ATP diffusional gradients are sufficient to maintain bioenergetic homeostasis in synaptic boutons lacking mitochondria

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Abstract

Experimental work on mitochondria distribution in axons indicates that approximately half of the presynaptic release sites do not contain mitochondria. This raises the question of how the boutons that do not contain a mitochondrion are supplied with ATP. We developed a mathematical model that simulates ATP transport by diffusion between a bouton containing a mitochondrion and a bouton lacking a mitochondrion. We investigated whether diffusive transport of ATP is sufficient to support the ATP concentration in a bouton lacking a mitochondrion above the minimum concentration required for endocytosis. Our computational results demonstrate that the difference in ATP concentration between a bouton containing a mitochondrion and a neighboring bouton lacking a mitochondrion is approximately 0.4%. We established that due to diffusion transport, the ATP concentration in the bouton lacking a mitochondrion is 3.75 times larger than the ATP concentration minimally required to support synaptic vesicle release.

Keywords: axons; mitochondria; ATP; diffusion-driven transport; mathematical modeling

1. Introduction

Mitochondria's main purpose is to produce easily accessible chemical energy in the form of ATP [1], [2]. In addition to stationary mitochondria, there are also anterogradely and retrogradely moving mitochondria [3], [4], [5], [6]. ATP supply to energy demand sites, such as presynaptic boutons, was thought to be mainly provided by stationary mitochondria that dock in these boutons [7], [8], [9].

However, experimental research indicates that approximately half of the presynaptic release sites do not contain mitochondria [10], [11], [12]. An interesting question is how ATPs are supplied into a bouton that lacks a mitochondrion. There are three competing hypotheses in the literature explaining how this can happen. 1) ATP is transported to boutons lacking mitochondria by diffusion [13]. 2) ATP is synthesized in boutons lacking a mitochondrion by glycolysis [13]. 3) ATP needs are partly met by ATP production in neighboring axons, dendrites, and glial cells [11], [14]. The correct explanation can also be a combination of these hypotheses.

The main novelty of our paper is the explanation why axons do not need a stationary mitochondrion in every energy demand site. To explain this, we developed a mathematical model of diffusion-driven ATP transport from a bouton containing a mitochondrion to a bouton lacking a mitochondrion. Using the developed model, we investigated whether diffusion-driven transport of ATP is sufficient to maintain the ATP concentration in a bouton lacking a mitochondrion above the minimum ATP concentration required to support synaptic vesicle release. If diffusion is sufficiently fast, the ATP concentration will be approximately uniform; if it is slow, the ATP concentration will decay with the distance from a mitochondrion [11], [14].

2. Materials and models

2.1. Equations governing ATP concentration in a single periodic unit control volume containing half of a mitochondrion

For the development of the model, we assumed that a mitochondrion is present in the center of every second varicosity (bouton) in the axon. The assumption of periodicity made it possible to define a periodic unit control volume containing half of a mitochondrion (the other half belongs to a neighboring periodic control volume), see Fig. 1. The width of axonal varicosity is 2δ; intervaricosity average spacing is L. Mitochondria are assumed to be located at every second varicosity. The distance between mitochondria is $2L + 4\delta$.

In the potion of varicosity occupied by a mitochondrion, the conservation of ATP molecules gives the following equation:

$$\frac{\partial C_1}{\partial t} = D\frac{\partial^2 C_1}{\partial x^2} - aC_1 + m\,, \qquad 0 \le x \le x_m\,, \tag{1}$$

where t is the time and x is the linear coordinate along the axon.

In the portion of varicosity not occupied by a mitochondrion, the ATP conservation is given by the following equation:

$$\frac{\partial C_2}{\partial t} = D\frac{\partial^2 C_2}{\partial x^2} - aC_2\,, \quad x_m \le x \le \delta\,. \tag{2}$$

In the intervaricosity spacing, the ATP conservation results in the following equation:

$$\frac{\partial C_3}{\partial t} = D\frac{\partial^2 C_3}{\partial x^2}\,, \delta \le x \le \delta + L\,. \tag{3}$$

In empty varicosity, not occupied by a mitochondrion (but still consuming ATP), the ATP conservation gives the following equation:

$$\frac{\partial C_4}{\partial t} = D\frac{\partial^2 C_4}{\partial x^2} - aC_4\,, \quad L + \delta \le x \le L + 2\delta\,. \tag{4}$$

Dependent variables utilized in the model are summarized in Table 1 and model parameters are summarized in Table 2.

Eqs. (1)-(4) were solved at steady-state subject to the following boundary conditions:

$$\text{At } x = 0: \qquad \left.\frac{dC_1}{dx}\right|_{x=0} = 0\,. \tag{5}$$

$$\text{At } x = x_m: \qquad C_1\big|_{x=x_m-0} = C_2\big|_{x=x_m+0}\,, \left.\frac{dC_1}{dx}\right|_{x=x_m-0} = \left.\frac{dC_2}{dx}\right|_{x=x_m+0}\,. \tag{6}$$

$$\text{At } x = \delta: \qquad C_2\big|_{x=\delta-0} = C_3\big|_{x=\delta+0}\,, \left.\frac{dC_2}{dx}\right|_{x=\delta-0} = \left.\frac{dC_3}{dx}\right|_{x=\delta+0}\,. \tag{7}$$

$$\text{At } x = L + \delta: \quad C_3\big|_{x=L+\delta-0} = C_4\big|_{x=L+\delta+0}\,, \left.\frac{dC_3}{dx}\right|_{x=L+\delta-0} = \left.\frac{dC_4}{dx}\right|_{x=L+\delta+0}\,. \tag{8}$$

$$\text{At } x = L + 2\delta: \left.\frac{dC_4}{dx}\right|_{x=L+2\delta} = 0\,. \tag{9}$$

Table 1. Dependent variables in the model of ATP transport and accumulation in the axon.

Symbol	Definition	Units
C	Linear concentration of ATP in the axon	μm^{-1}

Table 2. Parameters characterizing ATP transport and accumulation in the axon.

Symbol	Definition	Units	Value or range	Reference(s)	Value used in computations
D	Diffusivity of ATP in the cytosol	$\mu m^2\ s^{-1}$	710 – 720 in water, ~300 in the cytosol	[15], [16]	300
L	Intervaricosity average spacing	μm	3	[11]	3
$2x_m$	Length of mitochondria	μm	0.5-10	[17], Fig. 2G in [11]	1
δ	Half-width of axonal varicosity	μm	1	Fig. 2E of [11, 18]	1
A_c	Cross-sectional area of a neuron	μm^2	0.79 [a]		0.79 [a]
$\hat{C}_0$	ATP volume concentration in the axon	mM	2-4	[13]	3
C_0	ATP linear concentration in the axon	μm^{-1}	1.42×10^6 [b]	[13]	1.42×10^6 [b]
$\dot{m}$	ATP production rate per unit mass of a tissue	mol/(min kg)	6.6×10^{-3}	[19]	8×10^{-3} [b]

			-11×10^{-3}		
γ	Percent of mitochondria by volume in tissues	%	2-10	[20]	5
ϖ	Mass of a single mitochondrion	kg	322×10^{-15}	[21]	322×10^{-15}
m	ATP production rate per unit length of a mitochondrion	$\mu m^{-1}s^{-1}$	1.85×10^{5} [c]	[19], [21], [20]	1.85×10^{5} [c]
a	Kinetic constant characterizing the rate of mitochondria consumption to power endocytosis in varicosities	s^{-1}	0.085 [d]		0.23 [d]

[a] We estimated A_c as follows:

$A_c = \frac{\pi d^2}{4}$. We used 1 μm for the diameter of the axon, d.

[b] We calculated this value as follows:

$C_0 = \hat{C}_0 \times 10^{-18} N_A A_c$ ATP/ μm.

[c] The number of ATP produced by mitochondria per unit length of the axon is estimated as:

$m = \varpi\left(\gamma / 100\%\right)\left(\dot{m} / 60\frac{\text{s}}{\text{min}}\right) / \left(2x_m\right)$.

[d] We determined the value of a from the condition that the maximum value of ATP concentration, occurring at $x = 0$, is equal to C_0.

3. Results

3.1. Distribution of ATP concentration between varicosities containing a mitochondrion and varicosities lacking a mitochondrion

The analytical solution of Eqs. (1)-(4) subject to boundary conditions (5)-(9) for the steady-state situation is given by Eqs. (S1)-(S4) in Supplementary Materials.

The maximum concentration of ATP occurs in the center of a bouton occupied by a mitochondrion (Fig. 2). Away from the mitochondrion, the ATP concentration decreases and reaches its minimum value in the center of varicosity that lacks a mitochondrion (Fig. 2). If an ATP concentration in the bouton containing a mitochondrion is 3 mM, the drop of the ATP concentration between the bouton containing a mitochondrion and a bouton lacking a mitochondrion is approximately 0.4%. The increase of intervaricosity spacing makes the ATP concentration drop between varicosity containing a mitochondrion and neighboring varicosity without a mitochondrion. According to [13], to sustain vesicle endocytosis from boutons, the minimum ATP concentration of approximately 0.8 mM needs to be maintained in boutons. This corresponds to

$$C_{\min} = 0.8 \times 10^{-18} N_A A_c = 3.78 \times 10^5 \text{ ATP/ µm.} \tag{10}$$

The concentration displayed in Fig. 2a is 3.75 times greater than the minimally required ATP concentration given by Eq. (10). This result is consistent with experimental observations reported in [13], which concluded that diffusive transport of ATP is sufficiently fast for ATP distribution to be uniform, even in boutons lacking mitochondria.

Computations with a smaller value of ATP diffusivity predict a larger drop between the ATP concentration in a bouton containing a mitochondrion and a bouton lacking a mitochondrion (approximately 1% for $D = 100$ µm^2 s^{-1}, Fig. 2b). Computations with a greater value of ATP diffusivity predict a more uniform ATP distribution (the concentration drop is approximately 0.14% for $D = 500$ µm^2 s^{-1}, Fig. 2b).

3.2. Sensitivity of the concentration of ATP to ATP diffusivity and intervaricosity spacing

This investigation was performed by computing local sensitivity coefficients, which are first-order partial derivatives of the observables with respect to model parameters [22], [23], [24], [25]. For example, the sensitivity coefficient of the ATP concentration to ATP diffusivity D at steady-state (ss) was calculated as follows:

$$\frac{\partial C}{\partial D} \approx \left. \frac{C(D+\Delta D) - C(D)}{\Delta D} \right|_{\text{other parameters kept constant}} \quad (i=1,\ldots,N), \tag{11}$$

where $\Delta D = 10^{-2} D$ (we tested the accuracy by using various step sizes).

The sensitivity coefficient was non-dimensionalized to make it independent of the magnitude of the parameter whose sensitivity was tested [23], [26], as follows (for example):

$$S_D^C = \frac{D}{C}\frac{\partial C}{\partial D} \qquad (i=1,\ldots,N). \tag{12}$$

The dimensionless sensitivity of ATP concentration to the ATP diffusivity is positive and increases with the distance from the mitochondrion (Fig. 3a). This is because the increase of ATP diffusivity increases the rate of diffusion-driven transport of ATP, making the ATP concentration in the axon more uniform, closer to that in the region occupied by the mitochondrion.

The dimensionless sensitivity of ATP concentration to the intervaricosity spacing is negative and becomes more negative with the increase of the intervaricosity spacing (Fig. 3b). This is because diffusion becomes a less efficient transport mechanism with the increase of the distance to which ATP must be transported.

4. Discussion

We investigated a variation of the ATP concentration between a bouton containing a mitochondrion and a bouton lacking a mitochondrion. An analytical solution of equations governing the ATP diffusion is obtained. We established that providing the lack of mitochondria in every second bouton, the drop in the ATP concentration between a bouton containing a mitochondrion and a bouton lacking a mitochondrion is only 0.4%. This means that the ATP concentration in the axon is practically uniform due to the large diffusivity of ATP. This explains why axons do not need a stationary mitochondrion in every energy demand site.

The sensitivity of the ATP concentration to the ATP diffusivity is positive, which means that the increase of the ATP diffusivity makes the ATP concentration in the axon more uniform. Conversely, the sensitivity of ATP concentration to the intervaricosity spacing is negative, which means that the increase of the intervaricosity spacing makes the ATP concentration less uniform.

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Ethical Statement

None.

References

1. Fan T, Xia L, Han Y. Mitochondrion and apoptosis. *Acta Biochimica Et Biophysica Sinica* 2001; **33**(1): 7-12.

2. Picard M, McEwen BS. Psychological stress and mitochondria: A systematic review. *Psychosomatic Medicine* 2018; **80**(2): 141-153.

3. Mogre SS, Brown A, I., Koslover EF. Getting around the cell: Physical transport in the intracellular world. *Physical Biology* 2020; **17**(6): 061003.

4. Melkov A, Abdu U. Regulation of long-distance transport of mitochondria along microtubules. *Cellular and Molecular Life Sciences* 2018; **75**(2): 163-176.

5. Kruppa AJ, Buss F. Motor proteins at the mitochondria cytoskeleton interface. *Journal of Cell Science* 2021; **134**(7): jcs226084.

6. Narayanareddy BRJ, Vartiainen S, Hariri N, O'Dowd DK, Gross SP. A biophysical analysis of mitochondrial movement: Differences between transport in neuronal cell bodies versus processes. *Traffic* 2014; **15**(7): 762-771.

7. Hollenbeck PJ, Saxton WM. The axonal transport of mitochondria. *Journal of Cell Science* 2005; **118**(23): 5411-5419.

8. Lees RM, Johnson JD, Ashby MC. Presynaptic boutons that contain mitochondria are more stable. *Frontiers in Synaptic Neuroscience* 2020; **11**: 37.

9. Lewis TL, Jr., Turi GF, Kwon S, Losonczy A, Polleux F. Progressive decrease of mitochondrial motility during maturation of cortical axons in vitro and in vivo. *Current Biology* 2016; **26**(19): 2602-2608.

10. Obashi K, Okabe S. Regulation of mitochondrial dynamics and distribution by synapse position and neuronal activity in the axon. *European Journal of Neuroscience* 2013; **38**(3): 2350-2363.

11. Shepherd G, Harris K. Three-dimensional structure and composition of CA3 -> CA1 axons in rat hippocampal slices: Implications for presynaptic connectivity and compartmentalization. *Journal of Neuroscience* 1998; **18**(20): 8300-8310.

12. Chang DTW, Honick AS, Reynolds IJ. Mitochondrial trafficking to synapses in cultured primary cortical neurons. *Journal of Neuroscience* 2006; **26**(26): 7035-7045.

13. Pathak D, Shields LY, Mendelsohn BA, Haddad D, Lin W, Gerencser AA, Kim H, Brand MD, Edwards RH, Nakamura K. The role of mitochondrially derived ATP in synaptic vesicle recycling. *Journal of Biological Chemistry* 2015; **290**(37): 22325-22336.

14. Engl E, Attwell D. Non-signalling energy use in the brain. *Journal of Physiology-London* 2015; **593**(16): 3417-3429.

15. Bowen WJ, Martin HL. The diffusion of adenosine triphosphate through aqueous solutions. *Archives of Biochemistry and Biophysics* 1964; **107**(1): 30-36.

16. Macdonald CL, Yu D, Buibas M, Silva GA. Diffusion modeling of ATP signaling suggests a partially regenerative mechanism underlies astrocyte intercellular calcium waves. *Frontiers in Neuroengineering* 2008; **1**: 1.

17. Trushina E. A shape shifting organelle: Unusual mitochondrial phenotype determined with three-dimensional electron microscopy reconstruction. *Neural Regeneration Research* 2016; **11**(6): 900-901.

18. Agrawal A, Koslover EF. Optimizing mitochondrial maintenance in extended neuronal projections. *PLOS Computational Biology* 2021; **17**(6): e1009073.

19. Wibom R, Hultman E. Atp production-rate in mitochondria isolated from microsamples of human muscle. *American Journal of Physiology* 1990; **259**(2): E204-E209.

20. Willingham TB, Ajayi PT, Glancy B. Subcellular specialization of mitochondrial form and function in skeletal muscle cells. *Frontiers in Cell and Developmental Biology* 2021; **9**: 757305.

21. Fuchs P, Rugen N, Carrie C, Elsaesser M, Finkemeier I, Giese J, Hildebrandt TM, Kuehn K, Maurino VG, Ruberti C, Schallenberg-Ruedinger M, Steinbeck J, Braun H, Eubel H, Meyer EH, Mueller-Schuessele SJ, Schwarzlaender M. Single organelle function and organization as estimated from arabidopsis mitochondrial proteomics. *Plant Journal* 2020; **101**(2): 420-441.

22. Beck JV, Arnold KJ. *Parameter Estimation in Science and Engineering.* Wiley: New York, 1977.

23. Zadeh KS, Montas HJ. A class of exact solutions for biomacromolecule diffusion-reaction in live cells. *Journal of Theoretical Biology* 2010; **264**(3): 914-933.

24. Zi Z. Sensitivity analysis approaches applied to systems biology models. *Iet Systems Biology* 2011; **5**(6): 336-346.

25. Kuznetsov IA, Kuznetsov AV. Investigating sensitivity coefficients characterizing the response of a model of tau protein transport in an axon to model parameters. *Computer Methods in Biomechanics and Biomedical Engineering* 2019; **22**(1): 71-83.

26. Kacser H, Burns J, Fell D. The control of flux. *Biochemical Society Transactions* 1995; **23**(2): 341-366.

Figure captions

Fig. 1. (a) Schematic diagram of a single periodic unit control volume containing half of a mitochondrion (the other half belongs to a neighboring periodic control volume). The width of axonal varicosity is 2δ; intervaricosity average spacing is *L*. Mitochondria are assumed to be located at every second varicosity. The distance between mitochondria is $2L + 4\delta$. *Figure generated with the aid of servier medical art, licensed under a creative commons attribution 3.0 generic license. http://Smart.servier.com.*

Fig. 2. Linear concentration of ATP in the axon vs the distance from a mitochondrion for (a) different intervaricosity spacing; (b) different ATP diffusivities.

Fig. 3. (a) Dimensionless sensitivity of the ATP concentration to the ATP diffusivity. Computations were performed with $\Delta D = 10^{-2} D$. A close result was obtained for $\Delta D = 10^{-1} D$. (b) Dimensionless sensitivity of the ATP concentration to the intervaricosity spacing. Computations were performed with $\Delta L = 10^{-2} L$. A close result was obtained for $\Delta L = 10^{-1} L$. Sensitivities are analyzed around $D = 300$ μm^2 s^{-1}, $L = 3$ μm.

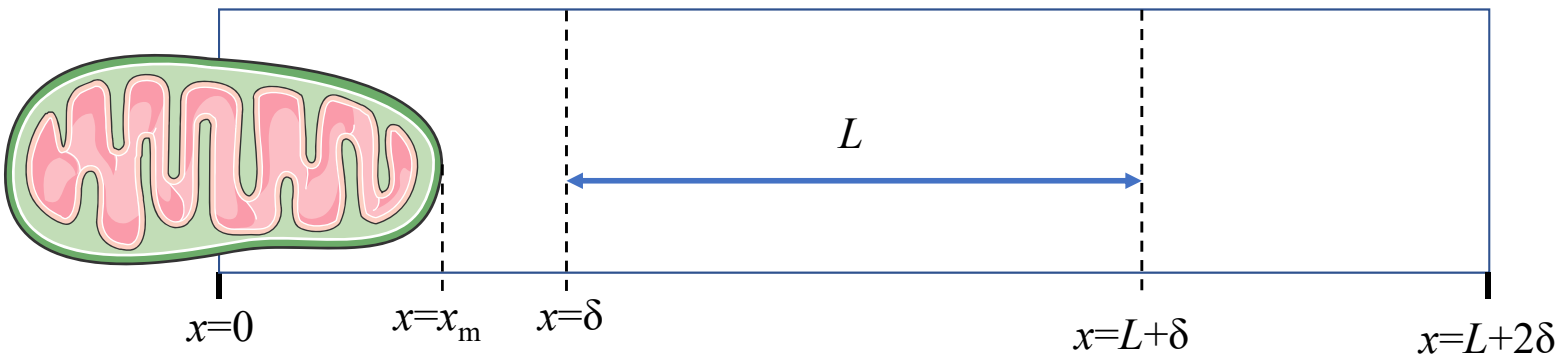
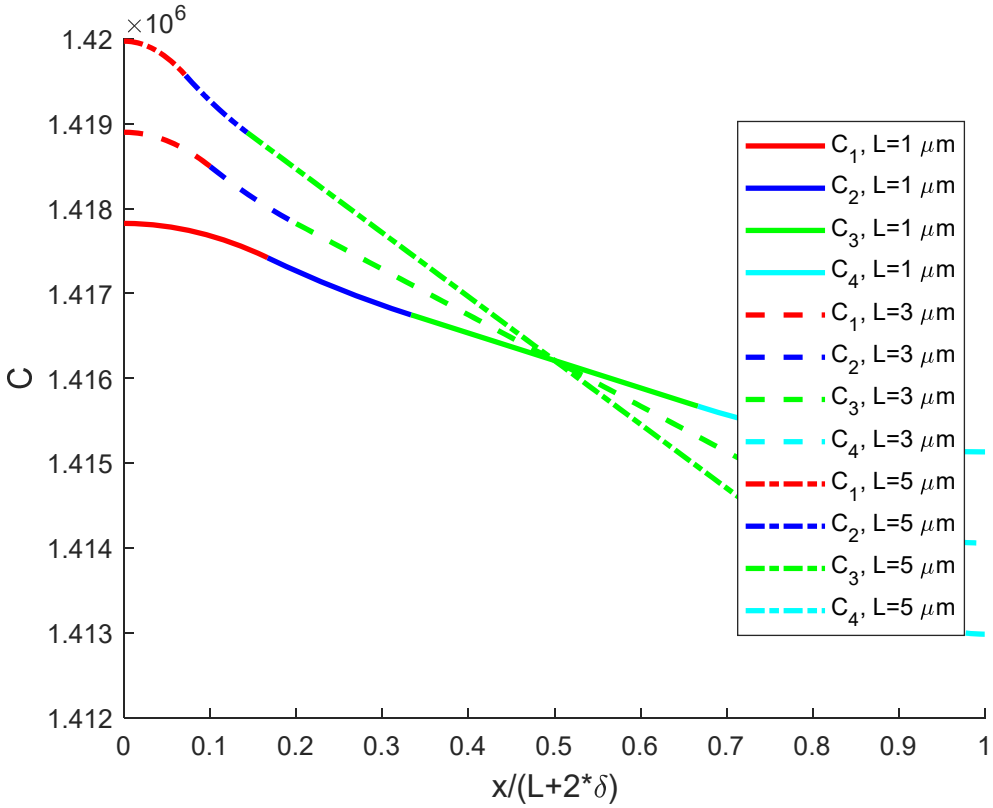


Figure 1

(a)



(b)

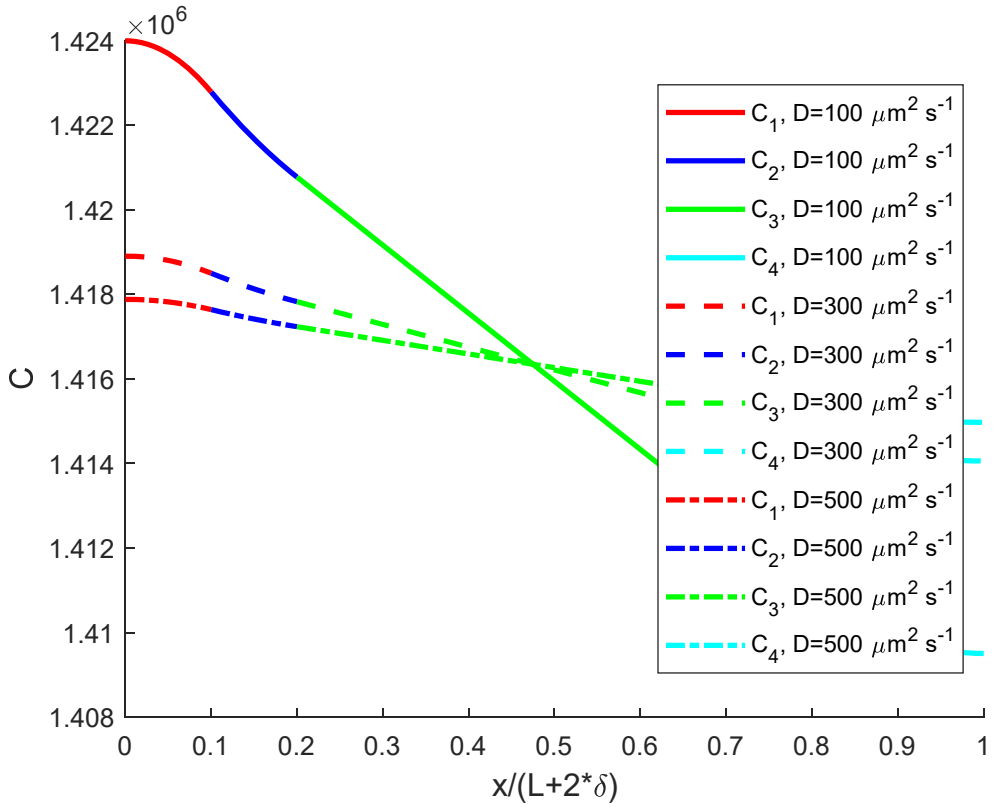
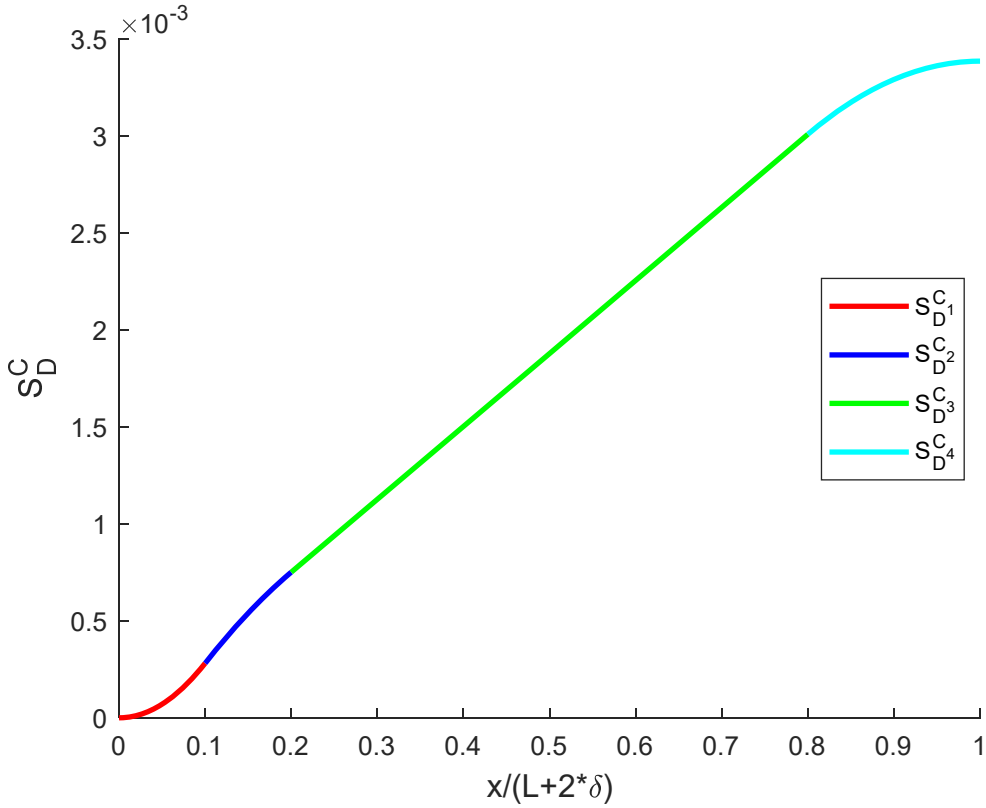


Figure 2a,b

(a)



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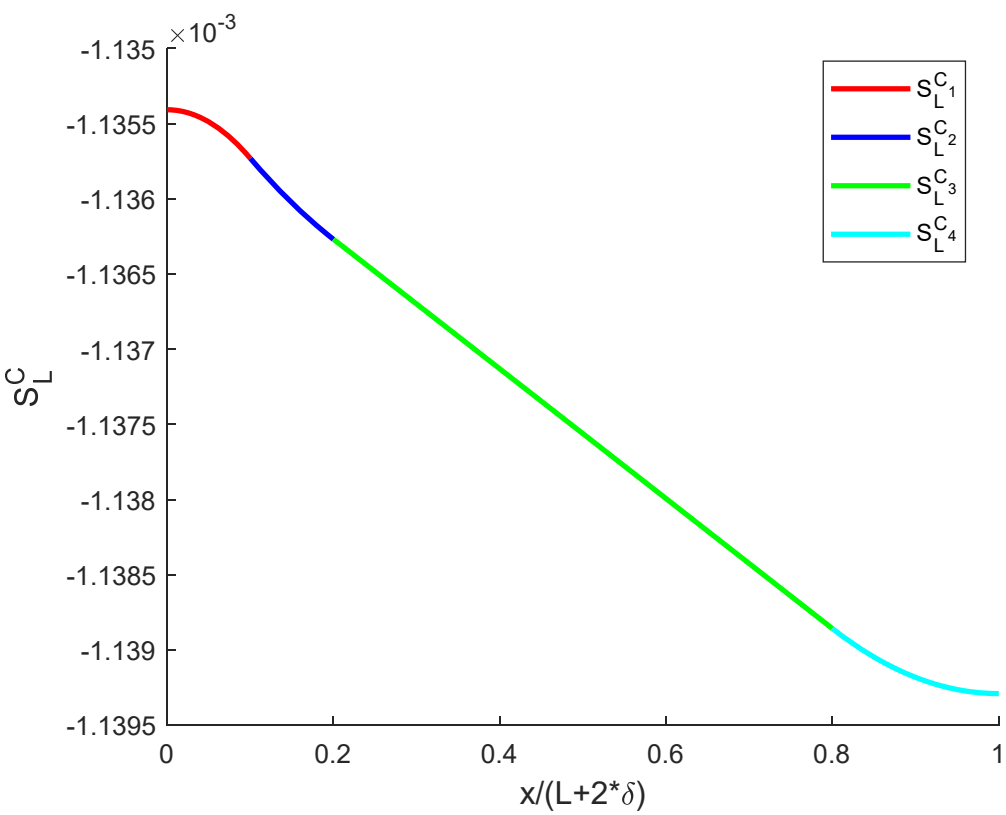

Figure 3a,b

ATP diffusional gradients are sufficient to maintain bioenergetic homeostasis in synaptic boutons lacking mitochondria

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Supplementary Materials

S1. Analytical solutions of governing equations

Analytical solutions of Eqs. (1)-(4) with boundary conditions (5)-(9) at steady-state are given by the following equations:

$$C_1 = -\left[m\left(\coth\left(\frac{\delta\sqrt{a}}{\sqrt{D}}\right) - 1\right)\exp\left(-\frac{\sqrt{a}\left(2\delta+2L+x+x_m\right)}{\sqrt{D}}\right)\right.$$

$$\times\left\{2\sqrt{D}\left[-\exp\left(\frac{2\sqrt{a}\left(\delta+L+x+x_m\right)}{\sqrt{D}}\right)+2\exp\left(\frac{\sqrt{a}\left(2\delta+2L+x+x_m\right)}{\sqrt{D}}\right)-2\exp\left(\frac{\sqrt{a}\left(6\delta+2L+x+x_m\right)}{\sqrt{D}}\right)\right.\right.$$

$$\left.+\exp\left(\frac{2\sqrt{a}\left(3\delta+L+x\right)}{\sqrt{D}}\right)-\exp\left(\frac{2\sqrt{a}\left(\delta+L+x_m\right)}{\sqrt{D}}\right)+\exp\left(\frac{2\sqrt{a}\left(3\delta+L\right)}{\sqrt{D}}\right)\right]$$

$$+L\sqrt{a}\left(\exp\left(\frac{2\delta\sqrt{a}}{\sqrt{D}}\right)-1\right)\exp\left(\frac{2\sqrt{a}\left(\delta+L\right)}{\sqrt{D}}\right)\left[-2\exp\left(\frac{\sqrt{a}\left(2\delta+x+x_m\right)}{\sqrt{D}}\right)+\exp\left(\frac{2\sqrt{a}\left(\delta+x\right)}{\sqrt{D}}\right)+\exp\left(\frac{2\delta\sqrt{a}}{\sqrt{D}}\right)\right.$$

$$\left.\left.\left.+2\exp\left(\frac{\sqrt{a}\left(x+x_m\right)}{\sqrt{D}}\right)-\exp\left(\frac{2\sqrt{a}\left(x+x_m\right)}{\sqrt{D}}\right)-\exp\left(\frac{2x_m\sqrt{a}}{\sqrt{D}}\right)\right]\right\}\right]$$

$$\left.\times\left[4a\left(\exp\left(\frac{2\delta\sqrt{a}}{\sqrt{D}}\right)\left(L\sqrt{a}+2\sqrt{D}\right)-L\sqrt{a}+2\sqrt{D}\right)\right]^{-1}, \qquad \text{(S1)}$$

$$C_2(x)=\left[m\left(\exp\left(\frac{2xm\sqrt{a}}{\sqrt{D}}\right)-1\right)\left(2\sqrt{D}\left(\exp\left(\frac{2\sqrt{a}\left(\delta+L+x\right)}{\sqrt{D}}\right)+\exp\left(\frac{2\sqrt{a}\left(3\delta+L\right)}{\sqrt{D}}\right)\right)\right)+\sqrt{a}L\left(\exp\left(\frac{2\delta\sqrt{a}}{\sqrt{D}}\right)-1\right)\right.$$

$$\left.\times\exp\left(\frac{2\sqrt{a}\left(\delta+L\right)}{\sqrt{D}}\right)\left(\exp\left(\frac{2\delta\sqrt{a}}{\sqrt{D}}\right)+\exp\left(\frac{2x\sqrt{a}}{\sqrt{D}}\right)\right)\exp\left(-\frac{\sqrt{a}\left(4\delta+2L+x+x_m\right)}{\sqrt{D}}\right)\right]$$

$$\times\left[8\left(a^{3/2}L\sinh^2\left(\frac{\delta\sqrt{a}}{\sqrt{D}}\right)+a\sqrt{D}\sinh\left[\frac{2\delta\sqrt{a}}{\sqrt{D}}\right]\right)\right]^{-1}, \tag{S2}$$

$$C_3(x)=\frac{m\left(\sinh\left(\frac{\delta\sqrt{a}}{\sqrt{D}}\right)\right)^{-1}\sinh\left(\frac{xm\sqrt{a}}{\sqrt{D}}\right)\left(\sqrt{D}\coth\left(\frac{\delta\sqrt{a}}{\sqrt{D}}\right)+\sqrt{a}\left(\delta+L-x\right)\right)}{a^{3/2}L+2a\sqrt{D}\coth\left(\frac{\delta\sqrt{a}}{\sqrt{D}}\right)}, \tag{S3}$$

$$C_4(x)=\frac{m\sqrt{D}\left(\exp\left(\frac{2xm\sqrt{a}}{\sqrt{D}}\right)-1\right)\left(\exp\left(\frac{2\sqrt{a}\left(2\delta+L\right)}{\sqrt{D}}\right)+\exp\left(\frac{2x\sqrt{a}}{\sqrt{D}}\right)\right)\exp\left(-\frac{\sqrt{a}\left(2\delta+L+x+xm\right)}{\sqrt{D}}\right)}{4\left(a^{3/2}L\sinh^2\left(\frac{\delta\sqrt{a}}{\sqrt{D}}\right)+a\sqrt{D}\sinh\left(\frac{2\delta\sqrt{a}}{\sqrt{D}}\right)\right)}. \tag{S4}$$