How old are dense-core vesicles residing in *en passant* boutons: simulation of the mean age of dense-core vesicles in axonal arbours accounting for resident and transiting vesicle populations

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In neurons, neuropeptides are synthesized in the soma and are then transported along the axon in dense-core vesicles (DCVs). DCVs are captured in varicosities located along the axon terminal called *en passant* boutons, which are active terminal sites that accumulate and release neurotransmitters. Recently developed experimental techniques allow for the estimation of the age of DCVs in various locations in the axon terminal. Accurate simulation of the mean age of DCVs in boutons requires the development of a model that would account for resident, transiting-anterograde and transiting-retrograde DCV populations. In this paper, such a model is developed. The model is applied to simulating DCV transport in *Drosophila* type II motoneurons. The model simulates DCV transport and capture in the axon terminals and makes it possible to predict the age density distribution of DCVs in *en passant* boutons as well as DCV mean age in boutons. The predicted prevalence of older organelles in distal boutons may explain the ‘dying back’ pattern of axonal degeneration observed in dopaminergic neurons in Parkinson’s disease. The predicted difference of two hours between the age of older DCVs residing in distal boutons and the age of younger DCVs residing in proximal boutons.

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is consistent with an approximate estimate of age difference deduced from experimental observations. The age density of resident DCVs is found to be bimodal, which is because DCVs are captured from two transiting states: the anterograde transiting state that contains younger DCVs and the retrograde transiting state that contains older DCVs.

1. Introduction

Neuropeptides play an important role in the regulation of mood, motivation, sleep and drug addiction [1]. Alterations in neuropeptide Y levels may be linked to neurodegenerative and neuroimmune diseases [2]. Neuropeptides are synthesized in the neuron body and then are transported in dense-core vesicles (DCVs) through the axon towards the axon terminals (figure 1a). Anterograde transport of DCVs is driven by kinesin-1 and kinesin-3 motors while retrograde transport is driven by cytoplasmic dynein motors [4].

*Drosophila melanogaster* is a popular model for investigating DCV transport [3,5–7]. The above papers investigated DCV transport in axons of *Drosophila* motoneurons with type I, II and III endings. These axonal endings have different morphologies with different numbers and sizes of *en passant* boutons (hereafter boutons). Boutons are varicosities located along the axon terminals; they are sites that accumulate and release neurotransmitters. Type II terminals have the largest number of boutons in *Drosophila*. The neurons that produce type II boutons in *Drosophila* and the neurons that die in humans with Parkinson’s disease (PD) share two properties: they are monoaminergic, which confers sensitivity to autooxidation, and they have extensive axonal arbours [7,8]. Thus a study of neurons with type II terminals may be useful to better understand PD.

Understanding the age distribution of organelles in axons is important because older organelles may have impaired function due to accumulated oxidative damage [9]. Investigating the DCV age distribution may also give clues on how the DCV addressing and delivery system to boutons is designed [3]. Investigating the DCV mean age distribution in various boutons of type II terminals, Levitan and colleagues [7] used a photoconvertible construct that switches from green to red fluorescence over a period of hours (red corresponds to older DCVs and green corresponds to younger DCVs). Their results demonstrate that in type II terminals, DCVs residing in distal boutons are older than those residing in proximal boutons.

Models simulating DCV transport in type Ib and type III axon terminals were developed in our previous papers [10–13]. The model of DCV transport in type II terminals, which contain a much larger number of boutons, was developed in [14]. We established that a model that simulates only the resident DCV population cannot correctly predict experimental results of [7] with respect to DCV age (older DCVs in more distal boutons). It was proposed that a model capable of simulating the resident and transiting DCV populations needs to be developed. In the present paper, we develop a model that simulates resident, transiting-anterograde and transiting-retrograde DCV populations and apply it to the analysis of DCV age distribution in type II terminals.

2. Material and models

(a) Governing equations

We simulated a terminal with 26 boutons (figure 1a). The model developed in this paper accounts for DCV concentrations in two different types of kinetic states in each bouton, the transiting and resident states. In the resident states, DCVs stay captured in boutons while in the transiting states DCVs can move between the boutons (figure 1b). The transiting states are further divided into anterograde and retrograde (figure 2). This is done for all transiting states except in bouton 1 (because DCVs turn around in bouton 1, this transiting state in neither anterograde nor retrograde). Our previous model, developed in [14], accounts only for DCVs in the resident
states and contains 26 ordinary differential equations (ODEs). The new model accounts for 26 residents and 51 transiting kinetic states and contains 77 ODEs. There are coupling terms between the transiting and resident DCVs in the new model (a transiting DCV can be captured and become a resident DCV, and then it can be re-released and become a transiting DCV again). Therefore, we need to state the governing equations for the new model.

We numbered the boutons in the axon terminal from the most distal #1 to the most proximal #26, following the convention introduced in [3] (figure 1a). Since the length of an axon is much greater than its width, we used the linear number density to characterize the DCV concentration.
We thus defined the DCV concentration as the number of DCVs (resident or transiting) per unit length of the axon.

We used a multi-compartment model [15–17] to formulate equations expressing the conservation of the number of DCVs in the compartments. Most boutons have three compartments (corresponding to resident, transiting-anterograde and transiting-retrograde states) while bouton 1 has two compartments (corresponding to resident and transiting states), see Figure 2. The number of DCVs is the conserved quantity.

The conservation of resident DCVs in the most proximal bouton gives the following equation (Figure 2):

\[
L_{26} \frac{dn_{26}}{dt} = \min[h_{26}^r(n_{sat0,26} - n_{26}, j_{ax\rightarrow26}) + \min[h_{26}^r(n_{sat0,26} - n_{26}, j_{25\rightarrow26})] - L_{26} \frac{n_{26} \ln(2)}{T_{1/2}},
\]

where \( n_i \) is the concentration of resident DCVs in bouton \( i \) \((i = 1, \ldots, 26); t \) is the time; \( n_{sat0,i} \) is the saturated concentration of resident DCVs in bouton \( i \) at infinite DCV half-life or at infinite DCV residence time \((i = 1, \ldots, 26); L_i \) is the length of a compartment occupied by bouton \( i \) (defined in Figure 1a, \( i = 1, \ldots, 26); h_i^a \) and \( h_i^r \) are the mass transfer coefficients characterizing the rates of DCV capture into the resident state in bouton \( i \) \((2, \ldots, 26)\) as DCVs pass through bouton \( i \) in the anterograde and retrograde directions, respectively; \( j_{ax\rightarrow26} \) is the anterograde flux of new DCVs from the axon to the most proximal bouton \((#26)\); and \( T_{1/2} \) is the half-life or half-residence time of DCVs captured into the resident state in boutons. In terms of DCV conservation in the resident states in boutons, it does not matter whether DCVs (and their content) are destroyed or released back to the transiting pool. If DCVs are destroyed (this case also includes the situation of DCV consumption in boutons due to neuropeptide release by exocytosis), \( T_{1/2} \) is interpreted as the half-life. If DCVs are released back to circulation, \( T_{1/2} \) is interpreted as the half-residence time. At a first approximation, we assume that processes such as DCV destruction in boutons, DCV consumption due to neuropeptide exocytosis, and DCV release back to circulation are described by linear kinetics, and hence can be modelled by a term similar to the last term on the right-hand side of equation (2.1) (the term involving \( T_{1/2} \)).

The term \( \min[h_{26}^r(n_{sat0,26} - n_{26}, j_{ax\rightarrow26}) \) on the right-hand side of equation (2.1) simulates the fact that the rate at which a bouton captures anterogradely moving DCVs cannot exceed the flux of anterogradely moving DCVs into the bouton. A similar role is played by the term \( \min[h_{26}^r(n_{sat0,26} - n_{26}, j_{25\rightarrow26}) \).
Stating the conservation of anterograde transiting DCVs in the most proximal bouton results in the following equation (figure 2):
\[
L_{26} \frac{d n_{26,t}}{dt} = j_{25 \rightarrow 26} - j_{26 \rightarrow ax} - \min[h^d_{26}(n_{sat0, 26} - n_{26}), j_{ax \rightarrow 26}] + \varepsilon \delta L_{26} \frac{n_{26} \ln(2)}{T_{1/2}},
\] (2.2)
where \(n_{26,i}^a\) is the concentration of anterograde transiting DCVs in bouton \(i (i = 2, \ldots, 26)\), \(j_{j \rightarrow k}\) is the flux of DCVs from compartment \(j\) to compartment \(k\) (figure 1b), \(j_{26 \rightarrow ax}\) is the retrograde flux of DCVs from the most proximal bouton back to the axon and \(\delta\) is the parameter which determines what happens to DCVs captured into the resident state of the boutons. \(\delta = 0\) means that all DCVs are eventually destroyed in boutons (or the DCV content leaves the boutons by spontaneous exocytotic events) while \(\delta = 1\) means that DCVs, after spending some time in the resident state, are released back to the transiting pool.

In equation (2.2), \(\varepsilon\) is the parameter indicating how DCVs released from the resident state are split between the anterograde and retrograde components. DCVs released from the resident state first join the transiting state in the corresponding bouton. \(\varepsilon\) is the portion of released DCVs that join the anterograde component and \((1 - \varepsilon)\) is the portion of DCVs that join the retrograde component.

The term release is used in our model as a term opposing capture. In biological literature, release also refers to releasing the contents inside of vesicles to the extracellular milieu by exocytosis. This is different from release of a captured DCV back to the transiting pool, which is the meaning of the term release in the context of our model.

Stating the conservation of retrograde transiting DCVs in the most proximal bouton gives the following equation (figure 2):
\[
L_{26} \frac{d n_{26,r}}{dt} = j_{25 \rightarrow 26} - j_{26 \rightarrow ax} - \min[h^r_{26}(n_{sat0, 26} - n_{26}), j_{ax \rightarrow 26}] + (1 - \varepsilon) \delta L_{26} \frac{n_{26} \ln(2)}{T_{1/2}},
\] (2.3)
where \(n_{26,i}^r\) is the concentration of retrograde transiting DCVs in bouton \(i (i = 2, \ldots, 26)\).

In boutons 25 through 2, stating the conservation of resident DCVs gives the following equations (figure 2):
\[
L_i \frac{d n_i}{dt} = \min[h^r_l(n_{sat0,i} - n_i), j_{i+1 \rightarrow i}] + \min[h^r_l(n_{sat0,i} - n_i), j_{i-1 \rightarrow i}] - L_i \frac{n_i \ln(2)}{T_{1/2}} (i = 25, \ldots, 2).
\] (2.4)

Stating the conservation of anterograde transiting DCVs in boutons 25 through 2 gives the following equations (figure 2):
\[
L_i \frac{d n_{i,t}}{dt} = j_{i+1 \rightarrow i} - j_{i-1 \rightarrow i} - \min[h^a_l(n_{sat0,i} - n_i), j_{i+1 \rightarrow i}] + \varepsilon \delta L_i \frac{n_i \ln(2)}{T_{1/2}} (i = 25, \ldots, 2).
\] (2.5)

Also, stating the conservation of retrograde transiting DCVs in boutons 25 through 2 gives the following equations (figure 2):
\[
L_i \frac{d n_{i,r}}{dt} = j_{i-1 \rightarrow i} - j_{i \rightarrow i+1} - \min[h^r_l(n_{sat0,i} - n_i), j_{i-1 \rightarrow i}] + (1 - \varepsilon) \delta L_i \frac{n_i \ln(2)}{T_{1/2}} (i = 25, \ldots, 2).
\] (2.6)

Stating the conservation of resident DCVs in the most distal bouton gives the following equation (figure 2):
\[
L_1 \frac{d n_1}{dt} = \min[h_l(n_{sat0,1} - n_1), j_{2 \rightarrow 1}] - L_1 \frac{n_1 \ln(2)}{T_{1/2}},
\] (2.7)
where \(h_l\) is the mass transfer coefficient characterizing the rate of DCV capture into the resident state in bouton 1 (DCVs pass bouton 1 only once).

There are no separate anterograde and retrograde transiting states in bouton 1 because DCVs turn around in this bouton. The conservation of transiting DCVs in the most distal bouton is used
Table 1. Approximate time, $t_{\infty}$, required for resident DCVs to reach a steady-state concentration in four representative boutons (1, 5, 13, and 26). We assumed that steady state is reached when the DCV concentration in a particular bouton reaches 99% of $n_{sat,i}$, which is defined in electronic supplementary material, equations (S4) and (S5). Since the DCV fluxes in and out of the transiting states are assumed to be the same, concentrations of transiting DCVs stay constant (equal to the value postulated by equation (2.13)) throughout the process of filling the terminal. It should be noted that the duration of Drosophila third instar (used in experiments of [7]) is approximately 48 h. $\delta = 1$.

| $n_{sat}$ | $\varepsilon$ | $t_{\infty}$ for $n_1$ (h) | $t_{\infty}$ for $n_5$ (h) | $t_{\infty}$ for $n_{13}$ (h) | $t_{\infty}$ for $n_{26}$ (h) |
|-----------|--------------|-----------------|-----------------|-----------------|-----------------|
| 1         | 0.8          | 1.82            | 39.42           | 19.99           | 7.58            |
| 0.1       | 0.5          | 1.82            | 39.42           | 19.99           | 7.58            |
| 1         | 0            | 1.82            | 39.42           | 20.02           | 7.49            |
| 1         | 1            | 1.82            | 39.42           | 19.98           | 7.60            |

to produce the following equation (figure 2):

$$L_1 \frac{dn_{1,t}}{dt} = j_{2\rightarrow 1} - j_{1\rightarrow 2} - \min[h_{1}(n_{sat,0,1} - n_{1}), j_{2\rightarrow 1}] + \delta L_1 \frac{n_1 \ln(2)}{T_{1/2}},$$  (2.8)

where $n_{1,t}$ is the concentration of transiting DCVs in bouton 1. In this paper, we assumed that $j_{ax\rightarrow 26}$ remains constant during the process of filling the terminal. Modelling the time dependence of $j_{ax\rightarrow 26}$ would require simulating the DCV concentration in the axon, which can be done as described in electronic supplementary material, S1.

Equations (2.1)–(2.8) include fluxes in the terminal (figure 2), which need to be modelled. The DCV fluxes have units of vesicles per second. The anterograde flux of transiting DCVs between the most proximal bouton (bouton 26) and bouton 25 is simulated as follows:

$$j_{26 \rightarrow 25} = j_{ax \rightarrow 26} - \min[h_{26}^j(n_{sat,0,26} - n_{26}), j_{ax \rightarrow 26}] + \varepsilon \delta L_26 \frac{n_{26} \ln(2)}{T_{1/2}}. $$  (2.9)

We then used the following equations to model anterograde fluxes between boutons 25 through 2:

$$j_{i \rightarrow i-1} = j_{i+1 \rightarrow i} - \min[h_{i}^j(n_{sat,0,i} - n_{i}), j_{i+1 \rightarrow i}] + \varepsilon \delta L_i \frac{n_i \ln(2)}{T_{1/2}} \quad (i = 25, \ldots, 2).$$  (2.10)

We modelled the retrograde flux from bouton 1 into bouton 2 by the following equation:

$$j_{1 \rightarrow 2} = H[t - t_1]j_{2 \rightarrow 1} - \min[h_{1}(n_{sat,0,1} - n_{1}), j_{2 \rightarrow 1}] + \delta L_1 \frac{n_1 \ln(2)}{T_{1/2}},$$  (2.11)

where $H$ is the Heaviside step function and $t_1$ is the time required for DCVs to change the direction in the most distal bouton if they are not captured. The Heaviside step function thus delays the onset of retrograde DCV flux by the time $t_1$. The effect of this initial delay is expected to be small because $t_1 = 300 \text{ s}$ (electronic supplementary material, table S1), which is small compared with how long it takes to fill the boutons with DCVs (hours), see table 1.

We modelled retrograde fluxes between boutons 2 through 25 by using the following equations:

$$j_{i \rightarrow i+1} = H[t - t_1]j_{i-1 \rightarrow i} - \min[h_{i}^j(n_{sat,0,i} - n_{i}), j_{i-1 \rightarrow i}] + (1 - \varepsilon) \delta L_i \frac{n_i \ln(2)}{T_{1/2}} \quad (i = 2, \ldots, 25).$$  (2.12)

The retrograde flux leaving the terminal from the most proximal bouton is modelled as

$$j_{26 \rightarrow ax} = H[t - t_1]j_{25 \rightarrow 26} - \min[h_{26}^j(n_{sat,0,26} - n_{26}), j_{25 \rightarrow 26}] + (1 - \varepsilon) \delta L_{26} \frac{n_{26} \ln(2)}{T_{1/2}}.$$  (2.13)

Equations (2.1)–(2.8) describe a system of 77 first-order ODEs; 77 initial conditions are thus required.
We assumed that initially there are no DCVs in the resident state in the terminal
\[ n_1(0) = 0, \ldots, n_{26}(0) = 0. \] (2.14)

We also assumed that the initial DCV concentration in the transiting states is constant and uniform
\[ n_{1,t}(0) = n_{0,t}, n_{2,t}(0) = n_{0,t}, \ldots, n_{26,t}(0) = n_{0,t}, n_{26,t}(0) = n_{0,t}. \] (2.15)

We investigated the sensitivity of the solution to various values of \( n_{0,t}. \) Values of parameters involved in the model are estimated in electronic supplementary material, S2.

(b) Model of age distribution of dense-core vesicles and mean age of dense-core vesicles in boutons

We used the method developed in [18,19] to compute the age distributions in the resident and transiting states in boutons. We recast governing equations (2.1)–(2.8) into the following matrix form:
\[ \frac{d}{dt} \mathbf{N}^*(t) = \mathbf{B}(\mathbf{N}^*(t), t) \mathbf{N}^*(t) + \mathbf{u}(t), \] (2.16)

where \( \mathbf{N}^* \) is the state vector defined by the following components:
\[ N_1^* = n_1 L_1, \ldots, N_{26}^* = n_{26} L_{26}, N_{27}^* = n_{1,t} L_1, N_{28}^* = n_{2,t} L_2, \ldots, \]
\[ N_{52}^* = n_{26,t} L_{26}, N_{53}^* = n_{2,t} L_2, N_{77}^* = n_{26,t} L_{26}. \] (2.17)

The first 26 components of the state vector represent the number of DCVs in the resident states and the last 51 components represent the number of DCVs in the transiting states (figure 2 and electronic supplementary material, section S3). Vector \( \mathbf{u}(t) \) is defined in equations (2.41) and (2.42) below.

Matrix \( \mathbf{B}(77,77) \) in our case is as follows. In simulating DCV fluxes in the most proximal bouton (the left-hand side diagram in figure 2), we accounted for the internal fluxes between the compartments, the external DCV flux entering the terminal from the axon, the DCV flux leaving the terminal back to the axon, as well as possible destruction of DCVs in the resident state in boutons. Based on the analysis of the DCV fluxes to and from the resident and transiting states in the most proximal bouton, equations for the following elements of matrix \( \mathbf{B} \) were obtained:
\[ b_{26,26} = -L_{26} \frac{n_{26} \ln(2)}{T_{1/2}} / (L_{26} n_{26}) = -\frac{\ln(2)}{T_{1/2}}, \] (2.18)
\[ b_{52,26} = \epsilon \delta \frac{\ln(2)}{T_{1/2}}, \] (2.19)
\[ b_{77,26} = (1 - \epsilon) \delta \frac{\ln(2)}{T_{1/2}}, \] (2.20)
\[ b_{52,52} = -\min\{h^d_{26}(n_{\text{sat}0,26} - n_{26}), j_{\text{ax} \rightarrow 26}\} + j_{26 \rightarrow 25}, \] (2.21)
\[ b_{26,52} = \frac{\min\{h^d_{26}(n_{\text{sat}0,26} - n_{26}), j_{\text{ax} \rightarrow 26}\}}{L_{26} n_{26,t}}, \] (2.22)
\[ b_{77,76} = \frac{j_{25 \rightarrow 26}}{L_{25} n_{25,t}}, \] (2.23)
\[ b_{26,77} = \frac{\min\{h^r_{26}(n_{\text{sat}0,26} - n_{26}), j_{25 \rightarrow 26}\}}{L_{26} n_{26,t}}, \] (2.24)
\[ b_{77,77} = -\frac{\min\{h^r_{26}(n_{\text{sat}0,26} - n_{26}), j_{25 \rightarrow 26}\} + j_{26 \rightarrow \text{ax}}}{L_{26} n_{26,t}}. \] (2.25)
By repeating a similar analysis for bouton \( i \) (\( i = 2, \ldots, 25 \)) (the middle diagram in figure 2), equations for the following elements of matrix \( B \) were obtained:

\[
b_{i,i} = - L_i n_i \ln(2) / (L_i n_i) = - \ln(2) / T_{1/2},
\]

\[
b_{i+26,i} = \varepsilon \delta \ln(2) / T_{1/2},
\]

\[
b_{i+51,i} = (1 - \varepsilon) \delta \ln(2) / T_{1/2},
\]

\[
b_{i+26,i+26} = - \min[h^d_i(i_{sat0,i} - n_i), j_{i+1 \rightarrow i}] + j_{i \rightarrow i-1}
\]

\[
b_{i,i+26} = \frac{\min[h^d_i(i_{sat0,i} - n_i), j_{i+1 \rightarrow i}]}{L_i n_i},
\]

\[
b_{i,i+51} = \frac{\min[h^d_i(i_{sat0,i} - n_i), j_{i-1 \rightarrow i}]}{L_i n_i},
\]

\[
b_{i+51,i+51} = - \frac{\min[h^d_i(i_{sat0,i} - n_i), j_{i-1 \rightarrow i}] + j_{i \rightarrow i+1}}{L_i n_i},
\]

and

\[
b_{i+26,i+1+26} = \frac{j_{i+1 \rightarrow i}}{L_{i+1} n_{i+1}},
\]

Additionally, the element \( b_{53,27} \) is assigned the following value (note that the flux \( j_{1 \rightarrow 2} \) leaves the compartment that simulates the transiting state in bouton 1 (with DCV concentration \( n_{1,t} \)), and enters the compartment that simulates the transiting-retrograde state in bouton 2 (with DCV concentration \( n_{2,t} \)), figure 2):

\[
b_{53,27} = \frac{j_{1 \rightarrow 2}}{L_1 n_{1,t}}.
\]

For \( i = 3, \ldots, 25 \), the element \( b_{i+51,i-1+51} \) is assigned the following value:

\[
b_{i+51,i-1+51} = \frac{j_{i-1 \rightarrow i}}{L_{i-1} n_{i-1,t}}.
\]

Finally, by repeating a similar analysis for the most distal bouton (the right-hand side diagram in figure 2), the following equations were obtained:

\[
b_{1,1} = - L_1 n_1 \ln(2) / (L_1 n_1) = - \ln(2) / T_{1/2},
\]

\[
b_{27,1} = \delta \ln(2) / T_{1/2},
\]

\[
b_{1,27} = \frac{\min[h_1(n_{sat0,1} - n_1), j_{2 \rightarrow 1}]}{L_1 n_{1,t}},
\]

\[
b_{27,27} = - \frac{\min[h_1(n_{sat0,1} - n_1), j_{2 \rightarrow 1}] + j_{1 \rightarrow 2}}{L_1 n_{1,t}}
\]

and

\[
b_{27,28} = \frac{j_{2 \rightarrow 1}}{L_2 n_{2,t}}.
\]

All other elements of matrix \( B \) are equal to zero.

The only flux entering the terminal is the anterograde flux from the axon to the most proximal bouton, \( j_{ax \rightarrow 26} \). We assumed that all DCVs which leave the terminal (their flux is described by \( j_{26 \rightarrow ax} \)) return to the soma for degradation, and that none of them reenter the terminal (allowing some DCVs to reverse direction near the soma, as has been seen in motor neurons [3], would shift the age distribution of DCVs in boutons towards an older age, but would not qualitatively
affect the results). Thus, the DCVs that enter the terminal (their flux is described by $j_{\text{ax}\rightarrow 26}$) are all newly synthesized in the soma, and their age at the time of entry to the terminal is set to zero. This means that our simulations neglect the time that it takes for DCVs to transit from the soma to the terminal. Thus, the DCV age computed here should be interpreted as the age of DCVs since their entry into the terminal. The 52nd element of vector $u$ is then given by the following equation:

$$u_{52} = j_{\text{ax}\rightarrow 26}. \quad (2.41)$$

Since no other external fluxes enter the terminal,

$$u_i = 0 \quad (i = 1, \ldots, 51, 53, \ldots, 77). \quad (2.42)$$

According to [18], the state transition matrix, $\Phi$, can be found by solving the following matrix equation:

$$\frac{d}{dt} \Phi(t, t_0) = B(N^*(t), t)\Phi(t, t_0). \quad (2.43)$$

Equation (2.43) must be solved subject to the following initial condition:

$$\Phi(t_0, t_0) = I, \quad (2.44)$$

where $I$ denotes an identity matrix.

We assumed that initially the resident states in boutons do not contain any DCVs and that all DCVs in the transiting states are new. We also assumed that all DCVs entering the terminal are new. Then the age density of DCVs that entered the terminal after $t = 0$ can be calculated as

$$p(c, t) = 1_{[0, t - t_0)}(c)\Phi(t, t - c)u(t - c), \quad (2.45)$$

where $1_{[0, t - t_0)}$ is the indicator function which is equal to 1 if $0 \leq c < t - t_0$, otherwise, $1_{[0, t - t_0)}$ equals 0. The age density of DCVs can be understood as the ratio of the number of DCVs having an age between $T$ and $T + dT$ over the duration of this interval $dT$. The integral from 0 to infinity with respect to time of the age density of DCVs in a certain kinetic state gives the number of DCVs in this kinetic state. More generally, an integral over any time period gives the number of DCVs having an age within that time range.

Following [20], the mean age of DCVs in boutons is defined as

$$\bar{c}_i(t) = \int_0^\infty \frac{cp_i(c, t)dc}{\int_0^\infty p_i(c, t)dc} \quad (i = 1, \ldots, 77), \quad (2.46)$$

where $1_{[0, t - t_0)}$ is the indicator function which is equal to 1 if $0 \leq c < t - t_0$, otherwise, $1_{[0, t - t_0)}$ equals 0. The age density of DCVs can be understood as the ratio of the number of DCVs having an age between $T$ and $T + dT$ over the duration of this interval $dT$. The integral from 0 to infinity with respect to time of the age density of DCVs in a certain kinetic state gives the number of DCVs in this kinetic state. More generally, an integral over any time period gives the number of DCVs having an age within that time range.

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where $1_{[0, t - t_0)}$ is the indicator function which is equal to 1 if $0 \leq c < t - t_0$, otherwise, $1_{[0, t - t_0)}$ equals 0. The age density of DCVs can be understood as the ratio of the number of DCVs having an age between $T$ and $T + dT$ over the duration of this interval $dT$. The integral from 0 to infinity with respect to time of the age density of DCVs in a certain kinetic state gives the number of DCVs in this kinetic state. More generally, an integral over any time period gives the number of DCVs having an age within that time range.

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$$\bar{c}_i(t) = \int_0^\infty \frac{cp_i(c, t)dc}{\int_0^\infty p_i(c, t)dc} \quad (i = 1, \ldots, 77), \quad (2.46)$$

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3. Results

(a) Assumptions concerning the fate of dense-core vesicles captured into the resident state in boutons

In this paper, we investigate two possible scenarios with respect to DCV destruction (consumption) in boutons. (i) $\delta = 1$. This scenario simulates the situation when all captured DCVs, after spending some time in the resident state, are re-released back to the transiting state. This scenario is supported by the scarcity of the organelle degradation machinery in axons (E. S. Levitan 2017, personal communication), which suggests that instead of being destroyed in boutons, captured DCVs may be released back to the transiting pool and re-enter the circulation. Old DCVs may then exit the circulation and travel back to the soma, where they may be destroyed in lysosomes, which are plentiful in the soma. In this scenario, since older DCVs are returning to the transiting state, the average age of DCVs is expected to increase faster from the proximal to distal boutons than in the second scenario. The increase of the DCV age from proximal to distal boutons is supported by experimental observations reported in [7]. The results for $\delta = 1$ are reported in figures 3–6 and electronic supplementary material, S4–S7. It should be noted that the results for the DCV concentrations in the resident states in boutons, shown in figures 3 and 4 and in table 1, are almost independent of the value of $\delta$ because it does not matter how DCVs leave the resident state, by re-release of DCVs back to the transiting state or by DCV destruction. The value of $\delta$ has some effect at small times because capture into the resident state is limited by the number of DCVs that enter the boutons, and DCV fluxes between the boutons are larger for $\delta = 1$ than for $\delta = 0$. (ii) $\delta = 0$. This scenario simulates the situation in which all captured DCVs are eventually destroyed or consumed in boutons. This scenario is supported by the following argument. Neuropeptides released from DCVs may be marked by ubiquitination for degradation in proteasomes. Also, neuropeptides may be released from boutons by spontaneous secretory events, which will also lead to DCV consumption in boutons. The results for $\delta = 0$ are reported in electronic supplementary material, figures S2 and S3.

(b) Verifying values to which concentrations of resident dense-core vesicles converge as $t \to \infty$

The solution of equations (2.1)–(2.8) was verified by comparing DCV concentrations in the resident states at $t \to \infty$ (at steady state) with the estimates of these parameters given by electronic supplementary material, equations (S4) and (S5). The concentrations converge to correct estimates of steady-state values (figure 3a). Also, figure 3a shows that we were able to incorporate into our model the drop-off in the DCV content (reported in [7]) that appeared suddenly at the farthest ends of the arbour. The model suggests that the drop-off could be explained by the assumption that approximately 20% of the most distal boutons are characterized by different parameter values. According to our hypothesis, these boutons have more limited capacity than other boutons; compare equations (S4) and (S5) given in the electronic supplementary material.

Another possible explanation is that the drop-off is caused by a depletion of the anterograde flux so that the most distal boutons do not receive enough DCVs. The fact that the drop-off distally is so sudden (figure 3a) may be indicative of an instability caused by an imbalance between anterograde and retrograde DCV fluxes. This hypothesis is supported by an observation that the drop-off does not occur in all branches, but rather is more common in the most distal branches [7].

More experimental research is needed in order to understand whether the position of the drop-off advances over time or remains between the same boutons (in our case, between boutons 5 and 4). If the latter is true, the explanation of the drop-off by limited capacity of the farthest boutons is plausible. An interesting physiological question is whether the drop-off means that the most distal boutons remain unused for neuropeptide release.
Figure 3. (a) Saturated DCV concentrations in the resident state in boutons, from the most proximal (#26) to the most distal (#1) bouton. Estimated values of saturated concentrations, calculated using equations (S4) and (S5) given in the electronic supplementary material, are compared with numerically obtained values of these concentrations (obtained from the numerical solution of equations (2.1)–(2.8) with boundary conditions (2.14), (2.15) at $t \to \infty$). $n_{0,t} = 1$ and $\varepsilon = 0.8$. Results are independent of the value of $\delta$. (b) DCV concentrations in the resident state at different times. $\delta = 1$, $n_{0,t} = 1$ and $\varepsilon = 0.8$. (Online version in colour.)

We assumed that the rate at which DCVs enter an anterograde transiting state of a bouton equals the rate at which DCVs leave the anterograde transiting state, see equations (2.2) and (2.5) and figure 2. The same is assumed about the retrograde transiting state, see equations (2.3) and (2.6) and figure 2. Also, the same is assumed about the transiting state in bouton 1, see equation (2.8) and figure 2. Therefore, the DCV concentrations in the transiting states are equal to their
Figure 4. The buildup towards steady state: concentrations of DCVs in the resident state in various boutons. (a) Boutons 26 through 14. (b) Boutons 13 through 1. $\delta = 1$, $n_{0,t} = 1$ and $\varepsilon = 0.8$. (Online version in colour.)

initial concentrations, $n_{0,t}$, over the whole duration of the process of filling the terminal with DCVs. Values of $n_{0,t}$, postulated in equation (2.15), are the same for all boutons (data not shown).

To check the order in which the boutons are filled, we plotted concentrations of resident DCVs in boutons at three representative times ($t = 1, 2$ and $5\, \text{h}$). The results reported in figure 3b agree with [7] which reported that in type II terminals DCVs first accumulate in proximal boutons; accumulation in distal boutons occurs much slower (and later) than in proximal boutons. It is interesting that the modelling results show that this behavior is more pronounced later in the process. A dip in the curve for $t = 1\, \text{h}$, for example, suggests that in the beginning some boutons (for example, bouton 19) accumulate fewer DCVs than more distal boutons (figure 3b).
Figure 5. (a) Age density of DCVs in the resident state in various boutons at steady state. (b) Age density of DCVs in the anterograde transiting state in various boutons at steady state. (c) Age density of DCVs in the retrograde transiting state in various boutons at steady state. $\delta = 1$, $n_{0,t} = 1$ and $\varepsilon = 0.8$. Age density is shown in every second bouton to make the figure less cluttered. (Online version in colour.)

(c) The buildup of dense-core vesicle concentrations in the resident and transiting states in boutons

It takes less than 40 h for the DCV concentrations in the resident state in boutons to reach their steady-state values. Interestingly, bouton 5 takes the longest amount of time to fill while bouton 1 takes the shortest amount of time (figure 4 and table 1). This is because we explained the drop-off in the DCV content, reported in [7], by assuming a smaller DCV capacity of the four most distal boutons (electronic supplementary material, equation (S5)). Since capacity of boutons 1–4 is assumed to be small, it takes a short time to fill them. Bouton 5 is the most distal bouton with a large capacity, and since the anterograde flux is reduced with distance by capture in more proximal boutons, it takes the longest time to fill bouton 5. The time to reach steady-state concentration in the resident state does not depend on the value of $n_{0,t}$ and only slightly depends on the value of $\varepsilon$ (table 1).

(d) Dense-core vesicle age density distributions and mean dense-core vesicle ages for the case when all captured dense-core vesicles are re-released to the circulation, $\delta = 1$

At steady state, the age of resident vesicles is distributed between 0 and 60 h. The age density of resident DCVs is bimodal (figure 5a), which occurs because DCVs are captured into the resident state from anterograde (younger) and retrograde (older) transiting pools (figure 2).

The age density of transiting DCVs exhibits a peak at a certain age, which shifts towards an older age from more proximal to more distal boutons for anterograde transiting DCVs (figure 5b)
and from more distal to more proximal boutons for retrograde transiting DCVs (figure 5c). The shift of the peak density thus occurs in the direction in which DCVs travel, as older DCVs travel to the next bouton.

Intriguingly, at steady state, the peak on the age density curve for DCVs in the transiting states does not coincide with the mean ages of DCVs in the transiting states (compare figures 5b,c and 6b,c). For example, the peak on the curve showing the age density of DCVs in the retrograde transiting state in bouton 2 (see the line marked by hollow circles in figure 5c) occurs at the DCV age of 1.94 h, while the mean DCV age in the retrograde kinetic state in bouton 2 is 6.88 h (figure 6c).

This is explained by the fact that although the age density distributions of transiting DCVs in figure 5b,c look similar to bell-shaped curves resembling normal distributions, they are in fact rightward skewed. Note that the age density in figure 5b,c takes a small positive value even at large values of $t$, which shifts the mean of the age density distribution to the right. For example, for $\delta = 1$, which corresponds to the situation when DCVs captured into the resident state are eventually released back to the circulation (the case displayed in figures 5 and 6), the age density of transiting-retrograde DCVs in bouton 2 at 6 h equals 0.2100 vesicles $\mu m^{-1} h^{-1}$. This small positive value corresponds to older DCVs released from the resident state in boutons. Note that this effect is absent if $\delta = 0$, which corresponds to the situation when DCVs captured into the resident state are eventually destroyed in boutons or leave the boutons by exocytosis. This situation is displayed in electronic supplementary material, figure S2b,c. Indeed, the age density of transiting-retrograde DCVs in bouton 2 at 6 h is equal to 0.0001 vesicles $\mu m^{-1} h^{-1}$ (see the line marked by hollow circles in electronic supplementary material, figure S2c). The peaks on the age

Figure 6. (a) Mean age of resident DCVs in various boutons versus time. (b) Mean age of anterograde transiting DCVs in various boutons versus time. (c) Mean age of retrograde transiting DCVs in various boutons versus time. $\delta = 1$, $n_{0s} = 1$ and $\varepsilon = 0.8$. (Online version in colour.)
Table 2. The mean age of resident and transiting DCVs (in hours) in four representative boutons (1, 5, 13 and 26) at steady state. \( \delta = 1 \).

| \( n_{0,t} \) | \( \varepsilon \) | mean age of resident DCVs in bouton | mean age of anterograde transiting DCVs in bouton |
|---|---|---|---|
| | | #1 (h) | #5 (h) | #13 (h) | #26 (h) | #1, t (h) | #5, ta (h) | #13, ta (h) | #26, ta (h) |
| 1 | 0 | 11.32 | 11.45 | 12.32 | 13.38 | 2.67 | 2.18 | 1.30 | 0.08 |
| 1 | 0.8 | 15.46 | 15.47 | 14.98 | 13.55 | 6.81 | 6.51 | 4.69 | 0.43 |
| 1 | 1 | 16.24 | 16.23 | 15.53 | 13.59 | 7.59 | 7.32 | 5.37 | 0.51 |
| 0.1 | 0.8 | 13.21 | 13.22 | 12.77 | 11.58 | 4.56 | 4.52 | 3.29 | 0.30 |

| \( n_{0,t} \) | \( \varepsilon \) | mean age of retrograde transiting DCVs in bouton |
|---|---|---|
| | | #2, tr (h) | #5, tr (h) | #13, tr (h) | #26, tr (h) |
| 1 | 0 | 2.79 | 3.40 | 6.04 | 9.37 |
| 1 | 0.8 | 6.88 | 7.12 | 7.96 | 9.37 |
| 1 | 1 | 7.66 | 7.85 | 8.38 | 9.37 |
| 0.1 | 0.8 | 4.57 | 4.62 | 4.95 | 5.54 |

density distributions in figure S2b,c exactly correspond to the mean ages of DCVs displayed in figure S3b,c.

The accuracy of the computed age density distributions was checked by integrating the densities with respect to the DCV age in boutons (which should give the number of DCVs) and comparing the result with the number of DCVs computed by solving equations (2.1)–(2.8) with boundary conditions (2.14), (2.15) (electronic supplementary material, figure S1). The number of DCVs is equal to the DCV concentration (found by solving equations (2.1)–(2.8)) multiplied by the length of a compartment.

The mean age of resident DCVs changes from 13.55 h in the most proximal bouton (#26) to 15.46 h in the most distal bouton (#1) (figure 6a, second line in table 2 corresponding to \( n_{0,t} = 1 \) and \( \varepsilon = 0.8 \)). The mean age of DCVs in the resident state thus increases gradually from the most proximal to the most distal bouton. This is consistent with the experimental findings of [7], who investigated the age of DCVs by marking the DCVs with a photoconvertible construct that changes fluorescence colour depending on its age. It takes DCVs longer to reach distal boutons because DCVs are likely to be captured several times along the way.

Based on results reported in fig. 3B of [7] for terminals with type II boutons, the ratio of red/green fluorescence between distal and proximal boutons is approximately 1.4. From fig. 1D of [21], the increase of red/orange to green fluorescence ratio in the first 10 h is approximately linear. Using the slope of the curve in fig. 1D of [21] and given that in the first 5 h the red/orange/green ratio increased approximately 4.2 times, we concluded that the difference between the age of DCVs in distal boutons and the age of DCVs in proximal boutons is approximately 2 h. This estimate should be taken with caution due to such factors as the difference in temperatures between the mammalian cells used in [21] and the fly cells used in [7] as well as the pH difference between mammalian and fly vesicles. These factors can influence the time course of ageing of the timer protein. Also, fluorescence ratio measurements would be sensitive to the exact optics used and pH (E. S. Levitan 2020, personal communication).

That said, the obtained estimate is consistent with 1.91 h (15.46 h in bouton 1 – 13.55 h in bouton 26) as predicted by our model. It should also be noted that the animals used in the experiments described in [7] were 4–5 days old at the time of the experiments and that DCVs are not made during the first day (E. S. Levitan 2019, personal communication). Since figure 5a suggests that the oldest DCVs in boutons are approximately 60 h old, DCV ages predicted by the model are within the ages of animals used in [7].

The mean age of anterograde transiting DCVs changes from 0.43 h in the most proximal bouton (#26) to 6.81 h in the most distal bouton (#1) (figure 6b, second line in table 2 corresponding to
The increase is due to release of older, previously captured DCVs into the transiting state from the resident state. The mean age of retrograde transiting DCVs changes from 6.88 h in the second most distal bouton (2) to 9.37 h in the most proximal bouton (26) (figure 6c, second line in table 2 corresponding to \( n_{0,1} = 1 \) and \( \varepsilon = 0.8 \)). The increase of the age of DCVs as the DCVs, after turning around in bouton 1, move retrogradely toward proximal boutons, is again due to release of older DCVs from the resident state.

An increase of parameter \( \varepsilon \), which indicates the portion of DCVs that join the anterograde component after being re-released from the resident state, increases the mean DCV age in the resident states in boutons (compare first three rows in table 2). This is because the release of older, previously captured DCVs into the anterograde transiting state increases the average DCV age in that state, and as DCVs continue moving toward more distal boutons, they can be recaptured, which in turn increases the age of DCVs in the resident state.

A decrease in the initial concentration of transiting DCVs, \( n_{0,1} \), causes a reduction in the mean age of the resident DCVs in boutons (compare rows 2 and 4 in table 2). This happens because in this situation, before reaching a particular bouton \( M \), fewer DCVs have been captured previously and resided in more proximal boutons (26, ... , \( M + 1 \)). This leads to younger DCVs in bouton \( M \).

The sensitivity analysis of the mean age of DCVs in boutons to parameters \( n_{0,1} \) and \( \varepsilon \) is presented in electronic supplementary material, S5.

4. Discussion, limitations of the model and future directions

Our model predicts that in type II terminals proximal boutons are filled before distal boutons (figure 3b). This prediction agrees with [7]. Resident DCVs exhibit a bimodal age density distribution (figure 5a), which is explained by the fact that DCVs are captured into the resident state from two pools: the anterograde transiting pool that contains younger vesicles and the retrograde transiting pool that contains older vesicles (figure 2). If it is assumed that captured DCVs, after spending some time in the resident state, are re-released back to circulation (\( \delta = 1 \)), our modelling results also show that resident DCVs are older in distal boutons than in proximal boutons (figure 6). The mean age of DCVs in the resident state of the most distal bouton is approximately 15.5 h while in the most proximal bouton it is approximately 13.5 h (table 2, line 2). The difference between the mean age of DCVs in proximal (younger DCVs) and distal (older DCVs) boutons (figure 6a) is explained by the fact that as they travel to distal boutons; for \( \delta = 1 \) DCVs experience several capture–re-release cycles along the way, spending some time in the resident state before being re-released to the transiting state.

The situation is different if DCVs are destroyed in boutons or leave the boutons by exocytosis (\( \delta = 0 \)). In this case, at steady state, resident DCVs in more proximal boutons are older than in more distal boutons (electronic supplementary material, table S4). Since this contradicts experimental results reported in [7], the model prediction supports the scenario when most captured DCVs are released back to circulation instead of being destroyed in boutons.

The predicted prevalence of older organelles in distal boutons for \( \delta = 1 \) may give important clues for establishing molecular mechanisms of the degeneration of axons of dopaminergic neurons in PD. This may explain ‘dying back’ degeneration of axons that begins in the distal axon [22,23]. The prevalence of older organelles in distal boutons is a consequence of large and extensive arbours of dopaminergic neurons [24]. Older organelles in such neurons may be subject to damage by reactive oxygen species [22].

The model generates predictions for the mean age of DCVs and the DCV age distribution, which could be tested experimentally, giving the model predictive value. The model thus suggests future experiments that could be used to validate the model’s predictions.

Future development of the model should address the following. (i) Possible change in the direction of DCV transport after DCVs have exited the terminal should be accounted for [3]. This would shift the age distribution in the terminal toward older DCVs. (ii) The alteration of capture efficiency by activity should also be included in the model [3,6,25]. (iii) Ideally, the delay of 300 s in bouton 1 required to change anterograde to retrograde motors should be applied to each vesicle...
arriving to bouton 1. The utilization of $H[t - t_1]$ function in equations (2.11)–(2.13) only provides an initial delay of the retrograde flux by 300 s. This factor will not affect any DCV arriving after $t_1$ time. Future models should overcome this limitation of the compartmental model, which can be done by treating DCVs as individual vesicles [26]. The utilization of a discrete model would also provide much more detailed information on the vesicle trajectories and age. (iv) Recent experimental results indicate that DCVs release some of their contents by kiss and run exocytosis, in contrast to traditional full collapse exocytosis, which fully empties DCVs [27]. This should also be addressed in future model development.

The current version of the model assumes that at $t = 0$, DCVs start flowing into an empty type II terminal, which is assumed to be post-development. Experiments reported in [7] investigated animals at the end of their third instar larva stage, before the animals retracted their neurons to start the transformation to adulthood. In order to better simulate the experimental results, future versions of the model should consider coupling DCV transport with terminal development as the animals grow to reach the third instar larva stage. Accounting for the terminal growth could affect the predicted mean age of DCVs in distal boutons. This is because older boutons will already be filled with DCVs, which will reduce the chances for DCVs to get captured (and subsequently re-released) as they travel toward distal boutons. In particular, the model should address whether type II boutons are growing by adding new boutons either at the ends of axons or between existing boutons. This may be important because DCVs may bypass fully occupied (older) boutons, but be intensively captured in new boutons, which would affect the order in which the boutons are filled. The discussed questions would be interesting to address through a combination of modelling and experimentation in future research.

Data accessibility. Additional data accompanying this paper, including references to sources [28–32], are available in the electronic supplementary material.

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