Oscillations in a neurite growth model with extracellular feedback

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HIGHLIGHTS

- A new model of neurite outgrowth accounting extracellular signalling is proposed.
- The model predicts three different scenarios of the neurite development.
- It has been demonstrated that the elongation dynamics is angle-specific.

ABSTRACT

We consider the influence of extracellular signalling on neurite elongation in a model of neurite growth mediated by building proteins (e.g., tubulin). The tubulin production dynamics were supplied by a function describing the influence of extracellular signalling, which can promote or depress neurite elongation. We found that this extracellular feedback could generate neurite length oscillations consisting of a periodic sequence of elongations and retractions. The oscillations prevent further outgrowth of the neurite, which becomes trapped in the non-uniform extracellular field. We analysed the characteristics of the elongation process for different distributions of attracting and repelling sources of the extracellular signalling molecules. The model predicts three different scenarios of neurite development in the extracellular field, including monotonic and oscillatory outgrowth, localised limit cycle oscillations and complete growth depression.

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1. Introduction

Neural development and dendritic morphogenesis underlie the formation of specific network structures, synaptic and information processing in the brain [1–4]. Abnormalities in neuronal development and regeneration are implicated in several neurological disorders, such as autism, schizophrenia and epilepsy [5–9]. The generation of certain morphological patterns involves complex intracellular molecular cascades that are modulated by extracellular signalling. Neurite elongation and branching lead to the formation of specific dendritic patterns, guided by extracellular growth factor molecules released by the other cells. The most significant neurotrophic factors that stimulate neurite outgrowth are GDNF, BDNF and NGF [10,11,12]. An inverse process called retraction is also important for the development and functioning of the nervous system. Retraction may be caused by lysophosphatidic acid [13] and some types of signalling molecules, such as semaphorins, netrins, and ephrins [14,15]; glutamate [16]; and others [17]. The elongation process depends on building proteins (e.g., tubulin, actin) produced in the cell soma. Those proteins are transported to the growth cone by diffusion and active transport and then assembled in microtubules, resulting in elongation of the neurite. In the extracellular space, the neurite is guided by growth factors, which provide a direction for its growth. Neurite growth can also be influenced by many other factors, including cell adhesion and binding to extracellular matrix components [18,19].

Many mathematical models have been proposed to simulate neural development features (reviewed in [4]). Based on microtubule assembly and depolymerisation, these models describe the production, degradation, and transport processes of proteins construction. The neurite length dynamics of these models are based on the evolution of tubulin concentration in the neurite compartments including the growth cone [20–22] or the evolution of the tubulin spatial concentration profile along the neurite [23,24], or the models have focused on the description of the mechanical properties of the neurite outgrowth [19,25,26]. The model of neurite outgrowth...
proposed in [27] is based on the assumption that the elongation can be controlled by membrane expansion and endocytosis. Depending on the coupling between the microtubules and the vesicle dynamics, different regimes corresponding to dendritic and axonal growth were found. The models mentioned above merely incorporated intracellular processes that underlie the outgrowth. The extracellular signalling described in other modelling studies accounts for different aspects of neural morphogenesis, such as neurite branching and navigation (e.g., [28]). However, the extracellular influence on the elongation and retraction processes remains poorly understood.

In this letter, we propose a neurite growth model that is capable of neurite elongation and retraction, driven by extracellular signalling. Such signals coming from the extracellular space may activate specific pathways that regulate different aspects of neuronal development (axon and dendrite growth), synapse formation and plasticity [29]. To model the intracellular dynamics, we used the tubulin-based compartmental model [22]. The extracellular signals are treated as a non-uniform field of molecules released by neighbouring cells and sensed by the growth cone. We assume that these molecules generate a feedback signal, which changes the rate of tubulin production. These changes depend on the type of extracellular molecules (whether they attract or repel the growth and, hence, promote or depress it). We analysed the computational consequences of this extracellular feedback and found that it could significantly affect the growth dynamics. In particular, certain extracellular concentration profiles may induce spontaneous neurite length oscillations. These oscillations may delay neurite growth, or the neurite may be completely trapped, oscillating with a particular frequency in a specific region of space.

2. Materials and methods

We investigate the tubulin-based compartmental model proposed in [22], supplied with extracellular feedback as follows:

\[
\begin{align*}
\frac{dc_i}{dt} & = l - \gamma_0 c_i + D_{01}(c_1 - c_i); \\
\frac{dc_i}{dt} & = D_{1,i-1}(c_{i-1} - c_i) + D_{1,i+1}(c_{i+1} - c_i); \\
\frac{dc_n}{dt} & = -\gamma_n c_n + D_{n,n-1}(c_{n-1} - c_n) - \alpha \cdot c_n + \beta; \\
\frac{dl}{dt} & = \alpha \cdot c_n - \beta; \\
\frac{dt}{dt} & = -\delta(l - l_0) + F(S(x, y, t)).
\end{align*}
\]

(1)

Here, the variable \(c_i\) describes the concentration of available (free) tubulin in the \(i\)th segment of the neurite \((i = 0, 1, \ldots, n)\), and \(L\) is the variable length of the neurite. The parameter \(\gamma\) determines the tubulin degradation rate, \(\alpha\) is an association constant, \(\beta\) is a dissociation constant, \(D_{ij} = (DA_{ij}/V_i\Delta x_{ij})\) is the diffusion rate from segment \(j\) to segment \(i\), \(A_{ij}\) is the cross-section area between segments, \(V_i\) is the volume of segment \(i\), \(\Delta x_{ij}\) is the distance between the centres of the nearest-neighbour segments, and \(D\) is the diffusion constant.

Neurotrophic factors affect the cell metabolism via the growth cone. After binding to the receptor, they form a complex that activates PI3-kinase and Akt. Akt activation leads to the CREB-mediated synthesis of actin and tubulin, accelerating outgrowth. In the growth cone, PI3-kinase induces Ras, which is a pathway that stimulates actin polymerisation [11,12,17]. In our model, the extracellular feedback is provided by the last equation. We assume that the rate of tubulin production, \(l\), is variable relative to an equilibrium level, \(l^*\), with a characteristic time scale, \(1/\delta\). The influence of extracellular signalling is described by the function \(F(S(x, y, t))\), where \(S(x, y, t)\) is an effective (average) concentration of the growth factors in the growth cone at a point \((x, y)\) in the 2D area at a time point \(t\). We suppose that the sources of extracellular molecules (e.g., other cells) are uniformly distributed in the 2D area and characterised by a parameter \(p\). These sources are divided into those that promote the growth of the neurite \((p = +1)\) and those that depress it \((p = -1)\), each occurring with an equal probability.

The concentration of signalling molecules diffusing from each source to the growth cone can be calculated by the following formula [30]:

\[
C'(r, t) = \frac{q}{4\pi D_t r} \left( 1 - \frac{2}{\sqrt{\pi}} \int_0^r e^{-x^2} dx \right),
\]

(2)

where \(q\) describes the constant rate of growth factor production, \(r\) is the distance between the source and the growth cone, and \(D_t\) is the diffusion rate of the extracellular signalling molecules. Examples of the extracellular signal distributions are shown in Fig. 2A–C as two-dimensional colour maps. Next, we calculate the overall influence of the molecules coming from all sources, accounting for these molecules at the growth cone location as a sum weighted by the signs, \(p_k\), of the corresponding sources:

\[
S(x, y, t) = \sum_{k=1}^{N} p_k \cdot C_k(r(x, y), t).
\]

(3)

We estimate the action of this effective concentration on the growth cone by the activation function \(F(S)\), here given the form of logistical curve for illustration:

\[
F(S) = A \left( \frac{1}{1 + \exp \left( \frac{-2\pi(S - \theta)}{C} \right)} - 1/2 \right),
\]

(4)
where $A$ is the maximal rate of the extracellular feedback, $B$ is the midpoint of the feedback activation, $C$ is the width of the active concentration interval.

The models (1)–(4) are initiated with a definite distribution of the growth factor sources. Simulation of neurite growth (1) starts simultaneously with the diffusion of signalling molecules (2)–(4). Then, the concentration $S(x,y,t)$ is calculated in the reference frame, moving with the growth cone.

In the absence of the extracellular feedback, e.g., for $I=I'=const$, the elongation dynamics are quite simple and are explained in detail in [22]. The neurite length grows monotonically with the concentration in the terminal segment, decaying to a specific value defined by the tubulin production rate (Fig. 1). In our computations, we use the following elongation schema. The last segment is considered to be the growth cone, with a fixed length $d$. The neurite length changes due to the elongation of the next-to-last segment. Its length is initially set to $l_1$, which is the minimal segment length. If the tubulin concentration is sufficient, the length of the segment increases to a value of $d+l_1$, after which, the segment is divided into two parts, one segment of length $d$ and the other, next to the growth cone, with length $l_1$.

3. Results

Let us now investigate the influence of the extracellular molecules on the growth dynamics. For illustration, we first consider three different growth factor source distributions (Fig. 2A–C) and choose the direction of neurite growth (shown by arrows). The models (1)–(4) do not account for neurite navigation, and the growth direction remains fixed during the simulations.

Fig. 3A illustrates the growing neurite dynamics in the profile shown in Fig. 2A. After a certain initial increase time, the neurite length begins to oscillate when the influence of the extracellular diffusive field becomes significant. The amplitude of the fluctuation decreases, and when the neurite enters the region with a positive extracellular signal, tubulin production is promoted and neurite growth continues.

An unexpected effect occurs in the second case (the distribution is shown in Fig. 2B), where the length fluctuation converges to self-sustained limit cycle oscillations (Fig. 3B). In fact, the neurite is trapped within a non-uniform pattern in the extracellular field of signalling molecules. With elapsed time, the profile of the field tends to its equilibrium (Fig. 2B); however, the neurite continues to oscillate with a particular amplitude and frequency (Fig. 3B).

The mechanism of the oscillatory dynamics can be explained by the interplay between the promoting and depressing extracellular signals. If the effective concentration is positive at a point of the growth cone $(x,y,t)$, the feedback is positive, and the tubulin production rate increases, which increases the elongation rate according to Eq. (1). Conversely, when the effective concentration $S$ is negative, the tubulin production rate decreases, and the neurite slows its growth. The most interesting case occurs when the growth cone concentration of tubulin reaches its threshold value, $C_{cr}^* = \beta/\alpha$. In this case, the neurite begins to shorten, e.g., $d\,dt < 0$, following the fourth equation of Eq. (1). Then, if the length satisfies the condition:

$$L = d(n-1) + l_1,$$

we assume that the length of the next-to-last segment becomes less than the minimal length $l_1$, and the $(n-1)$th and $(n-2)$th segments are merged as one, with the following concentration:

$$C_{n-1}(t) = \frac{C_{n-2} \cdot d + C_{n-1}(t-0) \cdot l}{d+l}$$

![](Image)
where $C_{a1}(t - 0)$ is the concentration in the next-to-last segment before the merge; therefore, the total neurite length decreases. This phenomenon corresponds to the retraction phase of the length oscillations, shown in Fig. 3.

In the case corresponding to Fig. 2C, the neurite is completely suppressed by the extracellular influence (Fig. 3C). The biological mechanisms underlying neurite pruning remain poorly understood. Similar to neurite elongation, the process of retraction is based on cytoskeleton modifications. These transformations are regulated by signalling pathways that are activated by the extracellular environment or cell-intrinsic programmes [14]. Recent studies have shown that a high concentration of glutamate triggers axonal reduction [16]. Additionally, lysophosphatidic acid induces neurite retraction via actin-dependent microtubule rearrangement [13]. Recent experimental data indicate that some signalling molecules are involved in neurite retraction (for example Sema3F through neuropilin-2, plexin-A3, and plexin-A4 [15]).

Next, we illustrate the dependence of neurite elongation dynamics on different initial angles $\theta$ for the set of growth factor source configurations shown in Fig. 2. As one may expect, for certain configurations, the ratio between promoting and depressor influences depends on the growth direction and, hence, each angle entails different neurite length dynamics. This finding is consistent with the modern hypothesis that this inhomogeneity of the attractant concentration gradient (e.g., neurotrophic factor) determines the specificity of the cellular interactions. However, in the configuration presented in Fig. 2B, neurites with any initial direction become locked at their specific length and begin to oscillate, as illustrated in Fig. 4B. Interestingly, the oscillation frequency is angle-dependent and decreases monotonically with increasing neurite length. Length fluctuation dynamics have been reported in earlier experimental and theoretical studies [27,31].

Finally, let us consider how the other two configurations shown in Fig. 2A and C provide directional specificity in the elongation dynamics. Fig. 4A shows that depending on the growth angle, the neurite may display either the outgrowth or the oscillations trapped by the profile of the extracellular field (Fig. 2C). As shown in Fig. 4C, in addition to outgrowth and oscillations, there are specific angle sectors for which all neurites are completely suppressed.

4. Discussion

Neurite growth represents a key process in the formation of morphological patterns in neural development. Several experimental and theoretical models have demonstrated that the neurite length dynamics are driven mostly by intracellular signalling based on the production and transport of building proteins. In large scale computations, these processes are often modelled by simple phenomenological functions [32,33]. Recent experiments have suggested an important role of extracellular factors in the modulation of the neurite development. Most of these studies typically discuss the guiding role of extracellular signals in the navigation of the growth cone and their influence on the probability of neurite branching. In addition, our model predicts that extracellular signalling can be crucial in the growth dynamics. Supplying basic compartmental model by extracellular feedback affecting tubulin production rate, we found that growing neurites can generate spontaneous oscillations defined by a non-uniform extracellular diffusive field. These extracellular molecules induce the polymerization of building proteins in the growth cone and an increase in cytoskeletal protein synthesis. Additionally, neurotrophic factors activate cell metabolism and accelerate the transport of building proteins. In contrast, increasing the concentration of the agents that activate the protease pathway and degrade tubulin and actin can trigger the axon retraction process.

Another interesting prediction of the model concerns the angle-dependent specificity of the neurite elongation dynamics. In fact, the extracellular space is structured by the growth factor concentration on the areas that are preferable or non-preferable for the neurite outgrowth. This phenomenon underlies the formation of the specific neurite morphology in development, along with growth cone navigation and branching.

5. Conclusion

A neurite growth model based on the description of building protein production and supplied with extracellular feedback was proposed to explain neurite elongation and retraction processes. We showed that depending on the extracellular feedback and on neurite space orientation, the proposed model generated different types of growth dynamics, including monotonic outgrowth, oscillations locking the neurite in a specific region of space and complete growth inhibition. We also demonstrated that the elongation dynamics are angle specific, providing preferable and non-preferable directions for neurite outgrowth.

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