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The Regulation of Cell Motility Through an Excitable Network

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Abstract: Recent years have demonstrated that the actin cytoskeleton and other signaling elements in motile cells have many of the hallmarks of an excitable medium, including the presence of propagating waves, a refractory period, as well as a threshold for activation. Here we show how these behaviors can be explained by the presence of a signal transduction excitable network that integrates a number of signals and coordinates actin polymerization. In this model, spontaneous triggering of the excitable network accounts for the random migration of unstimulated cells. Moreover, internal and external signals both chemical and mechanical bias excitability spatially, thus providing a means by which cell motility is directed towards spatial cues. We also show how the model predicts that the set point of the excitable system can be altered by changing the threshold.

Keywords: Excitable systems, cell motility, thresholds.

1. INTRODUCTION

The study of excitable systems dates back to the work of Alan Hodgkin and Andrew Huxley and their revolutionary research on the mechanisms for excitation and inhibition in the nerve cell membrane. Through a series of experiments and mathematical models, they established that the dynamics of this system exhibited what is now referred to as *excitability* — the presence of a single stable equilibrium with two qualitatively different behaviors following perturbation. When subjected to small-scale disturbances, the system exhibits a monotonic return to its equilibrium. In contrast, for sufficiently strong perturbations, the system undergoes a non-monotonic large scale characteristic excursion before returning to its equilibrium. Together, the system appears to respond in an "all-or-nothing" manner. Moreover, when the system does respond, it possesses a *refractory* period during which it cannot trigger further responses. When the excitable system is distributed in space, the system is said to form an excitable *medium*. In this case, waves of excitation can propagate indefinitely. In the years since, the study of excitable systems and excitable media has been firmly established as a branch of applied mathematics. Moreover, biologists have demonstrated that excitable behavior exists in various physiological and biological processes (Süel et al., 2006; Reichenbach and Hudspeth, 2014).

1.1 Biological Background

Amoeboid cells move by periodically extending protrusions of the cell cortex formed by newly-polymerized actin filaments (Bray, 2001). In addition to amoebae, a number of human cells employ amoeboid motility, including *neutrophils* — white blood cells that form the first line of defense in the immune response.

Over the last decade, an increasing number of studies have suggested that cell migration in several amoeboid cells is regulated by an excitable system whose activity regulates actin polymerization (reviewed by Iglesias and Devreotes, 2012; Shi and Iglesias, 2013). In particular, actin polymerization was observed to propagate in waves reminiscent of those seen in excitable media (Vicker, 2000; Weiner et al., 2007). Since these first papers hinting at the presence of an excitable network, it has been shown that the signaling network that regulates the actin cytoskeleton is itself excitable (Huang et al., 2013). Moreover, this network shows a number of other hallmarks of excitable systems, including the presence of a threshold, an all-or-none response to suprathreshold perturbations, and a refractory period (Huang et al., 2013; Nishikawa et al., 2014).

1.2 Mathematical model

Throughout this paper we will use a simple two-state *activator-inhibitor* model of an excitable system (Fig. 1A). This class of models was first suggested by FitzHugh (1961) and Nagumo et al. (1962) as mathematically tractable models describing excitable behavior. The model consists of two state variables. The first, the *activator* (X), possesses a nonlinear positive feedback term (usually cubic) which allows for self-regeneration. The second, the *inhibitor* (Y), has linear dynamics and enables a negative feedback loop on the activator. The dynamics of the latter is considerably slower, so that the positive feedback is fast and the negative feedback is slow.

In particular, for our model of the excitable system regulating motility in amoeboid cells, we use the following pair of normalized equations:

$$\dot{X} = -\big(1 + a_1(Y - S)\big)X + \frac{a_2 X^2}{a_3^2 + X^2} + B \tag{1}$$

$$\dot{Y} = \epsilon\,(X - Y) \tag{2}$$

to describe the relative levels of both signals.

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The variable B represents a basal (constant) level of activation while S represents an external variable input to the system. This input can represent extrinsic stochastic perturbations, the effect of various chemoattractants that bias the excitability of the system spatially, or internal polarity cues that also regulate cell behavior. The first of these could give rise to spontaneous pseudopods that drive random migration in unstimulated cells (Hecht et al., 2010; Xiong et al., 2010). The second could represent a biasing signal that guides cell motion towards chemoattractants; see Xiong et al. (2010). Finally, the third can represent a slow-time-scale positive feedback system that enables the cell to *remember* the location of previous pseudopods. This memory enables unstimulated cells to perform a more efficient persistent random walk, enabling the cell to cover a greater territory in search of nutrients (Cooper et al., 2012; Shi et al., 2013; Wang et al., 2014; Skoge et al., 2014). In stimulated cells, this memory acts to magnify the effect of chemoattractant gradients, allowing cells to move directionally in gradients as small as 1–2% (Shi et al., 2013). It has to be emphasized that this is a simplified model for a system which consists of more than a hundred genes (Swaney et al., 2010). Nevertheless, simulations of cell movement using this system (or variants) have been shown to recreate many aspects of cell movement quite accurately; see, for example, Neilson et al. (2011), Hecht et al. (2011) and Shi et al. (2013).

1.3 Phase-plane analysis

Because the model of the system is of second order, there are several straightforward analyses that can be carried out.

Existence of equilibria. From the Y-nullcline, we set $Y = X$ into the equation for the X-nullcline. This gives us the equivalent equation

$$\big(1 + a_1(X - S)\big)X = \frac{a_2 X^2}{a_3^2 + X^2} + B$$

or

$$a_1X^4 + (1 - a_1S)X^3 + \big(a_1a_3^2 - a_2 - B\big)\,X^2 + a_3^2(1 - a_1S)X - a_3^2B = 0$$

for the equilibrium value $X^\star$ of the activator. With no input (S=0) and a sufficiently low basal level of activation such that $a_1a_3^2 > a_2 + B$, then Descartes's rule of signs states that there is only one positive real root. However, upon the application of sufficiently strong stimulus (S) so that $a_1S > 1$, three positive real roots are possible. This can occur as S increases and suggests the possibility of bifurcations. However, note that for small or very large values of S only one solution exists; in the first case, $X^\star$ is small; in the latter $X^\star$ is large.

Local stability analysis. The Jacobian for the system has the form:

$$J = \begin{bmatrix} -\big(1 + a_1(X^\star - S)\big) + \dfrac{2a_2a_3^2X^\star}{(a_3^2 + X^{\star 2})^2} & -a_1X^\star \\ \epsilon & -\epsilon \end{bmatrix}.$$

The only term that can change sign is the (1,1) element. The second term:

$$\frac{2a_3a_4^2X^\star}{(a_4^2 + X^{\star 2})^2}$$

is a biphasic function of $X^\star$ starting (when $X^\star = 0$) and ending (as $X^\star \to \infty$) at zero. In these two extremes, and in the absence of an external stimulus, the trace is negative, the determinant positive, and hence the equilibrium is stable. For intermediate values of $X^\star$, this element can be zero or positive and the system can undergo a Hopf bifurcation.

Phase-plane analysis. The properties of this system can be best understood using phase-plane analysis. In particular, note that for zero input, the two nullclines are

$$Y = (a_2 + B)\frac{X^2 + a_3^2\frac{B}{a_2+B}}{X(a_3^2 + X^2)} - \frac{1}{a_1}$$

and $Y = X$.

The first term in the X-nullcline has three effective regimes as a function of X. For sufficiently small X, the curve is proportional to $1/X$. Since

$$a_3^2\frac{B}{a_2 + B} < a_3^2,$$

the nuclline will start to increase when $X^2 \geq a_3^2B/(a_2+B)$ before decreasing once again, after $X^2 \geq a_3^2$. This gives the nullcline a "reverse N" shape reminiscent of a cubic curve. The second term of the X-nullcline simply moves the curve down. Similarly, a constant input ($S > 0$) moves the X-nullcline vertically.

The minimum and maximum of the cubic nullcline are crucial to the existence of excitability. If the Y-nullcline intersects the X-nullcline to the left of the minimum, as in Fig. 1B, then the equilibrium is stable. In this case, a small increase in S moves the eqilibrium from point a to b. As the system is no longer at equilibrium, the state changes, however the trajectory is small as the system settles to its new equilibrium. In contrast, a sufficiently large increase in S, from a to c (Fig. 1C) causes the state to undergo a large excursion in phase space as the activator increases greatly, then decreases below its new steady-state value, before settling to this equilibrium. The nature of the excursion, such as duration and amplitude, are determined by the maximum of the cubic nullcline, which is determined by the strength of the positive feedback loop. What differentiates these two classes of trajectories and which inputs constitute a sufficient trigger depends on the *threshold* for activation, and this is discussed in more detail below.

For the situation in Fig. 1D, when the intersection is shifted to the right of the minimum from a to d, either a saddle-node, or Hopf bifurcation can occur, depending on the specific parameter set (Xiong et al., 2010; Iglesias, 2013). In both cases, the equilibrium becomes unstable, implying that any slight disturbance will cause a large excursion in phase space. In the former case, multiple equilibria appear, and the system can become biphasic. In the latter, the trajectory is oscillatory, as shown in Fig. 1D.

These changes illustrate how cell migration can be driven by an excitable system (Iglesias and Devreotes, 2012). In its basal state, the system is at equilibrium, but subject to random fluctuations in S. Sufficiently large values of S trigger the excitable system, leading to the formation of pseudopods. Additionally, the application of external stimuli increases S and triggers further activity. The presence of a chemoattractant gradient leads to spatially