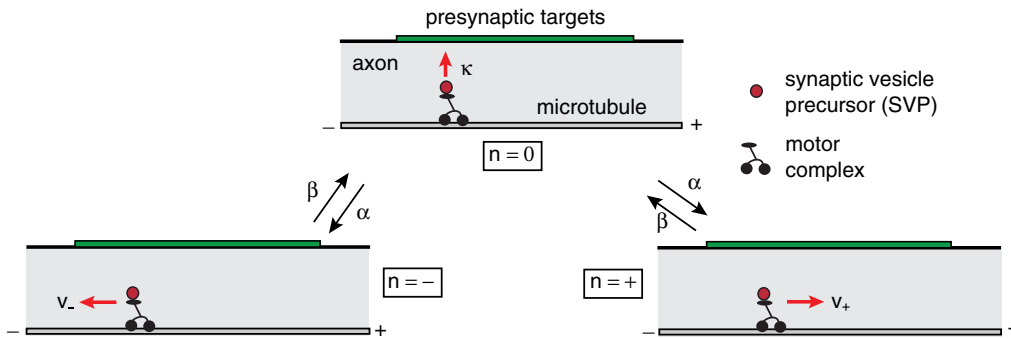


FIG. 1 (color online). Three-state model of the bidirectional transport of a single motor-cargo complex. The particle switches between an anterograde state ( $n = +$ ) of speed  $v_+$ , a stationary or slowly diffusing state ( $n = 0$ ), and a retrograde state ( $n = -$ ) of speed  $v_-$ . The motor complex can only deliver a SVP to a presynaptic target in the state  $n = 0$ .

simplification, let us take  $A = (0, \infty)$  (uniform distribution of synapses). Neglecting any interactions between motors, the population version of Eq. (2) is given by the advection-diffusion equation

$$\frac{\partial u}{\partial t} = -V \frac{\partial u}{\partial x} + D \frac{\partial^2 u}{\partial x^2} - ku, \quad x > 0, \quad (3)$$

where  $u(x, t)$  is the density of motor complexes carrying an SVP at  $x$  at time  $t$ . ( $u$  satisfies the same equation as  $p$ , Eq. (2), with  $\chi_A = 1$  and  $\int_0^\infty u(x, t) dx$  the total number of particles along the axon at time  $t$ .) We now assume that there is a constant flux of particles injected at the end  $x = 0$ , so that Eq. (3) is supplemented by the boundary condition

$$-D \frac{\partial u(0, t)}{\partial x} + Vu(0, t) = J_1. \quad (4)$$

Let  $c(x, t)$  denote the density of vesicles delivered to the presynaptic targets with

$$\frac{\partial c}{\partial t} = ku(x, t) - \gamma_c c(x, t), \quad (5)$$

where  $\gamma_c$  is the rate of vesicle degradation within a presynaptic target. (If we were to neglect degradation of vesicles, then it would be necessary to impose, by hand, a maximum capacity of presynaptic targets, otherwise,  $c(x, t)$  could become unbounded.) A basic limitation of this model follows from the observation that the steady-state distribution of vesicles decays exponentially with respect to distance from the soma with a correlation length  $\bar{\xi}$ . That is,

$$c(x) = \frac{k}{\gamma_c} \frac{J_1 e^{-x/\bar{\xi}}}{D/\bar{\xi} + V}, \quad \bar{\xi} = \frac{2D}{-V + \sqrt{V^2 + 4Dk}}. \quad (6)$$

Taking the typical values  $D = 0.1 \mu\text{m s}^{-1}$  for cytoplasmic diffusion [12] and  $v = 1 \mu\text{m s}^{-1}$  for molecular motors [2], and assuming that  $k < 1 \text{ s}^{-1}$ , we see that  $\bar{\xi} \approx (4/k) \mu\text{m}$ . Thus, in order to have correlation lengths comparable to axonal lengths of several millimeters, we would require delivery rates of the order  $k \sim 10^{-4} \text{ s}^{-1}$ , whereas measured rates tend to be of the order of a few minutes [13,14]. This is a conservative estimate, since molecular crowding is likely to result in diffusivities several orders of magnitude smaller than assumed above. Moreover, Eq. (5) implies that reducing  $k$  results in a lower steady-state density of vesicles in presynaptic targets. [Note that, if  $V = 0$ , then Eq. (3) is identical in form to the cable equation with  $u(x, t)$  interpreted as the voltage along a passive dendrite [7]. If a constant current is injected at a point  $X$  in the dendrite, then the resulting steady-state voltage decays exponentially towards the soma. Thus, more distal regions of a passive dendrite have a much smaller effect on triggering an action potential [5,6].]

We will show that a more uniform distribution of presynaptic vesicles can be achieved by taking the delivery of SVPs to be reversible, as has been observed experimentally in a number of organisms [8,9]. This requires generalizing the above advection-diffusion model in order to keep track of motor complexes that are no longer carrying an SVP. Therefore, let  $u_0(x, t)$  and  $u_1(x, t)$  denote the density of motor complexes without and with an attached SVP, respectively, and denote the forward and backward rates for cargo delivery by  $k_+$   $k_-$ , respectively, see Fig. 2. The transport of each motor population is described by an advection-diffusion equation, but we now include the transitions between the two populations due to the reversible exchange of SVPs with presynaptic targets. Thus,

$$\frac{\partial u_0}{\partial t} = D \frac{\partial^2 u_0}{\partial x^2} - V_0 \frac{\partial u_0}{\partial x} - \gamma_0 u_0 - k_- c u_0 + k_+ u_1, \quad (7a)$$

$$\frac{\partial u_1}{\partial t} = D \frac{\partial^2 u_1}{\partial x^2} - V_1 \frac{\partial u_1}{\partial x} - \gamma_1 u_1 + k_- c u_0 - k_+ u_1, \quad (7b)$$

for  $x > 0$ . We are allowing for the possibility that the mean speed of a motor complex with or without cargo may differ. We have also included the degradation terms  $\gamma_j u_j$ ,  $j = 0, 1$ , which account for the fact that motor complexes may dysfunction and no longer exchange cargo with synaptic targets. [One could also include such a term in the one population model by taking  $k \rightarrow k + \gamma_u$  in Eq. (2).] Equations (7) are supplemented by the following boundary conditions at  $x = 0$ :

$$J[u_0(0, t)] = J_0, \quad J[u_1(0, t)] = J_1,$$

where  $J(u) = -D \partial_x u + vu$ . That is, motor complexes without and with cargo are injected at the somatic end  $x = 0$  at constant rates  $J_0$ , and  $J_1$ , respectively. It is important to emphasize that the injected motor complexes are not necessarily newly synthesized from the cell body. For it has been found, experimentally, that motor complexes recycle between the distal and somatic ends of the soma [8,9]. In the case of a finite axon, we could model recycling by imposing an absorbing boundary condition at

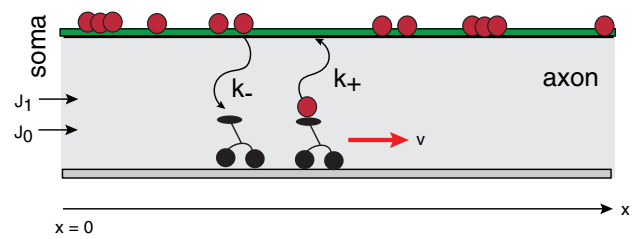


FIG. 2 (color online). Schematic diagram of the reversible exchange of vesicles between motor-cargo complexes and presynaptic targets. Each motor is modeled according to an advection-diffusion equation with average speed  $v$  and diffusivity  $D$ .

the distal end and reinjecting the distal flux into the somatic end. Since most of these complexes would be without an SVP, this would mainly contribute to  $J_0$ . Moreover, if the axon is much longer than the range of vesicular delivery necessary to supply *en passant* synapses, then the effects of the absorbing boundary can be ignored, and we can treat the axon as semi-infinite. Finally, in order to incorporate the reversible exchange between motor complexes and presynaptic targets, it is necessary to modify Eq. (5) according to

$$\frac{\partial c}{\partial t} = k_+ u_1(x, t) - k_- c(x, t) u_0(x, t) - \gamma_c c(x, t). \quad (8)$$

It is clear that, if  $k_- = 0$  and  $\gamma_c > 0$ , then we recover the previous single population model. Let us begin by considering the opposite limit for which  $k_- > 0$  and  $\gamma_c = 0$ . (The distribution  $c$  of presynaptic vesicles will remain bounded provided that  $J_0 > 0$ .) Equation (8) implies that, at steady state,

$$c(x) = \frac{k_+ u_1(x)}{k_- u_0(x)}, \quad (9)$$

Substituting Eq. (9) into the steady-state versions of Eqs. (7) then gives

$$u_j(x) = \frac{J_j e^{-x/\xi_j}}{D/\xi_j + V_j}, \quad \xi_j = \frac{2D}{-V_j + \sqrt{V_j^2 + 4D\gamma_u}}, \quad (10)$$

for  $j = 0, 1$ . Combining with Eq. (9) then yields the following result for the steady-state density of synaptic vesicles:

$$c(x) = \frac{k_+ J_1 D/\xi_0 + V_0}{k_- J_0 D/\xi_1 + V_1} e^{-\Gamma x}, \quad (11)$$

where  $\Gamma = \gamma_1^{-1} - \gamma_0^{-1}$ . In particular, if the transport properties of the motor complex are independent of whether or not it is bound to an SVP ( $V_0 = V_1$ ), then  $\gamma_0 = \gamma_1$ , and we have a uniform vesicle distribution  $c(x) = \bar{c} = (k_+/k_-)(J_1/J_0)$ .

To further explore the ability of this model to produce a democratic cargo distribution, we numerically solve Eqs. (7) for a range of parameter values. We focus on the biophysically relevant case in which  $\gamma_c$  is small (relative to  $k_{\pm}$ ) but nonzero. Hence, we consider how the normalized distribution  $c(x)/c(0)$  varies with  $\phi \equiv k_-/\gamma_c$ , which determines the proportion of vesicles that are recycled into the system after leaving the targets. Figure 3 displays the normalized concentration profiles for a variety of  $k_-/\gamma_c$  values with either  $J_0 = J_1$  or  $J_0 = 0$ . (We take the domain size to be much longer than  $600 \mu\text{m}$  in order to avoid boundary effects.) It can be seen that when  $J_0 > 0$ , the length scale over which nonexponential decay occurs is an increasing function of  $k_-/\gamma_c$ , while when  $J_0 = 0$  the model

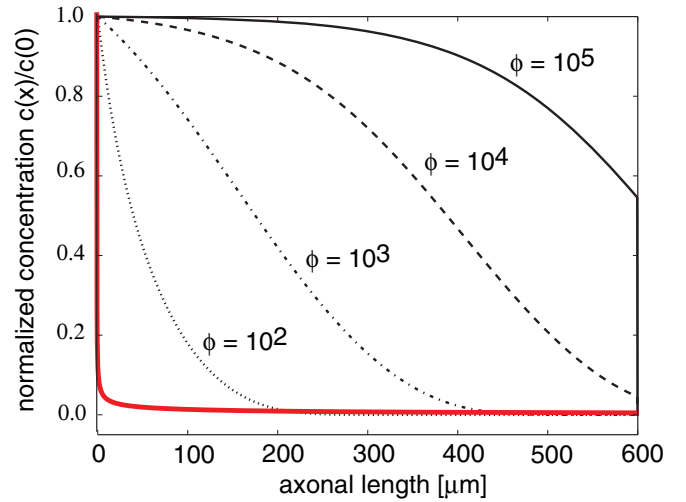


FIG. 3 (color online). Numerical solutions for steady state vesicle concentration as a function of axonal distance for different values of  $\phi = k_-/\gamma_c$  and  $J_0 = 1.5$ . For comparison, the corresponding concentration profile when  $J_0 = 0$  (which is insensitive to  $\phi$ ) is shown by the thick line (red line). We have also set  $\gamma_u = 10^{-2} \text{ s}^{-1}$ ,  $J_1 = 1.5$ ,  $k_+ = 0.5 \text{ s}^{-1}$ ,  $k_- = 1.0 \mu\text{m s}^{-1}$ ,  $v_0 = v_1 = 1 \mu\text{m s}^{-1}$  and  $D = 0.1 \mu\text{m}^2 \text{ s}^{-2}$ .

fails to distribute cargo across a substantial region of the axon. Hence, an additional component of a delivery mechanism that includes recapture is a source of motors which are able to receive vesicles. We emphasize that this does not require additional motors to be synthesized in the soma, instead, motors may return to the beginning of the axon after delivering their cargo. From the perspective of synaptic democracy, it seems desirable to maximize  $k_-$ ; however, increasing the recapture rate decreases the efficiency of the delivery mechanism and can result in an overall loss of vesicles due to motor degradation. The tradeoff that occurs between efficiency and equality can be seen in Fig. 4, where we have plotted the half-length ( $l_{1/2} = \max\{x: c(x) \geq 1/2\}$ ) as a function of  $k_-/k_+$  along with the total number of vesicles delivered at steady state. Our simulations confirm that these results are qualitatively insensitive to changes in all other parameter values.

To summarize, we have presented and analyzed a new model of vesicle delivery in axons by molecular motor transport. Our analysis suggests that a combination of stop-and-go transport and reversible interactions between motors and targets, both of which have been observed experimentally, provides a biophysically plausible mechanism for the democratic distribution of molecular cargo among synapses. We have also established that an increase in democracy comes at the expense of the quantity of cargo delivered and the ratio  $k_-/k_+$  serves as a measure of the efficiency of the delivery mechanism. While little is known about the specific mechanisms by which vesicles are removed from the motors and transported to the synapses, our results have identified some of the potential limitations

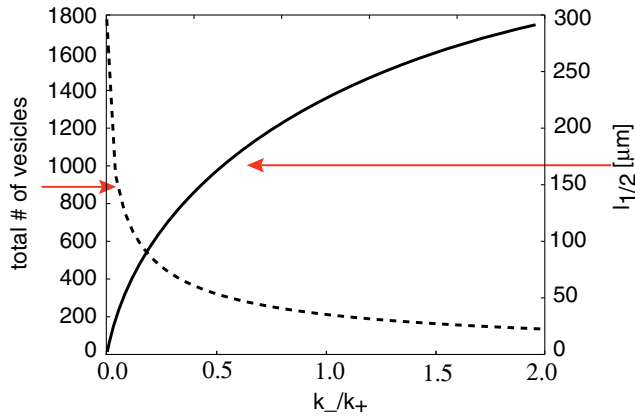


FIG. 4 (color online). The dashed line shows the total number of vesicles delivered, while the solid line shows the half-length  $l_{1/2}$  of the concentration profile. The norm of  $(k_-/L, k_+)$  is fixed at 0.5, but we find that the results are qualitatively independent of this quantity. Other parameter values as Fig. 3 with  $\gamma_c = 10^{-3}$ .

of any such mechanism. We also expect similar issues to apply to other intracellular transport processes, including vesicular transport in dendrites. Our model is based on experimental data regarding the nature of intracellular transport and the fact that neurons can maintain functional synapses at distal ends of axons and dendrites. One experimental test of our model would be to measure the parameter  $\phi$ , since we assumed  $\phi$  is sufficiently large, see Fig. 3. Another way to test the predictions of our model would be to pharmacologically manipulate the ratio  $k_-/k_+$  and to see how that affects the distribution of some green fluorescent protein-labelled synaptic protein.

There are several possible extensions of our model. First, each motor could be allowed to carry more than one SVP (or cluster of SVPs). We would then need to consider a multipopulation model with  $u_n, n = 0, 1, \dots, N$  denoting the concentration of motors carrying  $n$  SVPs and  $N$  the maximum capacity of a motor. Second, we could consider an inhomogeneous distribution of presynaptic targets. One source of inhomogeneity reflects the fact that *en passant* synapses are only distributed over a subinterval of an axon. Following Maeder *et al.* [9], this could be incorporated by considering a compartmental model of the axon, in which an *en passant* compartment is sandwiched between a proximal and a distal compartment, neither of which have

synaptic targets. As shown by Maeder *et al.* [9], the total number of vesicles delivered to the *en passant* compartment is sensitive to the distribution of motor velocities in the surrounding compartments. Another source of inhomogeneity is the discrete nature of individual presynaptic targets, the effects of which can be tackled using homogenization theory [15]. Finally, we could use a compartmental model to explicitly model the effects of motor recycling in order to determine its contribution to the currents  $J_0, J_1$ .

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