A SIMULATION OF GROWTH CONE FILOPODIA DYNAMICS BASED ON TURING MORPHOGENESIS PATTERNS

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INTRODUCTION

The neuronal growth cone is a dynamic, "shape changing" structure which guides the developing neurite to a distant target (Figure 1). The growth cone membrane is constantly creating **filopodia**, long, thin structures which grow and shrink into the extra–cellular space. The exact causes of filopodial excursions are unknown. However, experimental work by Davenport (1992) linked filopodial outgrowth to the local concentration of calcium, which Hentschel (1994) proposed as a morphogen regulating neuronal dendrite growth. We suggest that calcium acts as a morphogen to directly regulate the pattern of filopodial outgrowth and subsequent retraction. Turing (1952) developed a mathematical basis for "**morphogenesis**", the process underlying the development of the shape of an organism. This provides an appropriate framework for modelling the neuronal growth cone.

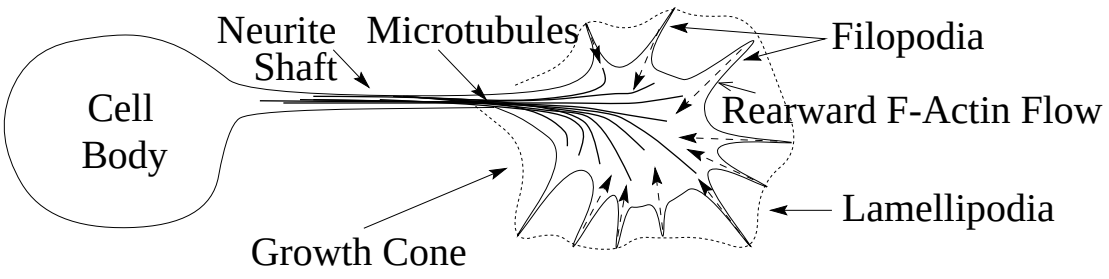

Figure 1. The growth cone.

TURING PATTERNS

Turing patterns can occur when two (or more) chemicals diffuse and interact. The two chemicals are normally an activator A and inhibitor B . A activates itself and B. B inhibits itself and A. When diffusion is included this can be written:

$$\frac{\delta A}{\delta t} = k_1 A - k_2 B + D_A \nabla^2 A \qquad \frac{\delta B}{\delta t} = k_3 A - k_4 B + D_B \nabla^2 B \tag{1}$$

k_1,k_2,k_3,k_4 are constants, and D_A and D_B are the diffusion constants. For pattern formation to occur, the activator A must diffuse slower than the inhibitor B. The events leading up to pattern formation are as follows (see Figure 2).

(1) The system is at steady state. (2) A random perturbation leads to an increase in activator A. (3) Inhibitor B increases. If there is no diffusion this halts the process. (4) The inhibitor diffuses faster than the activator. (5) The activator peak grows (6) Surround inhibition leads to a characteristic pattern width.

Turing patterns have been used to explain animal coat patterns such as tiger and zebra markings, seashell stripes, and drosophila wing prepatterns. The advantage of using Turing equations for this simulation is that they are able to generate a large number of different behaviours - stripes,blobs, oscillations, moving waves - with only a small change needed in any one of the parameters.

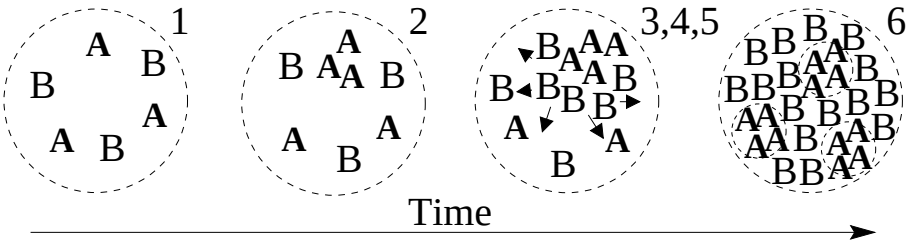

Figure 2. Reaction–diffusion events leading to pattern formation.

THE BIOLOGY OF THE GROWTH CONE

The growth cone is a structure which develops at the tip of the neurite to guide the growing axon to its target. When a growth cone reaches its correct target, it halts, and remodels itself to form a synapse. Figure 1 shows the major regions of the growth cone, the central, transitional and peripheral zones.

The central zone is dominated by microtubules, a polymerised protein made up of tubulin monomers which is important in giving structural support to the axon. Microtubules in the growth cone undergo rapid periods of growing and shrinking known as dynamic instability. The peripheral domain has few microtubules present and a high concentration of the **F–Actin** polymer. Actin normally polymerizes at the leading edge of the growth cone, and then flows rearward towards the central zone at a rate of 3–6 μm per minute. Growth cone advance is inversely proportional to retrograde F–Actin flow. Greater adhesion to the substrate changes the default retrograde movement into a forward movement of the growth cone (Lin, 1995). Filopodia growth and shrinkage is then due to localised variations in the binding of the F–Actin network to the substrate.

CALCIUM AS A MORPHOGEN

Calcium regulates many proteins which alter the F–Actin network. These include α-actinin an actin bundling protein, and gelsolin, an actin severing protein. Localised changes in growth cone calcium concentration alter F–Actin dynamics and consequently filopodial behaviour. Growth occurs with a bell shaped dependency on calcium, peaking at a certain

calcium concentration (Mattson, 1987) (Figure 3).

An increase in calcium can be due to either calcium influx across the membrane or calcium induced calcium release (CICR) from internal stores (Davenport 1992;1996). Calcium can be viewed as an activator as both mechanisms result in rapid filopodial extension from the membrane surface. A candidate molecule for an inhibitor is cAMP. The diffusive constant of cAMP $\approx 4.6 \times 10^{-7} cm^2 s^{-1}$ is faster than that of calcium $\approx 10^{-8} cm^2 s^{-1}$ (Safford, 1977). Thus calcium could act as a short range activator and cAMP as a long range inhibitor in the growth cone. Previously Goodwin (1985) proposed cAMP and calcium as a possible reaction-diffusion pair in the marine algae Acetabularia.

THE SIMULATION

The model chosen to simulate calcium and cAMP concentrations uses simplified equations based on a standard pattern generator developed by Gierer and Meinhardt (Edelstein, 1988, p. 531), with similar properties to (1).

$$\frac{\delta A}{\delta t} = \frac{k_1 A^2}{B} - k_2 A + D_A \Delta^2 A \qquad \frac{\delta B}{\delta t} = k_3 A^2 - k_4 B + D_B \Delta^2 B \tag{2}$$

In the equations, A=calcium, B=cAMP. Calcium activates itself and cAMP with rate constants k_1 and k_3. A rise in calcium levels due to CICR leads to a rise in cAMP levels, which inhibit further calcium increase. Both equations have inhibitory decay terms, with constants k_2 and k_4. (For calcium this can also be viewed as a term describing membrane influx and pumping). The equations are implemented on a finite element grid with e.g. 100×100 pixels, representing a square of side 10 to 100 μm (Figure 4). At the start of the simulation the growth cone is circular, with diameter half the grid size. Initially, all pixels within the growth cone have steady state concentration values for calcium and cAMP with $\pm 1\%$ random noise added. Changes in the values of calcium and cAMP are calculated and updated at each time step.

Pixels outside the growth cone represent the substrate and are initially empty. Pixels on the growth cone membrane have a moveable position co-ordinate which allows them to expand radially into the empty pixels. Calcium and cAMP can then diffuse into the new space. Maximum outgrowth of 6 μm/minute occurs at a concentration of 200 μM calcium. At very low and very high calcium concentrations, the flow is negative, representing lack of F–Actin coupling to the substrate. In the present simulation, outgrowth and shrinkage only occurs from a fixed number of membrane pixels, while the remainder are static.

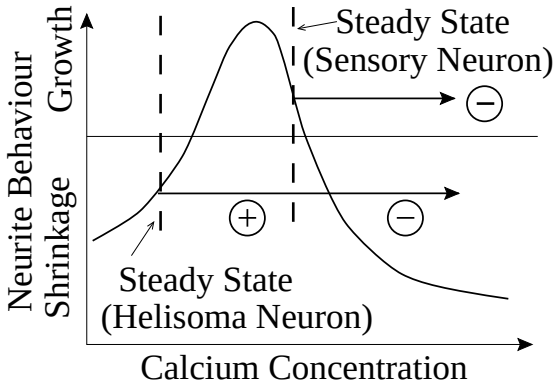

Figure 3. Calcium-dependent outgrowth.

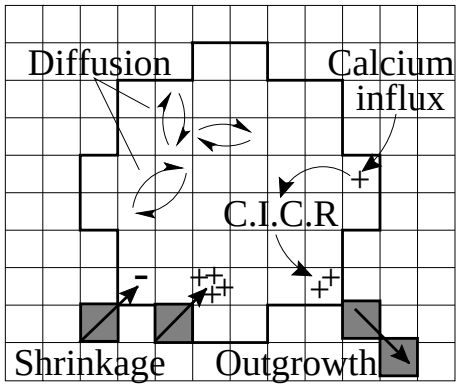

Figure 4. The model simulation.

RESULTS

Figure 5 shows the results of a simulation with k_1=k_2=k_4=1, k_3=0.01, $D_A = 10^{-8} cm^2 s^{-1}$, $D_B = 10^{-7} cm^2 s^{-1}$. Initially the growth cone has a calcium concentration of 100nM and filopodia sprout in all directions. After 40 seconds, a hot spot begins to develop, leading to extended growth in this area, and after 90s filopodia have retracted at the opposite pole.

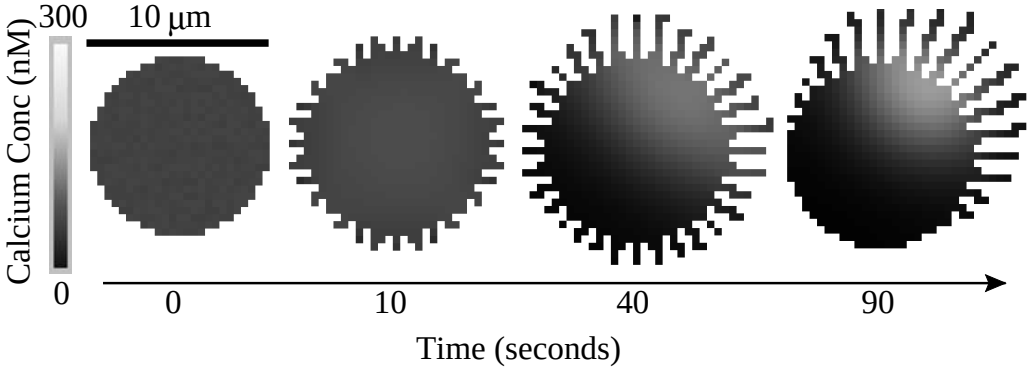

Figure 5. Time sequence of calcium concentration leading to stable pattern formation.

The growth cone pattern can be altered by increasing the size of the cell (Figure 6) or by altering the decay parameters k_2,k_4, and keeping the size fixed. In the present model, the width of the calcium hotspots determines both where the filopodia sprout, and their maximum extension. Explicitly modelling the calcium influx from the external medium would alter the maximum filopodia extension. As filopodia have a large surface membrane to volume ratio, a small calcium influx significantly affects the calcium concentration. Including this influx in the model may lead to narrow spacing of filopodia in the growth cone with long extensions.

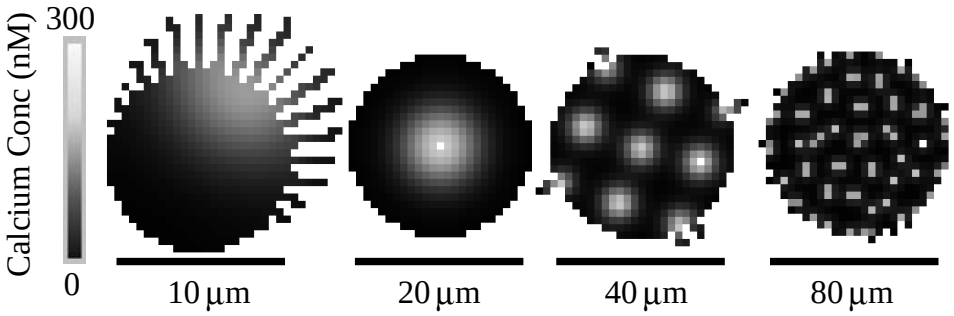

Figure 6. Stable patterns obtained at increasing length scales.

In both Figures 5 and 6, the patterns are stable once the hotspots are established, and existing filopodia remain extended. Continuous extension and retraction of the filopodia would require an underlying calcium concentration with temporal and spatial variations. Unstable oscillations generated by the system are shown in Figure 5 (k_1=k_2=150,k_3=1, k_4=100,D_A=$2.5 \times 10^{-8} cm^2 s^{-1}$). These oscillations are too rapid for stable outgrowth to occur but similar variations over a timescale of minutes would lead to filopodial extension and retraction.

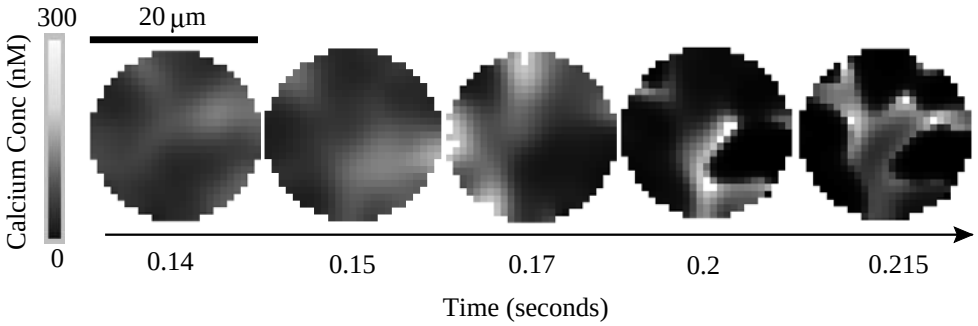

Figure 7. Time sequence of rapid, instable patterns.

CONCLUSION

This is the first model to simulate the causes of the "random and spontaneous" excursions of neurite growth cone filopodia. The simulation demonstrates that any mechanism which results in a slowly varying temporal and spatial calcium pattern would alter the local neurite geometry, resulting in filopodial creation, outgrowth and shrinkage. In the current model, the number of membrane points allowed to develop into filopodia are limited. Although it is possible that biological factors such as local calcium stores, and radially oriented microtubules may create discrete sites for filopodia creation, it is more likely that filopodia could develop continuously along the membrane. We aim to extend the present model to include this behaviour. This model provides a starting point for understanding filopodia dynamics from a new computational perspective.

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