

## Research article

# A simple dynamic model that accounts for regulation of neuronal polarity

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## Abstract

It has been shown that competing molecular interactions of atypical protein kinase C isoforms regulate neuronal polarity. For instance, silencing one particular isoform known as protein kinase M- $\zeta$  or overexpression of a second isoform known as protein kinase C- $\lambda$  in hippocampal neurons alters neuronal polarity, resulting in neurons with extra axons. In contrast, the overexpression of protein kinase M- $\zeta$  prevents axon specification. These data suggest that antagonistic competition between PKC isoforms could contribute to the development of polarity and axon specification. Here, an excitatory and inhibitory non-linear network model is employed to describe neuronal polarity under different conditions. The model shifts the balance of excitation and inhibition to replicate a variety of scenarios during axon outgrowth, which are then compared with experimental results.

## Keywords

Neurites; axon formation; neuronal polarity; excitatory-inhibitory network; winner-take-all network

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## 1. Introduction

Neuronal polarity develops through a poorly understood symmetry breaking mechanism by which only one of many minor neurites begins a period of sustained net outgrowth and differentiates into an axon [1–3]. The remaining minor neurites maintain alternating phases of extension and retraction that result in little, if any, net growth. A number of candidate gene products have been identified to influence the development of neuron polarity, generally acting to alter local actin and microtubule reorganization [1]. For example, the formation of an axon is mediated by localized concentrations of phosphatidylinositol 3-kinase (PI3K) and atypical protein kinase C (aPKC) [3]. Further, the aPKC isoforms  $\zeta$  (PKM- $\zeta$ ) and  $\lambda$  (aPKC- $\lambda$ ) interact with polarity complex protein protease-activated receptor-3 (Par3), all of which are evolutionarily conserved regulators of cell polarity [4]. In cultured hippocampal neurons, two aPKC isoforms, aPKC- $\lambda$  and PKM- $\zeta$ , have different patterns of expression [5]. The localization of each of these isoforms is spatially distinguishable in polarized neurons. PKM- $\zeta$  is distributed at non-axon-forming neurites, whereas aPKC- $\lambda$  localizes in the presumptive axon. PKM- $\zeta$  competes with aPKC- $\lambda$  for binding to Par3 and disrupts the aPKC- $\lambda$ -Par3 complex. Silencing of PKM- $\zeta$  or overexpression of aPKC- $\lambda$  in hippocampal neurons alters neuronal polarity, resulting in neurons with extra axons. In contrast, the overexpression of PKM- $\zeta$  prevents axon specification. These studies suggested a molecular model wherein mutually antagonistic intermolecular competition between aPKC isoforms directs the establishment of neuronal polarity.

Such intermolecular competition has been observed in the development of polarity through changes in the activity of the small GTPase HRas [1]. This upstream regulator of PI3K becomes increased

in the axonal growth cone as symmetry is breaking. The raised HRas concentration results from a positive feedback loop between PI3K and HRas. Simultaneously, as HRas increases within the axonal growth cone, there is a decrease in HRas concentration in the remaining neurites. This mechanism can be seen as a negative feedback loop that facilitates the formation of only one axon. Furthermore, a mix of positive signals help the gathering of aPKC-Par polarity complexes at the prospective axon. Simultaneously, an antagonistic negative signal, PKM- $\zeta$  moderates interruption of the emergence of the aPKC-Par polarity complex at the minor neurites and that is conducive to hippocampal neuron polarization [5].

From a theoretical point of view, neuronal polarization is an attractive biological problem because there are still no answers as to how molecular information is decoded differently to result in the growth of either one or more axons. Further, it is not known how stability and flexibility are achieved [6]. There are few theoretical models that address such questions. Fivaz *et al.* [1] have mathematically modeled and demonstrated that local positive feedback between HRas and PI3K, when coupled to recruitment of a limited pool of HRas, generates robust symmetry breaking and formation of a single axon. Furthermore, Naoki and colleagues [6, 7] developed a biophysical model in which the active transport of proteins can regulate neurite growth. These findings provide evidence suggesting that, as neurites elongate, transported factors accumulate in the growth cone but are degraded during retrograde diffusion to the soma. In this case, the system effectively operates as a local activation-global inhibition mechanism, resulting in both stability and flexibility. Similarly, Samuels *et al.* [8] developed another model of neuronal polarization based upon the existence of a ‘determinant

chemical' whose concentration at neurite tips influences the growth rate. The mathematical model describing the formation, transport and consumption of this chemical, and the resulting neurite growth undergo a winner-take-all instability, yielding rapid growth of one neurite (the axon) and diminished growth of all others [8]. An alternative approach is offered by Guo and Cheng [9], whereby a simple, inhibitor-free model incorporates known cytochemical and cyto-mechanical factors to produce features of neuronal polarization in environments provided with minimal extracellular regulators. That is because biochemical factors eliciting long-range inhibition, claimed in other studies, remain ambiguous. They proposed specifically that cyclic nucleotides and membrane-associated phospholipids are involved in the initiation, propagation, and integration of polarization signals. Although the behavior of these models agree well with the results of many experiments in which growth-modulating substances are applied to individual growth cones, where one axon is developed, they fail to describe more general scenarios such as neurons with either extra axons or no axons at all. Only the model described by Naoaki and colleagues [6, 7] is capable of explaining alternative axon growth scenarios, but the model is more complicated.

Activation by positive loops and inhibition by negative loops can be seen as excitatory and inhibitory mechanisms in non-linear networks, especially in a winner-take-all network in which each network's elements inhibit all other elements except for themselves [10]. This mechanism is known as mutual or global inhibition, and it is a form of competition where the network element with the largest excitatory input generally wins the competition by suppressing the activity of other elements that remain below their thresholds. If the excitatory or inhibitory input to each element were regarded as data supporting a physicochemical response to which that element would signal, then these networks would make transitions based upon the balance of these physicochemical reactions. For that reason it is proposed that a winner-take-all model can be used to simulate different experimentally observed axon growth scenarios.

It was found that the theoretical model described here matched the experimental scenarios well, showing that the model can be used to study the typical emergence of neuronal polarity, through which a single axon emerges from a neuron leaving the remaining neurites to develop into dendrites via a different growth mechanism. Importantly, the model may also be applicable to other scenarios where the system is out of balance, atypically resulting in either the absence of axons or the development of multiple axons.

## 2. Methods

### 2.1. Time lapse recording of a neuron during the development of polarity

The neuron culture was prepared using day 18 embryonic rat hippocampus plated onto polylysine coated coverslips as previously described [11]. Five hours after plating, a coverslip containing neurons was placed in a temperature controlled chamber (Warner) with unconditioned neuronal growth medium (MEM with N2.1 supplements, equilibrated to 37°C and 7% CO<sub>2</sub> for one hour prior to chamber set up). The recording was made as previously described [12] with a Leica DM-RXA microscope and phase contrast 40X objective, Micromax 5 Mhz CCD camera (Roper Scientific, Inc., Trenton, NJ), and MetaMorph software (Universal Imaging Co, Downingtown, PA). 155 Images were acquired in a period of 16.5 hours (see Fig. 1a).

In this video eight minor processes were observed, each with the potential to become the axon.

### 2.2. Software Development for axon outgrowth analysis in a neuron

MATLAB (R2012b) was used for analysis of axon outgrowth. The pattern of extension and retraction of all processes (minor processes and the developing axon) were determined from the video. The program generated a graph containing the maximum values of the vectors that follows the axon outgrowth in each frame.

This analysis is divided into 7 sections:

- Read frames from video.
- Threshold each frame.
- Obtain the centroid of the neuron.
- Locate and trace follower vectors for axon outgrowth.
- Extract value of the maximum follower vector.
- Plot maximum follower vector vs. video frame.

### 2.3. Tracking algorithm

For an optimal solution, a series of points were identified that repeat throughout each frame. Before applying any image processing technique, individual video frames were initially obtained. To make a correct segmentation to locate the centroid of the neuron, each frame is denoised. The centroid coordinates are obtained from the first frame, and the coordinates of the axons to be analyzed are identified with the help of image display graphic tools (see Fig. 1b). These coordinates are entered manually. Subsequently, the process to determine the length value of the vector from the centroid to the farthest point was started; the point where the axon begins growing and a set of coordinates are given to these points. These coordinates were analyzed in the next frame. By always taking as a reference the centroid, the farthest new point from the centroid was identified and this process repeated throughout the video.

### 2.4. Video frame analysis

To apply image processing techniques, the video was split into single frames. The frames were analyzed in pairs so as to avoid losing the coordinates of the previous points. The multimedia container format is Audio Video Interleave, and we used the MATLAB function "VideoReader" to analyze the contents of the video frames. The VideoReader function creates a VideoReader object to read files containing video data. The object includes information regarding the video file and allow you to read data from the video.

### 2.5. Image segmentation

Segmentation is the process of partitioning a digital image into multiple segments and it was used to remove background information. The video had a capture resolution of 30 frames per second and each frame was a monochromatic image of 480×640 pixels of eight bits per pixel. Thresholding is one of the simplest segmentation techniques; it separates the background from objects by use of an ideal threshold value. When pixel values are below and above the threshold they are changed to zero or one, respectively. To find the optimal threshold value for each frame, the MATLAB function "edge" was used. The edge function finds edges in intensity images. It returns a binary image in black and white having inside 1s where the function finds edges in the input image and 0s elsewhere. The output of a

segmented image is a monochromatic binary image. (see Fig. 1c). In the binary image, the centroid of the neuron was calculated; the coordinates are located and saved for later use. Once the centroid is set, the edges are removed to define the structure of the neuron by using the function and then a false color function is used to color the structure (see Fig. 1d). Once the image is segmented and shows the defined neuron structure, it is possible to locate the farthest point of the axon. This point is saved as a reference for the next video frame to be analyzed. The process was repeated until the last video frame is reached. At the end of the process a matrix, with the X, Y coordinates of each vector, is created.

## 2.6. Growth vector

For the growth vector, the distance between the centroid point and the farthest point of the axon was calculated. With this value of the size of the vector, it was possible from each analyzed frame to plot axon outgrowth throughout the video.

## 2.7. Axon outgrowth time series

Applying the aforementioned method the analysis for a time interval of 16.5 hours could be reported. Letters and colors identify the axons (see Fig. 1e and 1f). In Fig. 1 the growth vector value is given for each labeled neurite during the video. The plot shows the different axon outgrowths labeled with letters. Finally, in Fig. 2 the normalized growth is presented, where, all growth values are divided by the largest maximum growth value (axon (a) in Fig. 1b).

# 3. Results

## 3.1. Theoretical model

A modified Michaelis-Menten function [10] was employed. This function describes enzyme kinetics very well, and was used to design an excitatory-inhibitory network that might be predictive of neurite outgrowth during the development of neuron polarity. It relates a parameter  $P$ , which in this case represents the net chemical excitatory-inhibitory activity in a neurite, to the response  $S(P)$ :

$$S(P) = \begin{cases} \frac{MP^j}{\sigma^j + P^j} & P \geq 0 \\ 0 & P < 0 \end{cases}, \quad (1)$$

where  $M$  is the maximum chemical information threshold for axon determination (ITAD) for very intense excitatory-inhibitory activity, and  $\sigma$  generally sets the point where  $S(P)$  reaches its half maximum value. Finally  $j \in \mathbb{Z}^+$  establishes the maximum slope of the function, representing the sharpness of the transition between threshold and saturation. Here, without loss of generality,  $j = 2$  and none of the conclusions depend on the choice of this particular value. The neurite response equations that give the information threshold for determining axon dynamics are:

$$\tau \frac{dT}{dt} = -T + \frac{M(E_T - kND)_+^2}{\sigma^2 + (E_T - kND)_+^2}, \quad (2)$$

$$\tau \frac{dD}{dt} = -D + \frac{M(E_D - k(N-1)D - kT)_+^2}{\sigma^2 + (E_D - k(N-1)D - kT)_+^2}, \quad (3)$$

where  $T$  is the ITAD of whichever neurite excites first via chemical signals and  $D$  is the ITAD of the remaining neurites. Parameter  $\tau$  gives the time constant in milliseconds(ms). The constant  $k$  deter-

mines the strength of the inhibitory feedback, while  $()_+$  indicates that the expression in parenthesis is zero for negative arguments. That is, negative terms inside the brackets are neglected.  $T$  and  $D$  can be distinguished only by the level of their excitatory strengths  $E_T$  and  $E_D$ . These inputs may represent the strength of the positive feedback mechanism described in the Introduction. Here, a number of neurites are used with different values for  $E_T$ ,  $E_D$ , and  $k$  depending upon the simulated scenario. For example, if a neuron with two neurites and four axons is required, then four of or similar to equations (2) are solved with either a null or very small  $k$  values (low inhibition effects) and two of or similar to equations (3) with the same value for  $E_D$  and a high value for  $k$  (high inhibition). Equations (2) and (3) were solved by the fourth order Runge-Kutta method with different initial conditions for  $T$  and  $D$ .

## 3.2. Time lapse recording of a neuron during the development of polarity and its theoretical comparison

To test that the theoretical model described axon outgrowth, a rat hippocampal neuron growing on polylysine (see Methods) was analyzed as it developed a polarized phenotype within the time frame of a live cell recording. The pattern of growth was compared for eight neurites, including the axon, of the cell. Fig. 1a shows the normalized length of the eight neurites during the recording (duration 16.5 hours), with 155 images acquired (see Methods). Fig. 1b shows the normalized ITAD obtained from the theoretical model correlates very well the normalized length. Values were assigned based on the behavior of the cells. Excitatory Strength  $E_T = 85$  and seven excitatory strengths  $E_D = 79.8$ , the parameter  $k$  value is 0.1,  $\sigma = 120$  and  $\tau = 1$  ms. Normalized experimental initial length values were employed as initial conditions for  $T$  and  $D$  in the model.

## 3.3. Scenarios where the regulation of polarity is altered

Following reference [5], rodent hippocampal neurons prepared from embryonic day 18 were transfected with green fluorescent protein (GFP) alone, or with 3xFlag-PKM- $\zeta$ , 3xFlag-aPKC- $\lambda$ , and PKM- $\zeta$ - $\Delta$  Par3 to interfere with the normal course of polarity development. 3xFLAG system is composed of three tandem FLAG epitopes (the part of an antigen molecule to which an antibody attaches itself). This array enhances the sensitivity to detect fusion proteins up to 200 times. The consequences of these manipulations were: For GFP alone, 59% of the neurons ( $n = 168$ ) developed just one axon, 3% more than one axon, 20% indeterminate, and 18% no axons. These data provided a useful case study with which to further test the model. These data were used to predict the information threshold for axon determination under different experimental conditions, such as single axon, multiple axons, or elongated axon formation. The following key parameters were adjusted accordingly in the model:  $k$ ,  $E_T$ , and  $E_D$ . Fig. 3 shows the simulation for GFP alone where the majority of neurons have at least one axon. The values employed were,  $k = 3$  in all neurites,  $E_T = 120$  and  $E_{D1} = E_{D2} \dots = E_{D5} = 79.8$ ,  $M = 100$ ,  $\sigma = 120$ , and  $\tau = 20$  ms. Where  $E_{Di}$  ( $i = 1 \dots 5$ ) are the excitatory strengths of the neurites that were dominated by the excitatory strength of the winning neurite. Initial conditions were  $T = D = 0$ .

For 3xFlag-PKM- $\zeta$ , 24% of the neurons ( $n = 150$ ) presented just one axon, 2% more than one, 23% indeterminate and 51% no axons. PKM- $\zeta$  inhibited all axon formation in more than 50% of the hippocampal neurons within the time frame tested. Fig. 4 shows

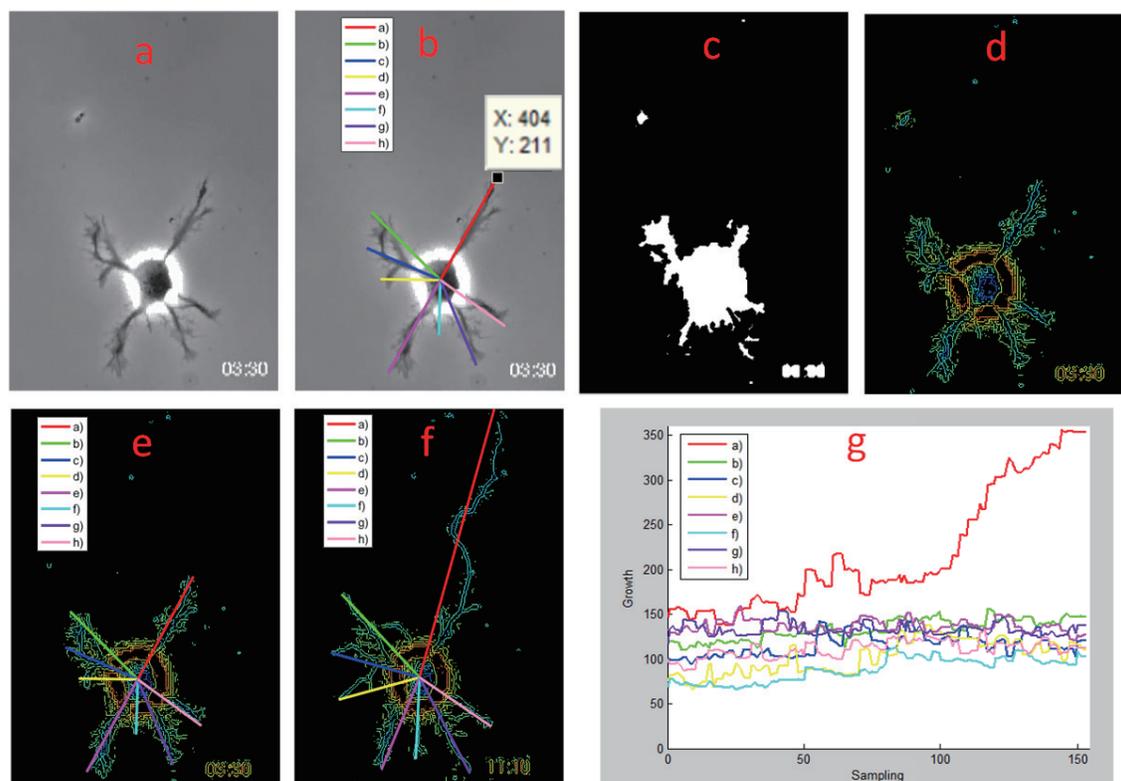


Fig. 1. Time lapse recording of a neuron during the development of polarity and the associated image processing. (a) Frame of original video. (b) Initial frame. (c) Initial frame thresholding. (d) Frame showing neuron structure in false color. (e) Initial frame with growth vectors, and (f) last frame with growth vectors, both in false color. (g) Growth information frame.

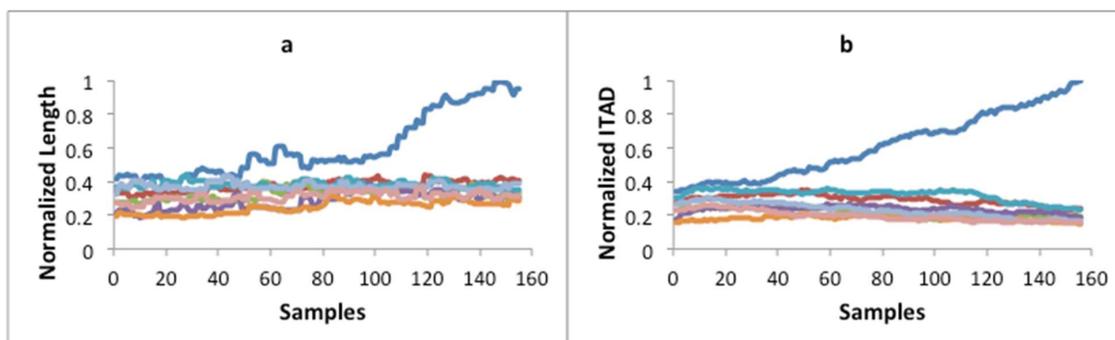


Fig. 2. Time lapse recording of a neuron during the development of polarity and its theoretical comparison. (a) Normalized neurite length for eight neurites within a recorded neuron, compared with (b) Information threshold for axon determination in eight neurites.

the 3xFlag-PKM- $\zeta$  experiment where most neurons have no axon.  $k = 3$  in all neurites,  $E_T = 29.8$ ,  $M = 100$ , and  $\sigma = 120$  and  $\tau = 20$  ms. Initial conditions were  $T = D = 0$ .

For 3xFlag-aPKC- $\lambda$ , 43% of neurons ( $n = 151$ ) presented just one axon, 23% more than one, 14% indeterminate, and 20% no axons. Undoubtedly 3xFlag-aPKC- $\lambda$  promotes axon formation with more than one axon per neuron in 23% of the analyzed population of neurons (in GFP alone it is found 3% only). Fig. 5 shows the 3xFlag-aPKC- $\lambda$  experiment where around a quarter of neurons has more than one axon. In two neurites  $k = 3$ ,  $E_T = 79.8$ ,  $E_{D1} = E_{D2} \dots = E_{D5} = 79.8$ ,  $M = 100$ ,  $\sigma = 120$ , and  $\tau = 20$  ms. Initial conditions were  $T = D = 0$ .

Lastly, for PKM- $\zeta$ - $\Delta$  Par3, 57% of the neurons ( $n = 149$ ) generated just one axon, 2% more than one, 16% indeterminate, and 25% no axons. Obviously PKM- $\zeta$ - $\Delta$  Par3 obtains similar percentages as for GFP alone but it appears that the final length of axons is larger. Fig. 6 shows the simulation for PKM- $\zeta$ - $\Delta$  Par3 where the majority of neurons have at least one axon but the final length of the axons seems longer than the GFP alone case. Here,  $k = 3$  was employed for all neurites,  $E_T = 540$ , and  $E_{D1} = E_{D2} \dots = E_{D5} = 79.8$ ,  $M = 100$ ,  $\sigma = 120$ , and  $\tau = 20$  ms. Initial conditions were  $T = D = 0$ .

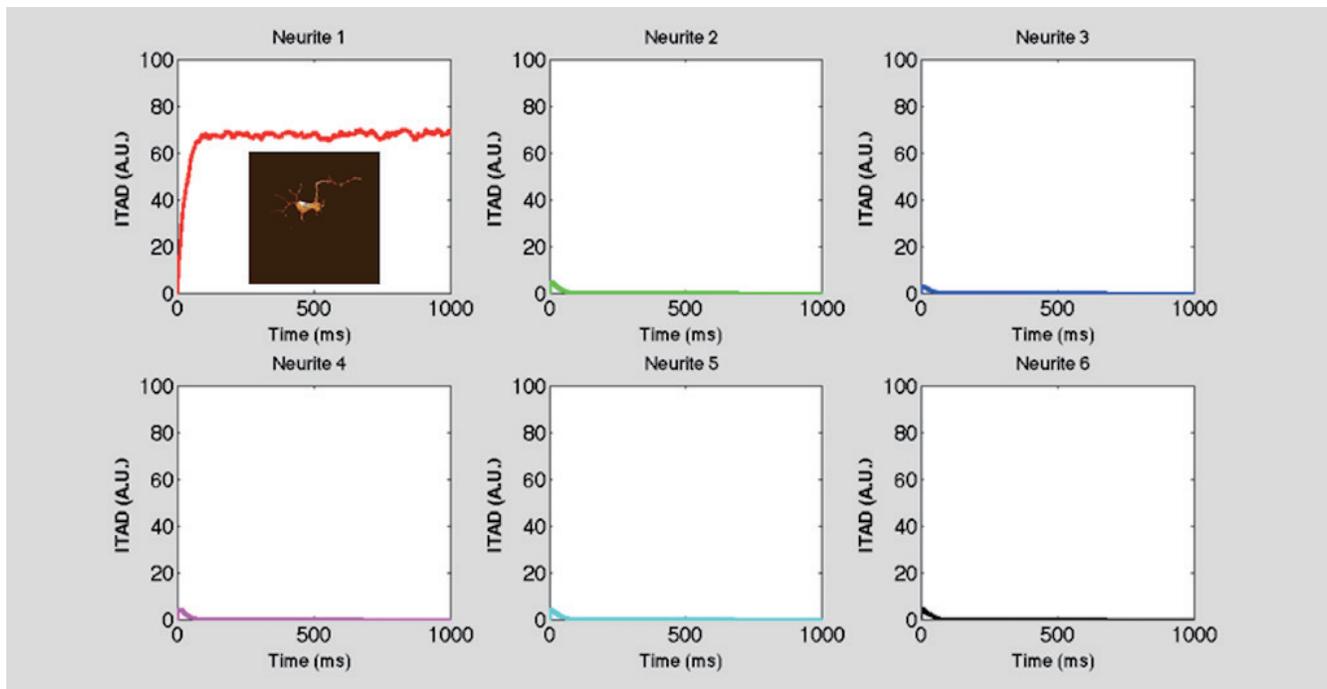


Fig. 3. Information threshold for axon determination in six neurites from cells expressing GFP(Green Fluorescent Protein) alone. In this case the majority of neurons have at least one axon (identified as “neurite 1”). The first neurite has an excitatory strength value of 120, which is larger than the strength value of the remaining five neurites ( $E_D = 79.8$ ). All neurites share the same level of inhibition. Initial conditions were  $T = D = 0$ . Insert image (adapted from [5]) shows a typical neuron used in this experiment.

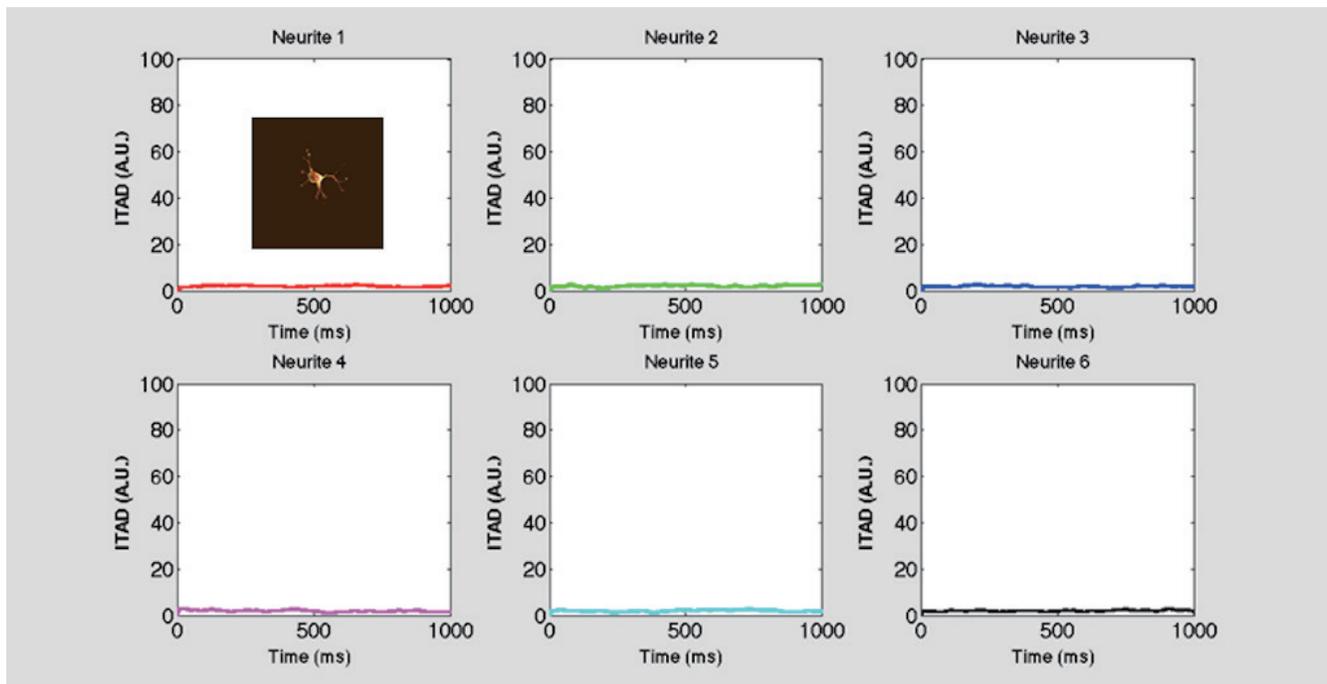


Fig. 4. Information threshold for axon determination in six neurites for 3xFlag-PKM- $\zeta$ . For cells overexpressing this construct, the majority of neurons did not form a defined axon. All the excitatory strength values are 29.8 ( $E_T = E_D$ ). All neurites share the same level of inhibition. Initial conditions were  $T = D = 0$ . Insert image shows a typical neuron used in this experiment. The image was adapted from [5].

#### 4. Discussion

By applying a simple excitatory-inhibitory network model to actual cases of axon formation, it was shown possible to reproduce the

experimental results of axon growth. The model used different excitatory and inhibitory equilibriums to simulate a variety of growth

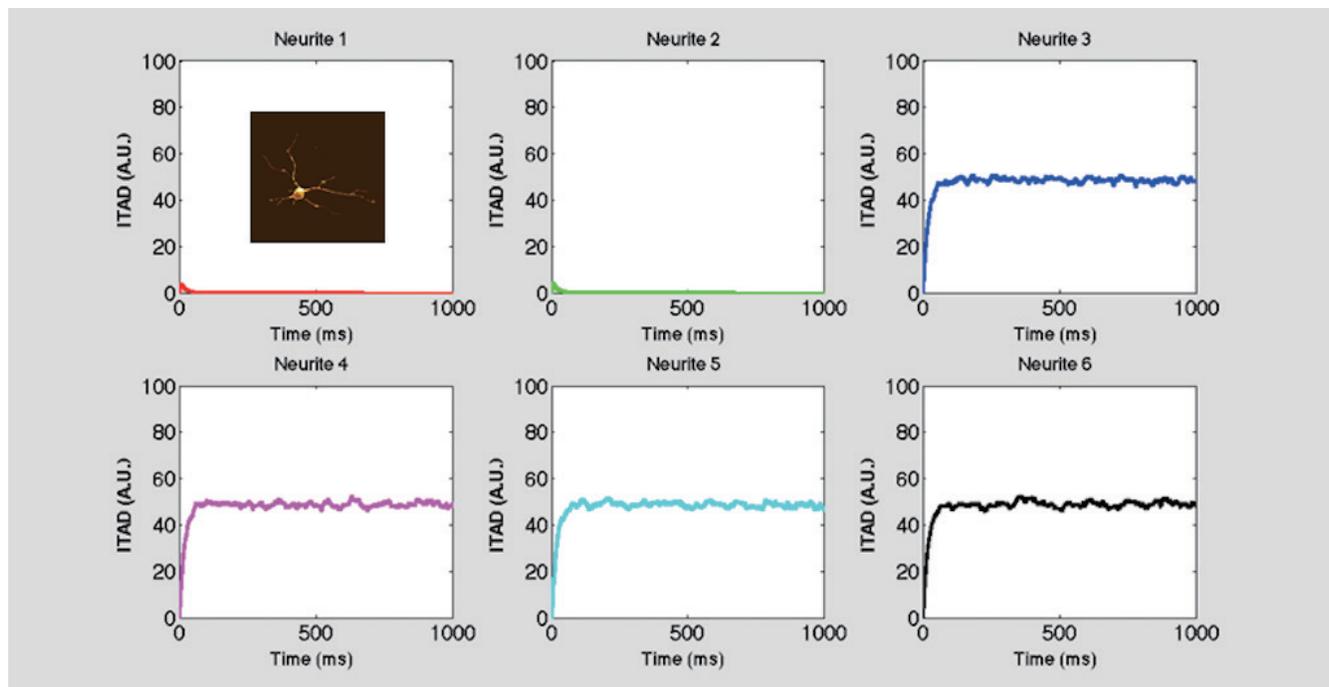


Fig. 5. Information threshold for axon determination in six neurites for 3xFlag-aPKC- $\lambda$ . In this case a quarter of neurons have more than one axon. All excitatory strength values are 79.8 ( $E_T = E_D$ ). Two neurites have  $k = 3$  and in four  $k = 0$ . Initial conditions were  $T = D = 0$ . Insert image (adapted from [5]) shows a typical neuron used in this experiment.

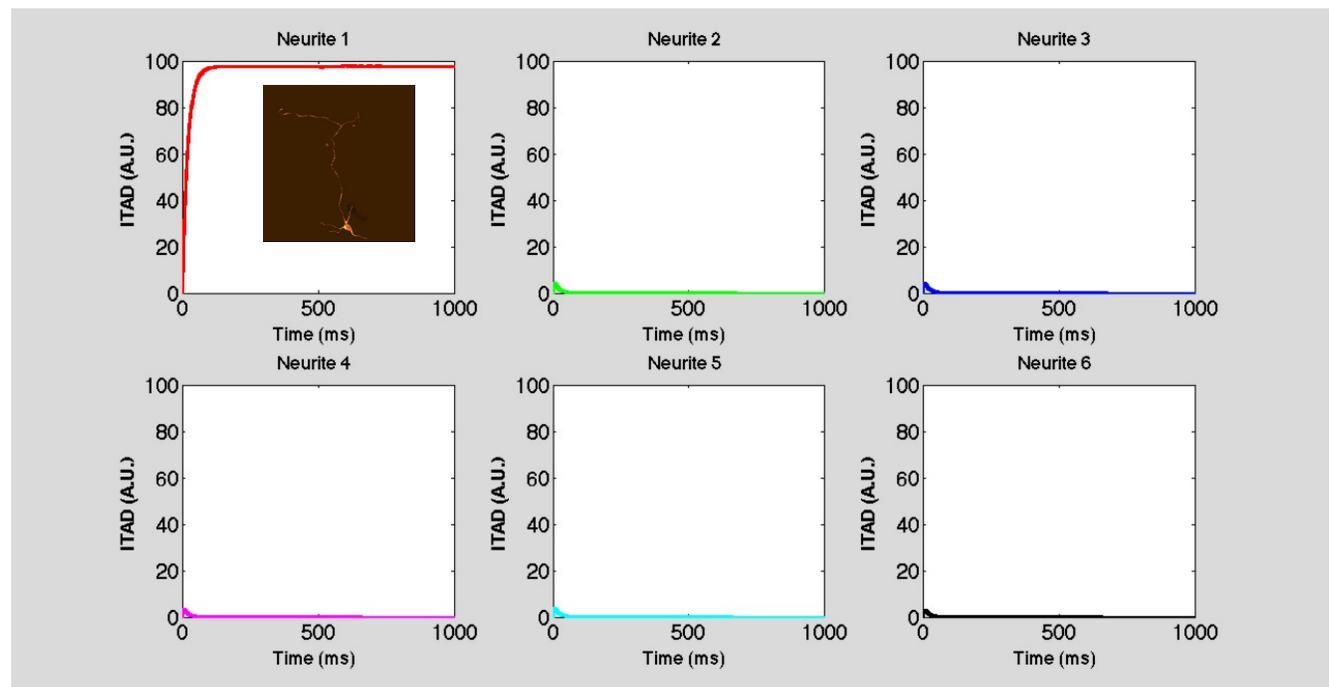


Fig. 6. Information threshold for axon determination in six neurites for PKM- $\zeta$ - $\Delta$  Par3. In this case the majority of neurons have at least one axon but the final length of axons seems longer than the experiment described in Fig. 3. The first neurite has an excitatory strength value of 540, which is bigger than the remaining five neurites ( $E_D = 79.8$ ). Initial conditions were  $T = D = 0$ . All neurites share the same level of inhibition. The insert image (adapted from [5]) shows a typical neuron used in this experiment.

scenarios, including the development of a typical polarized neuron, examples where either multiple or no axons form, as well as cases of

accelerated axon outgrowth. Further, when applied to a time lapse series, the model not only predicted the endpoint of the outgrowth

period (i.e. a single axon with several shorter immature neurites), but also modeled with high fidelity the temporal sequence of outgrowth events for all the neurites.

One advantage of this model is the ability to set different excitatory and inhibition parameter values. This control enables the model to be adapted to different scenarios associated with neurite outgrowth and axon formation. Thus, while the model can be used to study the typical emergence of neuronal polarity, whereby a single axon emerges from a neuron, leaving the remaining neurites to develop into dendrites via a different growth mechanism, it also can be applied to other scenarios. For example, in cases where hippocampal neurons produced at least one axon, this was simulated in the model by setting the value of one excitatory strength  $E_T$  higher than that the remaining excitatory strengths  $E_D$ . Experimental conditions that interfered with axon formation by modulating the intracellular concentration of 3xFlag-PKM- $\zeta$  could also be modeled. Simply by setting the inhibitory strength values equal for all neurites, then decreasing the excitatory strengths by approximately 4-fold, the model predicted that the formation of an axon would be blocked. Alternatively, when 3xFlag-aPKC- $\lambda$  was modulated, approximately a quarter of neurons produced more than one axon. This was simulated by setting the inhibitory strength  $k$  equal to zero in those neurites becoming axons while keeping the excitatory strength in all neurites equal. Finally when PKM- $\zeta$ - $\Delta$  Par3 was present, most of the neurons had at least one axon, but the net length of the axons produced was typically longer than in control cells. It was found that increased excitatory strength in one neurite gave a similar result. In that case,  $E_T$  was increased from 120 to 540.

Thus, it is concluded that this simple excitation-inhibition model can predict the outcomes observed as neurons become polarized under normal conditions [2], as well as under experimental manipulations that alter those conditions [1, 5]. Other models have offered a mechanism of local activation and global inhibition. Fivaz *et al.* [1] proposed that a local positive feedback between HRas and PI3K (local activation), coupled to recruitment of a limited pool of HRas in all the neurites (global inhibition), generates robust symmetry breaking and formation of a single axon. Samuels *et al.* [8] advanced the idea that neuronal polarization is based upon the existence of an unidentified chemical factor whose concentration at the neurite tips modifies the growth rate of each neurite. The formation, transport and consumption of this factor and the resulting neurite growth undergo a winner-take-all instability, yielding rapid growth of one neurite (local activation) and diminished growth of all others (global inhibition). In the model described here, this case corresponds to all neurites sharing the same inhibition level (global inhibition), while just one neurite exhibits an excitation level higher than the others (local excitation). One limitation of the other models is that they cannot account for more complex scenarios that deviate from single axon formation [1, 8, 9].

An alternative model presented by Naoki and colleagues [6, 7] does account for the process by which not only a single axon forms in a neuron, but also either extra axons form or neurons exhibit no axons at all. Nevertheless that model is more complicated than the model proposed here. Some factor, called X, is actively taken from the soma to the growth cone, and then activates another factor, called Y, which directly controls axon growth. Factor Y is activated by factor X, but its activation is characterized by hysteresis, consequently factor Y can be seen as a bi-stable switch. Thus, factor Y only adopts an “or” state, transitioning suddenly to the opposite state when the

concentration of factor X crosses a threshold. Such a mathematical model effectively works as a local activation-global inhibition mechanism as well. In the model presented in [6, 7], a supernumerary scenario is reached when the degradation rate of factor X is low and it has the same value in all neurites (global inhibition). In such a case, the winning neurite cannot easily suppress the elongation of other neurites and they also become axons. In the case of neurons that fail to form axons, Naoki’s model requires the factor X transport rate be low (low local excitation) and the degradation rate be the same in all neurites (same level of global inhibition), therefore no neurite is selected as an axon. In the model described here every individual neurite can have its own excitation or inhibition level, a mechanism that could be considered as local excitation-inhibition. In this manner it can be independently selected which neurite becomes an axon or remains as a neurite. There is an advantage to being able to select which neurites change due to predictive power. Predictive power can only be anticipated if the molecular state of each neurite is known. For example, complexity can be added to the model by introducing local changes in concentrations of polarity activators or inhibitors. It is known, for example, that the balance of gene products can vary by location within the cell. Such polarity activator or inhibitor concentrations would determine the excitatory and inhibitory inputs for the present model but that is beyond the scope of the current work.

Therefore, keeping the same excitatory levels in all neurites and considerably decreasing inhibitory levels but not necessarily to the same values, the model can predict supernumerary neurons, that is neurons with multiple axons neurons. Similarly, the model can predict an-axonal neurons by substantially decreasing the excitatory inputs (low local excitation) while keeping the same inhibitory input values in all neurites (same level of global inhibition). Note that in this model there are situations when there is local excitation in some neurites with no inhibitory inputs (see Fig. 5), reflecting a local inhibition-free condition. This particular state has been proposed in [9] where the cells use biophysical cues to form multiple neurites in the absence of local positive feedback and long-range chemical inhibition. Such physical forces can induce localization of second messengers at the neurite tip without involving any chemical inhibition. The localization, in turn, might initiate potential positive feedback (local activation) by accumulating second messengers. Consequently, the symmetry is broken. This model explains the formation of one axon but not extra axons.

## 5. Conclusion

In summary, application of the model to experimental data obtained from neurons demonstrated that it can reproduce experimental neuronal polarization behavior by scaling different excitatory or inhibitory strength values. The ability to account for these different scenarios suggests the model is robust. Additionally, regardless of the mechanism invoked during the development of polarity (e.g. a single stimulus, or an interplay between combinations of molecular pathways and mechanical cues), the cellular decision to extend an axon may in the end simply result from the balance between excitation and inhibition. How specific molecular complexes inhibit axon formation remains to be determined, e.g. the PKM- $\zeta$ -Par3 complex reported in [5]. That is why a better theoretical understanding of these balance mechanisms may be of importance, as different diseases could prevent or promote axon creation in currently unknown

ways.

From a mathematical point of view, models similar to the one presented here describe very well decision neuronal networks in humans [10]. In those models, when excitatory signals from sensory stimuli are contrasted against background noise, the network detects and selects the location of the target. Inhibitory levels are controlled by the noise presented in the background. These neuronal network results, taken together with the results reported here, imply that excitation-inhibition dynamics might be scale invariant, an idea that warrants future exploration.

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## Conflict of Interest

All authors declare no conflict of interest.

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