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# TRANSIENT FEEDBACK AND ROBUST SIGNALING GRADIENTS

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**Abstract.** Robust development of biological organisms in the presence of genetic and epi-genetic perturbations is important for time spans short relative to evolutionary time. Gradients of receptor bound signaling morphogens are responsible for patterning formation and development. A variety of inhibitors for reducing ectopic signaling activities are known to exist and their specific role in down-regulating the undesirable ectopic activities reasonably well understood. However, how a developing organism manages to adjust inhibition/stimulation in response to genetic and/or environmental changes remains to be uncovered. The need to adjust for ectopic signaling activities requires the presence of one or more feedback mechanisms to stimulate the needed adjustment. As the ultimate effect of many inhibitors (including those of the nonreceptor type) is to reduce the availability of signaling morphogens for binding with signaling receptors, a negative feedback on signaling morphogen synthesis rate based on a root-mean-square measure of the spatial distribution of signaling concentration offers a simple approach to robusness and has been demonstrated to be effective in a proof-of-concept implementation. In this paper, we complement the previous investigation of feedback in steady state by examining the effect of one or more feedback adjustments during the transient phase of the biological development.

**Key words.** Morphogen gradients, robustness, feedback mechanism.

## 1. Introduction

Robust development in the presence of genetic mutation and/or epigenetic perturbations is important for biological organisms. Gradients of receptor bound signaling morphogens are known to be responsible for pattern formation and development. The morphogen (aka *ligands*) Decapentaplegic (*Dpp)* in a Drosophila wing imaginal disc, for example, is synthesized at a localized source and transported downstream by active or passive diffusion for binding with signaling receptors Thickvein (*Tkv*) to form a signaling spatial gradient. Graded differences in receptor occupancy at different locations underlie the signaling differences that ultimately lead cells down different paths of development [1, 2, 3, 4].

Genetic and epigenetic changes often alter the ligand synthesis rate resulting in abnormal signaling. Experimental results by S. Zhou in A.D. Lander's lab [4] show that *Dpp* synthesis rate doubles when the ambient temperature is increased by $6°C$. With such an increase in Dpp synthesis rate, the simple models developed in [5, 6, 7] would predict signaling gradients qualitatively different from that at the lower ambient temperature. Yet, little abnormality in the actual development of the wild-type wing imaginal disc is seen under such a change of ambient temperature (see also [8, 9]). In effect, patterning of the Drosophila wing is largely insensitive to a significant increase in synthesis rate. In general, an important requirement

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for normal biological development is for the relevant signaling morphogen gradients not easily altered by genetic or environmental fluctuations that cause significant changes in the constitution of the developing organism. The development is said to be *robust* when the output of the biological system is insensitive to variations in input or system parameters.

A variety of agents for regulating signaling activities are known to exist and their specific role in down– or up–regulating the abnormal activities reasonably well understood. These include molecular entities (such as heparan sulfate proteoglycans [10]) that bind with signaling ligands but the resulting complexes do not signal. Such non-signaling molecular entities are called *nonreceptors*; their presence has been observed to reduce the amount of morphogens available for binding with signaling receptors. That nonreceptors down-regulate cell signaling has also been confirmed theoretically in [11] and references therein. (For other possible mechanisms for down-regulating signaling gradients, see discussion in [12] and references cited there.) Just how a developing organism manages to up-regulate inhibition (such as nonreceptor concentration) or activation (such as raising the binding rate) in response to genetic and/or environmental changes remains to be uncovered.

Evidence exists that adjustments for abnormal signaling activities require one or more feedback mechanisms to stimulate the needed level of correction. Feedback has long been seen as a mechanism for responding to an enhanced signaling gradient and stimulating up-regulation of inhibitors of morphogen signaling to achieve robustness (see [13, 14, 15, 16] for examples). Specific feedback loops identified in the literature include:

- BMP-2 causes significant up-regulation of Sox9 and the BMP antagonist Noggin expression [14, 17].
- High levels of Wingless signaling induce Notum expression and Notum modifies the heparan sulfate proteoglycans Dally-like and Dally that contribute to shaping Wingless gradient [18].

Just how a specific feedback is induced by ectopic signaling morphogen concentration has been an area of current research (see also [19, 20, 21] and references cited there).

In [12], we initiated a different approach to the role of feedback in ensuring robust signaling gradients. The overall goal of the project is to investigate the effectiveness of feedback mechanisms other than a negative feedback of the Hill's function type on signaling receptor synthesis (which is known to be ineffective [19, 20, 21]). With the ultimate effect of many inhibitors (of the nonreceptor type) being a reduction of the availability for signaling morphogens for binding with signaling receptors, we embarked in [12] a proof-of-concept examination of a new spatially uniform nonlocal feedback process (distinctly different from the conventional (spatially nonuniform) Hill function feedback) on the morphogen synthesis rate. This negative feedback is based on a root-mean-square measure of the spatial distribution of signaling concentration offers a simple approach to robustness and has been demonstrated to be effective in [12] for a signaling gradient in steady state. In this paper, we examine the corresponding transient problem with repeated feedback adjustments taking effect during the transient phase of the development.

## 2. Signaling Gradients and Pattern Formation

**2.1. The Initial-Boundary Value Problem for the Basic Model.** The basic process of biological developments is reasonably well understood by biologists.

Signaling protein molecules, collectively known as *morphogens* or *ligands*, are synthesized, possibly at some localized source, and bound reversibly to associated signaling receptors. Through endocytosis, the morphogen-receptor complexes, or *signaling gradient* for brevity, signal and degrade. The gradient of signaling morphogen concentration induces differential cell fates resulting in nonuniform patterns and functions. A simple example is the *Decapentaplegic* (*Dpp*) morphogen involved in the development of the structure of the Drosophila wing imaginal disc. With *Dpp* synthesized in a narrow region straddling the border between the anterior and posterior compartment of the wing imaginal disc and relatively uniform along the direction of that boundary, we may focus on a one-dimensional model for the *Dpp* gradient formation in the extracellular space of the posterior compartment first introduced and analyzed in [5, 6, 7]. The idealization of activities in the wing imaginal disc leading to this basic model and its applicability as well as insight to other morphogen gradient systems with similar characteristics have already been described in these references and references cited there. Here, we give only the dimensionless form of the initial-boundary value problem (IBVP) for this basic model consisting of the three differential equations

$$\frac{\partial a}{\partial t} = \frac{\partial^2 a}{\partial x^2} - h_0 ar + f_0 b - g_L a + v_L(x,t), \tag{1}$$

$$\frac{\partial b}{\partial t} = h_0 ar - (f_0 + g_0)b, \qquad \frac{\partial r}{\partial t} = v_R(x,t) - h_0 ar + f_0 b - g_R r, \tag{2}$$

for the dimensionless concentrations, $a$, $b$ and $r$, of free morphogen (e.g., *Dpp* in the Drosophila wing disc), bound morphogen (or the ligand-receptor complex) and unoccupied receptors (e.g., *Tkv* for *Dpp* in Drosophila wing disc), respectively. All three concentrations are normalized by the steady state receptor concentration $R_0$ prior to the onset of the normalized ligand synthesis $v_L(x,t)$. In this investigation, receptors are synthesized by a time independent synthesis rate (normalized as $v_R(x)$) long before the onset of ligand production. The dimensionless space and time variables $x$ and $t$ are normalized by $X_{\max}$ (the span of the wing imaginal disc in the distal direction from the boundary of the anterior and posterior compartment in the case of the wing imaginal disc) and the time constant $X_{\max}^2/D$ with $D$ being the diffusion coefficient for the diffusive ligand molecules, respectively. The various rate constants $\{k_{on}R_0, k_{off}, k_L, k_{\deg}, k_R\}$ for binding, dissociation, free ligand degradation, bound ligand degradation and unoccupied receptor degradation are also normalized by the same time constant,

$$\{h_0, f_0, g_L, g_0, g_R\} = \frac{X_{\max}^2}{D}\{k_{on}R_0, k_{off}, k_L, k_{\deg}, k_R\}. \tag{3}$$

where $R_0$ being the steady state receptor concentration prior to the onset of ligand synthesis. For typical biological organism, $g_0$ is typically smaller than $g_R$ for an adequate accumulation of signaling receptor-ligand complexes toward the formation of a signaling morphogen gradient and patterning. On the other hand, the free ligand degradation rate is usually negligibly low and is set to zero in most gradient models.

With ligand synthesized only in the narrow production region $(-X_{\min}, 0)$, we normalize the synthesis rate $V_L$ by setting $v_L(x,t) = (V_L/R_0)/(D/X_{\max}^2)$ to get

$$v_L(x,t) = e\bar{v}_L H(-x) = \frac{e\bar{V}_L/R_0}{D/X_{\max}^2} H(-x) = \begin{cases} e\bar{v}_L & (-x_m \le x < 0) \\ 0 & (0 < x \le 1) \end{cases} \tag{4}$$

where $\bar{V}_L$ is the uniform (wild-type) synthesis rate in the narrow production region, $x_m = X_{\min}/X_{\max}$. The constant $e$ is an *enhancement factor*; it is normally 1 but may take on other values due to environmental changes. For our purpose, it suffices to consider receptor synthesis to be at a uniform rate $\bar{V}_R$ in both time and space (throughout the spatial domain) with

$$v_R(x,t) = v_R(x) = \bar{v}_R = \frac{\bar{V}_R/R_0}{D/X_{\max}^2}. \tag{5}$$

Prior to the onset of ligand synthesis, unoccupied receptors should be in a steady state concentration determined by the second equation in (2) to be

$$R_0 = \frac{\bar{V}_R}{k_R} = \frac{\bar{v}_R}{g_R} R_0 \tag{6}$$

with

$$\bar{v}_R = \frac{\bar{V}_R/R_0}{D/X_0^2} = \frac{k_R}{D/X_0^2} = g_R. \tag{7}$$

The three differential equations are augmented by the following (normalized) boundary conditions

$$x = -x_m : \quad \frac{\partial a}{\partial x} = 0, \qquad x = 1 : \quad a = 0, \tag{8}$$

all for $t > 0$. For the wing imaginal disc, the no flux condition at the compartment border is a consequence of assumed symmetry of the two compartments of the wing disc. The kill end condition at the edge, $X = X_{\max}$, reflects the assumption of an absorbing end (which we may occasionally take to be infinitely far away to avoid making such an assumption). Until morphogens being generated at $t = 0$, the biological system is in quiescence so that we have the (normalized) initial conditions

$$t = 0 : \quad a = b = 0, \quad r = 1 \tag{9}$$

keeping in mind $\bar{v}_R = g_R$.

The initial-boundary value problem (IBVP) defined by (1), (2), (8) and (9) has been analyzed mathematically and computationally in [6, 12] and elsewhere. The model is generally applicable to other morphogen gradient systems with similar characteristics, at least for some insight to the qualitative behavior of such gradients.

**2.2. A Steady State Particular Solution.** With both ligand and receptor syntheses $v_L(x,t) = e\bar{v}_L H(-x)$ and $v_R(x,t) = \bar{v}_R$ independent of time for the basic model, it has been shown in [6] that a unique time independent particular solution of the IBVP exists with

$$\{\bar{a}_e(x), \bar{b}_e(x), \bar{r}_e(x)\} = \lim_{t\to\infty} \{a(x,t), b(x,t), r(x,t)\}, \tag{10}$$

that is linearly stable with respect to a small perturbation from the steady state. The subscript $e$ in these steady state quantities indicates the level of synthesis rate enhancement (ectopicity) with $e = 1$ corresponding to normal development and $e > 1$ corresponding to ectopic signaling gradients.

The steady state solution may be determined by the well-posed two-point boundary value problem (BVP) for $\bar{a}_e(x)$:

$$\bar{a}_e'' - \frac{g_0 \bar{a}_e}{\alpha_0 + \zeta_0 \bar{a}_e} - g_L \bar{a}_e + e\bar{v}_L H(-x) = 0, \tag{11}$$

$$\bar{a}_e'(-x_m) = 0, \qquad \bar{a}_e(1) = 0. \tag{12}$$

with

$$\bar{b}_e(x) = \frac{\bar{a}_e(x)}{\alpha_0 + \zeta_0 \bar{a}_e(x)}, \qquad \bar{r}_e(x) = \frac{\alpha_0}{\alpha_0 + \zeta_0 \bar{a}_e(x)} \tag{13}$$

where

$$\alpha_0 = \frac{f_0 + g_0}{h_0}, \quad \zeta_0 = \frac{k_{\deg}}{k_R} = \frac{g_0}{g_R}, \tag{14}$$

keeping in mind $\bar{v}_R = g_R$ (see (7)).

**2.3. A State of Low Receptor Occupancy.** The morphogen system is said to be in a state of *low receptor occupancy* (LRO) if

$$\max_{-x_m \le x \le 1} \zeta_0 a_e \ll \alpha_0. \tag{15}$$

For such a system, we may neglect terms involving $\zeta_0 \bar{a}_e$ in (11)-(13) to get an approximate set of solutions $\{\bar{A}_e(x), \bar{B}_e(x), \bar{R}_e(x)\}$ to be determined by

$$\bar{A}_e'' - \mu_L^2 \bar{A}_e + e\bar{v}_L H(-x) = 0, \qquad \mu_L^2 = \frac{g_0}{\alpha_0} + g_L \tag{16}$$

$$\bar{A}_e'(-x_m) = 0, \qquad \bar{A}_e(1) = 0. \tag{17}$$

The exact solution for $\bar{A}_e(x)$ is

$$\bar{A}_e(x) = \begin{cases} \frac{e\bar{\nu}_L}{\mu_L^2}\{1 - \frac{\cosh(\mu_L)}{\cosh(\mu_L(1+x_m))}\cosh(\mu_L(x+x_m))\} & (-x_m \le x \le 0) \\ \frac{e\bar{\nu}_L}{\mu_L^2}\frac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L(1-x)) & (0 \le x \le 1) \end{cases}, \tag{18}$$

with

$$\bar{b}_e(x) \simeq \bar{B}_e(x) = \frac{\bar{A}_e(x)}{\alpha_0}, \qquad \bar{r}_e(x) \simeq \bar{R}_e(x) = 1. \tag{19}$$

As indicated in a previous section, $g_0$ is typically smaller than $g_R$ so that $\zeta_0 < 1$. In that case, a state of LRO requires $a_e \ll \alpha_0$ with $\alpha_0 = O(g_0/h_0) \ll 1$ (so that degradation of signaling ligand complexes is not more rapid than their formation). It follows from (18) that $\bar{\nu}_L$ is necessarily small compared to unity for a gradient system to be in a state of low receptor occupancy.

**Remark 1.** *For $\mu_L \gg 1$, the expression for $\bar{A}_e(x)$ in the signaling range of $0 \le x < 1$ is effectively a boundary layer adjacent to $x = -x_m$, steep near $x = -x_m$ and dropping sharply to $\bar{\nu}_L/\mu_L^2$ (which is rather small) away from $x = -x_m$. Thus, even if a morphogen system is in a steady state of low occupancy, its signaling gradient may not be biologically useful for patterning if the condition $\mu_L^2 = O(1)$ is not met.*

## 3. Robustness of Signaling Gradient

**3.1. Ectopic Gradients.** Normal development of wing imaginal disc and other biological organisms may be altered by an enhanced morphogen synthesis rate stimulated by genetic or epigenetic changes. As mentioned earlier, *Dpp* synthesis rate in Drosophila imaginal disc doubles when the ambient temperature is increased by $6°C$. At a state of low receptor occupancy (LRO), a significant increase in morphogen synthesis rate is seen from the results given in (18)-(19) to increase proportionately the magnitude of the steady state signaling morphogen concentration and hence changing the cell fate at each spatial location. Without the restriction of LRO, the steady state free and signaling morphogen gradients are expected to

be an increasing function of synthesis rate (but not necessarily proportionately and now also with some gradient shape distortion) as shown below.

**Proposition 2.** *$\bar{a}_e(x;e)$ is a non-decreasing function of $e$ and increasing at least for some segment(s) of the solution domain $[-x_m, 1]$.*

Let $w(x,e) = \partial\bar{a}_e/\partial e$. Upon differentiating partially the BVP for $\bar{a}_e(x,e)$ with respect to $e$, we obtain

$$w'' - \frac{\alpha_0 g_0 w}{(\alpha_0 + \zeta_0 \bar{a}_e)^2} - g_L w + v_L H(-x) = 0,$$
$$w'(-x_m, e) = 0, \qquad w(1,e) = 0$$

It is straightforward to verify that $w_\ell(x,e) = 0$ is a lower solution (but not an exact solution) of the BVP for $w(x,e)$ and $w_u(x,e) = v_L\{(x_m(1-x) + (1-x^2)/2\}$ is an upper solution (and again not an exact solution). The monotone method of [22, 23, 24] assures us that $w(x,e)$ exists with

$$0 = w_\ell(x,e) \le w(x,e) \le w_u(x,e).$$

and $w(x,e)$ is not identically zero (since $w_\ell(x,e) = 0$ is not the solution of the BVP for $w(x,e)$).

**Corollary 3.** *$\bar{b}_e(x,e)$ is a non-decreasing function of $e$ and increasing in some segment(s) of $[-x_m, 1]$.*

*Proof.* Upon differentiating $\bar{b}_e(x,e)$ partially with respect to $e$, we obtain

$$\frac{\partial\bar{b}_e}{\partial e} = \frac{\partial}{\partial e}\left[\frac{\bar{a}_e(x)}{\alpha_0 + \zeta_0\bar{a}_e(x)}\right] = \frac{\alpha_0 g_0 w}{(\alpha_0 + \zeta_0\bar{a}_e)^2} \ge 0$$

and not identically zero in some segment of $[-x_m, 1]$. □

**3.2. A Robustness Index.** To calibrate the ectopicity of the enhanced signaling for $e > 1$, we introduced a robustness index $R_b(t)$ to measure the deviations of ectopic signaling gradient (from the wild-type gradient, i.e., $e = 1$) after ligand synthesis rate enhancement ($e > 1$). Let $b_1(x,t)$ be the normalized signaling morphogen concentration for a normal ($e = 1$) ligand synthesis rate $v_L(x,t) = \bar{v}_L H(-x)$ (after normalization). Let $b_e(x,t)$ be the same quantity for an enhanced (ectopic) synthesis rate $e\bar{v}_L H(-x)$ . A rather natural global measure of signaling gradient robustness is the following *signal robustness index* $R_b$ corresponding to the root mean square of the difference between $b_e(x,t)$ and $b_1(x,t)$ over the solution domain:

$$R_b(t) = \frac{1}{b_h - b_\ell}\sqrt{\frac{1}{x_\ell - x_h}\int_{x_h}^{x_\ell}[b_e(x,t) - b_1(x,t)]^2 dx} \tag{20}$$

where $0 \le b_\ell(t) < b_h(t) \le b(-x_m, t)$ and $-x_m \le x_h < x_\ell \le 1$. The quantities $x_\ell$, $x_h$, $b_\ell$ and $b_h$ may be chosen away from the extremities to minimize the exaggerated effects of outliers.

For a system in steady state with

$$\bar{b}_1(x) = \lim_{t\to\infty} b_1(x,t), \qquad \bar{b}_e(x) = \lim_{t\to\infty} b_e(x,t), \tag{21}$$

the robustness index $R_b(t)$ tends to a constant $\bar{R}_b$:

$$\bar{R}_b = \lim_{t\to\infty} R_b(t) = \frac{1}{\bar{b}_h - \bar{b}_\ell}\sqrt{\frac{1}{x_\ell - x_h}\int_{x_h}^{x_\ell}[\bar{b}_e(x) - \bar{b}_1(x)]^2 dx} \tag{22}$$

To be concrete, we have taken in previous investigation (such as [12]) $x_h = 0$ so that $\bar{b}_h = \bar{b}_1(0)$ for a representative magnitude as a measure of the extent of the root-mean-square deviation. For the other end, we have taken $x_\ell = 1$ so that $\bar{b}_\ell = \bar{b}_1(1) = 0$. In that case, (22) simplifies to

$$\bar{R}_b = \frac{1}{\bar{b}_1(0)} \sqrt{\int_0^1 [\bar{b}_e(x) - \bar{b}_1(x)]^2 dx} \tag{23}$$

In subsequent development, we may occasionally omit the subscript "1" for the special case of normal gradients (when $e = 1$) so that they are denoted by $\{\bar{a}(x), \bar{b}(x), \bar{r}(x)\}$ instead of $\{\bar{a}_1(x), \bar{b}_1(x), \bar{r}_1(x)\}$.

It should be noted that the robustness index $R_b(t)$ is not the only possible calibration of ectopicity of the signaling gradient. At least one other measure has been discussed in [9, 21] and elsewhere. Even for the present investigation, it would be simpler to replace the time dependent $b_h(x_h, t) - b_\ell(x_\ell, t)$ by $\bar{b}_h(x_h) - \bar{b}_\ell(x_\ell)$ or some representative magnitude for the signaling gradient even for the transient phase.

**3.3. Approximate Solution for Low Receptor Occupancy.** For a morphogen system in a state of LRO (before and after ligand synthesis rate enhancement) so that $g_0 a_e / g_R \ll \alpha_0$, we have from (18)-(19) the following approximate steady state expression for the signaling gradients of the normal (wild type) and (environmentally or genetically) perturbed system:

$$\bar{b}_e(x) \sim \frac{e\bar{v}_L}{\alpha_0 \mu_L^2} \frac{\sinh(\mu_L x_m) \sinh(\mu_L (1-x))}{\cosh(\mu_L (1+x_m))} = \frac{\bar{v}_L}{\alpha_0 \mu_L^2} \bar{B}_e(x) = \frac{\bar{v}_L}{\alpha_0 \mu_L^2} \left[ e \bar{B}_1(x) \right], \tag{24}$$

for $0 \le x \le 1$ where $\mu_L^2 = g_L + g_0/\alpha_0$ with $\mu_L^2 \simeq h_0 + g_L$ whenever $f_0 \ll g_0$ (as it is for *Dpp* in Drosophila wing imaginal disc).

For $x_h = 0$ , we have from (18) and (19)

$$\bar{b}_h = \bar{b}_1(0) \simeq \frac{\bar{v}_L}{\alpha_0 \mu_L^2} \frac{\sinh(\mu_L x_m) \sinh(\mu_L \ell_M)}{\cosh(\mu_L (\ell_M + x_m))} = \frac{\bar{v}_L}{\alpha_0 \mu_L^2} \bar{B}_1(0), \tag{25}$$

for LRO systems. By taking $x_\ell = 1$ and $\bar{b}_\ell = \bar{b}(1) = 0$ and with $e = 2$ for the enhanced synthesis rate, $\bar{R}_b$, in the absence of any feedback, was found in [12] to be approximately given by

$$\begin{aligned} \bar{R}_b &\simeq \bar{r}_b = \frac{1}{\sinh(\mu_L)} \sqrt{\int_0^1 [\sinh(\mu_L (1-x))]^2 dx} \\ &= \frac{1}{\sinh(\mu_L)} \sqrt{\frac{1}{2} \left( \frac{\sinh(2\mu_L)}{2\mu_L} - 1 \right)} \equiv \gamma. \end{aligned} \tag{26}$$

Numerical solutions for the steady state BVP and the corresponding robustness index $\bar{R}_b$ have been obtained for several sets of parameter values in [12] to assess the level of robustness of the corresponding *Dpp* gradients. We mentioned here only the results pertaining to the first example with the organism characterized by the parameter values shown in Table 1 there and reproduced below for later references.

We know from [6] that this system meets the condition for a state of LRO and is further confirmed to be so by comparison of the exact numerical solution with that of the linearized model.

TABLE 1

$$
\begin{aligned}
X_{\max} &= 0.01\ cm, \quad X_{\min} = 0.001\ cm, \quad k_{on}R_0 = 0.01/\sec./\mu M,\\
k_{deg} &= 2\times 10^{-4}/\sec., \quad k_R = 0.001/\sec., \quad k_{off} = 10^{-6}/\sec., \quad k_L = 0,\\
D &= 10^{-7}\ cm^2/\sec., \quad \bar{V}_L = 0.002\ \mu M/\sec., \quad \bar{V}_R = 0.04\ \mu M/\sec.
\end{aligned}
$$

| c | $\bar{R}_k$ | $\bar{R}_{k+1}$ | $\bar{b}_1(0)$ | $\tilde{b}(0;\bar{R}_k)$ | $\tilde{b}(0;\bar{R}_{k+1})$ | $\tilde{b}(0;0)$ |
|---|---|---|---|---|---|---|
| 0 | 0.39380 | 0.39380 | 0.05798 | 0.11533 | 0.11533 | 0.11533 |
| 1 | 0.24096 | 0.24099 | 0.05798 | 0.09327 | 0.09306 | 0.11533 |
| 2 | 0.18290 | 0.18296 | 0.05798 | 0.08451 | 0.08469 | 0.11533 |
| 4 | 0.11114 | 0.11163 | 0.05798 | 0.07422 | 0.07431 | 0.11533 |

## 4. Feedback on Ligand Synthesis Rate

**4.1. A Non-local Feedback with Delay.** With signaling gradient up-regulated by an elevation of ambient temperature, some feedback mechanism is needed to adjust for the developing organism to stimulate an appropriate level of inhibition. Down-regulation of (ectopic) signaling activities is known to be possible in different ways. Whether it is through more nonreceptors or higher degradation rate of free or bound ligands, the net effect is equivalent to a lower concentration of free ligand available for binding with signaling receptors. In a proof-of-concept investigation of a new approach to feedback initiated in [12], the effect of a negative feedback stimulated by a higher than normal signaling ligand concentration was taken to be simply a reduction of the ligand synthesis rate $V_L$. To implement this approach, we took the normalized synthesis rate $v_L(x,t)$ to include a negative feedback factor using the signaling robustness index $R_b(t)$ as an instrument for down-regulating the synthesis rate:

$$v_L(x,t) = \kappa(t;\tau)\bar{v}_L H(-x) \equiv \frac{e\bar{v}_L H(-x)}{1 + c\,[R_b(t-\tau)]^n} \tag{27}$$

where the *amplification factor* $e$ is as previously introduced in (1) and where $c$ and $n$ are two parameters to be chosen for appropriate feedback strength similar to those for a Hill's function. Two features of the feedback process in (27) should be noted. First, with $c = n = 1$, the feedback mechanism reduces the synthesis rate by a fraction that depends on the root-mean-square deviation over an appropriate spatial span (e.g., the distal span of the posterior compartment of the wing imaginal disc of the Drosophila). Second, the feedback may not be instantaneous as a delay of $\tau$ unit of dimensionless time is allowed for the feedback to become effective.

With $\tau > 0$ (and $v_R(x,t) = \bar{v}_R$ uniformly throughout the entire distal-proximal span of the wing imaginal disc), the IBVP for the three normalized concentration may be computed as we would for the problem without feedback except that the ligand synthesis rate now changes with time. In particular, for the period $[0,\tau]$, the problem is identical to the one without feedback. For the interval $[k\tau,(k+1)\tau]$ and with $t = k\tau\ +\eta,\ 0 \le \eta \le \tau$, the synthesis rate is modified to

$$v_L(x,t) = \frac{e\bar{v}_L H(-x)}{1 + c\,[R_b((k-1)\tau + \eta)]^n} \qquad (0 \le \eta \le \tau) \tag{28}$$

with all concentrations continuous at the junctions between the time intervals.

**4.2. Time Independent Steady State with Feedback.** It is known from [6] that the extracellular model system without feedback has a unique steady state that is linearly stable with respect to small perturbations from the steady state. It

was shown in [12] that a monotone decreasing steady state solution also exists and is unique for our model with feedback on the ligand synthesis rate taken in the form of (28). Suppose $\{a(x,t),\, b(x,t), r(x,t)\}$ of (1) - (9) tend to the time independent states $\{\tilde{a}_e(x), \tilde{b}_e(x), \tilde{r}_e(x)\}$ and therewith $R_b(t) \to \tilde{R}_b$ (see (20) and (22)). In that case, we have $v_L(x,t)$ of (28) tends to $e\bar{\kappa}(\tilde{R}_b)\bar{\nu}_L H(-x)$ where

$$\bar{\kappa}(\tilde{R}_b) = \lim_{t\to\infty} \kappa(t;\tau) = \frac{1}{1 + c\left(\tilde{R}_b\right)^n}. \tag{29}$$

Note that we have used $\bar{\kappa}(\tilde{R}_b)$ for $\kappa(t;\tau)$ in the steady state case since the *amplitude factor* $\kappa(t;\tau)$ is no longer time dependent and is only a function of $\tilde{R}_b$ (and of course of $n$ and $c$ in both cases).

The new BVP for the steady state free ligand concentration in the presence of feedback was shown in [12] to be :

$$\tilde{a}_e'' - \frac{g_0\tilde{a}_e}{\alpha_0 + \zeta_0\tilde{a}_e} - g_L\tilde{a}_e + e\bar{\kappa}(\tilde{R}_b)\bar{\nu}_L H(-x) = 0, \tag{30}$$

$$\tilde{a}_e'(-x_m) = 0, \qquad \tilde{a}_e(1) = 0. \tag{31}$$

where $\bar{\kappa}(\tilde{R}_b)$ is given by (29) with $\tilde{R}_b$ calculated from $\tilde{a}_e(x)$:

$$\tilde{R}_b = \frac{1}{\bar{b}_h}\sqrt{\int_0^1 [\tilde{b}_e(x;\tilde{R}_b) - \bar{b}_1(x)]^2 dx}. \tag{32}$$

where

$$\tilde{b}_e(x;\tilde{R}_b) = \frac{\tilde{a}_e(x;\tilde{R}_b)}{\alpha_0 + \zeta_0\tilde{a}_e(x;\tilde{R}_b)}, \qquad \bar{b}_1(x) = \frac{\bar{a}_1(x)}{\alpha_0 + \zeta_0\bar{a}_1(x)}. \tag{33}$$

with $c = 0$ in (30) for the calculation of the wild-type $\bar{a}_1(x)$ and $\bar{b}_1(x)$.

The solution of the BVP (30) - (31) is much less straightforward since the forcing term now involves an integral of a complicated expression of the unknown $\tilde{a}_e(x;\bar{R}_b)$. Methods of solution for this more complex BVP (including the LRO case) have already been discussed in [12] and some numerical results were obtained by an iterative algorithm there and summarized in Table 1 of this paper for $n = 1$ and $c$ =1, 2 and 4 to demonstrate the efficacy of our approach. For subsequent references, we mention here only the exact solution for the LRO case, denoted by $a_0(x;\bar{r}_b)$, for its dependence on the LRO approximation $\bar{r}_b$ of the exact robustness index $\bar{R}_b$:

$$\tilde{a}_0(x;\tilde{r}_b) = \begin{cases} \frac{e\bar{\kappa}(\tilde{r}_b)\bar{\nu}_L}{\mu_L^2}\{1 - \frac{\cosh(\mu_L\ell_m)}{\cosh(\mu_L(1+x_m))}\cosh(\mu_L(x+x_m))\} & (-x_m \le x \le 0) \\ \frac{e\bar{\kappa}(\tilde{r}_b)\bar{\nu}_L}{\mu_L^2}\frac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L(1-x)) & (0 \le x \le 1) \end{cases}, \tag{34}$$

where $\mu_L^2$ is as given by (16) and where $\bar{R}_b$ in the expression (29) for $\bar{\kappa}$ is now replaced by the corresponding approximate expression $\tilde{r}_b$

$$\tilde{r}_b = \frac{1}{\bar{b}_h}\sqrt{\int_0^1 [\tilde{b}_0(x;\tilde{r}_b) - \bar{b}_0(x)]^2 dx} \tag{35}$$

using the low receptor occupancy approximate solution $\tilde{b}_0(x;\tilde{r}_b)$ for $\tilde{b}(x;\tilde{R}_b)$:

$$\tilde{b}(x;\tilde{R}_b) \simeq \frac{\tilde{a}_0(x;\tilde{r}_b)}{\alpha_0} = \tilde{b}_0(x;\tilde{r}_b), \tag{36}$$

$$\alpha_0\tilde{b}(0;\tilde{R}_b) \simeq \tilde{a}(0;\tilde{R}_b) \simeq \tilde{a}_0(0;\tilde{r}_b) = \frac{e\bar{\kappa}(\tilde{r}_b)\bar{\nu}_L}{\mu_L^2}\frac{\sinh(\mu_L x_m)}{\cosh(\mu_L(1+x_m))}\sinh(\mu_L). \tag{37}$$

For $\mu_L \gg 1$, the expression for $\tilde{a}_0(x;\bar{r}_b)$ in the signaling range of $0 \leq x < 1$ is asymptotically

$$\tilde{a}_0(x;\bar{r}_b) \sim \frac{e\bar{\kappa}(\bar{r}_b)\bar{\nu}_L}{\mu_L^2} e^{-\mu_L x} \qquad (0 \leq x < 1)$$

so that the gradient is effectively a boundary layer adjacent to $x = 0$, steep near $x = 0$ and dropping sharply to near zero away from $x = 0$.

For $n = 1$, the expressions (33) for the enhanced and wild-type normalized signaling morphogen gradients, $\tilde{b}(x;\bar{R}_b)$ and $\bar{b}_1(x)$, in the range relevant for cell signaling are to be used, respectively, in the expression (35) as was done in (26) to obtain for a low receptor occupancy system

$$\tilde{R}_b \simeq \tilde{r}_b(c) = \gamma(\mu_L)\left[\frac{2}{1+c\tilde{r}_b} - 1\right] \tag{38}$$

with

$$\gamma(\mu_L) = \frac{1}{\sqrt{2}\sinh(\mu_L)}\sqrt{\left(\frac{\sinh(2\mu_L)}{2\mu_L} - 1\right)}. \tag{39}$$

For $c = 0$ (corresponding the case of no feedback), we have immediately

$$\left[\tilde{R}_b\right]_{c=0} \simeq \tilde{r}_b(0) = \gamma(\mu_L)$$

For the example in the discussion following (26), we have $\gamma(\mu_L) = 0.3938.....$ while accurate numerical solutions of the previous section give $\tilde{R}_b = 0.39380...$

For $0 < c < \infty$, the relation (38) may be written as the quadratic equation

$$c\tilde{r}_b^2 + (1 + c\gamma)\tilde{r}_b - \gamma = 0 \tag{40}$$

for $\bar{r}_b$ with one positive solution

$$\tilde{R}_b \simeq \tilde{r}_b = \frac{1}{2c}\left[-(1+c\gamma) + \sqrt{(1+c\gamma)^2 + 4\gamma}\right] > 0. \tag{41}$$

For the problem specified by the parameter values in Table 1 and $c = 1$, such a feedback process gives

$$\left[\tilde{R}_b\right]_{c=1} \simeq \tilde{r}_b(1) = 0.2410... \tag{42}$$

(which is nearly identical to the average 0.2410... of the $8^{th}$ and $9^{th}$ iterates found earlier for $\tilde{R}_b(c = 1)$).

## 5. Feedback in Transient Phase.

Generally, a feedback mechanism would not become effective until the enhanced signaling reaches a noticeable level of ectopicity. Typically, this occurs during the transient phase of the development (and not when the system is already in a quasi-steady state). It is also possible that there is a delay in sensing the excessive signaling and the actual initiation of the response with the delay time short compared to the time to quasi-steady state. For systems capable of repeated feedbacks, their impact prior to steady state may be cumulative toward eventual patterning and development. It is important then to investigate feedback effective during the transient phase of the signaling morphogen gradient formation.

In principle, the developing organism may adjust continuously with continuous feedback but is unlikely to function with such sensitivity or efficiency. It is more likely to make feedback adjustments at a few instants or perhaps only once at some threshold ectopicity. As a proof-of-concept investigation, we consider two models

of a feedback adjustment after each time interval $\tau$, both for the special case of $n = 1$, to illustrate the method of solution for this class of problems.

Let $t_k = k\tau, k = 0, 1, 2, 3, ...$ and suppose feedback adjustments are made only at $t_1, t_2, t_3, t_4,$ ..... and not in between these instants in time. We designate the time interval $[t_k, t_{k+1})$ as period $k$. For both models, an additional feedback adjustment is made during period $k$ on the enhanced ligand synthesis rate $v_L(x,t) \equiv v_e^{(k-1)}(x,t)$ of the previous time interval. The adjustment is by a factor $(1 + cR_b(\bar{t}_k))^{-1}$ for some constant $c$ and some instant $\bar{t}_k$ (keeping $n = 1$ for this first effort).

In this section, we investigate the *first model* in which feedback adjustment for period $k$ is made at the start time $t_k$ of that time interval on the basis of the robustness index $R_b$ at that moment so that $\bar{t}_k = t_k$. In that case, we have for period $k$

$$v_L(x,t) \equiv v_e^{(k)}(x,t) = \frac{v_e^{(k-1)}(x,t)}{1 + cR_b(t_k)} \equiv e\kappa_k \bar{v}_L H(-x).$$

We now examine the evolution of the signaling gradient with time in successive time intervals $[t_k, t_{k+1})$.

**5.1. The Interval $[0, t_1)$.** In this initial interval corresponding to $k = 0$, the enhanced ligand synthesis rate with (our nonlocal spatially uniform) feedback is

$$v_L = \frac{e\bar{v}_L H(-x)}{1 + cR_b(t_0)} = e\bar{v}_L H(-x) \equiv v_e^{(0)}(x,t) \qquad (0 \le t \le t_1 = \tau) \tag{43}$$

since

$$\begin{aligned} R_b(t_0) &= \frac{1}{\bar{b}_h}\int_0^1 [b_e(x,t_0) - b_1(x,t_0)]^2 dx \\ &= \frac{1}{\bar{b}_h}\int_0^1 [b_e^{(0)}(x,0) - b_1(x,0)]^2 dx = 0 \end{aligned}$$

given $b_e(x,0) = b_1(x,0) = 0$ by the initial conditions To emphasize that the result applies only to the initial time interval $[0, t_1)$, we adopt the notation

$$R_b(t_0) \equiv R_b^{(0)}(0) = 0.$$

It follows that feedback is not effective in this interval. In that case, the synthesis rate of the ectopic IBVP (1), (2), (8) and (9) in this time interval, $v_L = v_e^{(0)}(x,t) = e\bar{v}_L H(-x)$ with the subscript $e$ corresponding the magnitude of ectopicity, is the same as the problem without feedback. The solution, to be denoted by $a_e^{(0)}(x,t)$, $b_e^{(0)}(x,t)$ and $r_e^{(0)}(x,t)$ (including the wild type corresponding to $e = 1$ in (43)) can be obtained by numerical methods with the initial conditions (9) at $t = 0$ (as we have done previously in [6] for problems without feedback).

**5.2. The Interval $[t_1, t_2)$.** In this next interval $\tau = t_1 \le t < t_2 = 2\tau$ corresponding to $k = 1$, the enhanced ligand synthesis rate with feedback is

$$v_L = \frac{e\bar{v}_L H(-x)}{1 + cR_b(t_1)} \equiv v_e^{(1)}(x,t) \qquad (\tau \le t < \tau).$$

with

$$\begin{aligned} R_b(t_1) &= \frac{1}{\bar{b}_h}\int_0^1 [b_e(x,t_1) - b_1(x,t_1)]^2 dx \\ &= \frac{1}{\bar{b}_h}\int_0^1 [b_e^{(0)}(x,\tau) - b_1(x,\tau)]^2 dx \equiv R_b^{(1)}(t_1) \end{aligned}$$

since, by continuity, $b_e(x,t_1)$ is the same at the junction $t_1$ of the two adjacent intervals. $b_e^{(1)}(x,\tau) = b_e^{(0)}(x,\tau)$ by continuity. With $b_e^{(0)}(x,\tau)$ already known from the solution of the IBVP for the previous interval, the modified synthesis rate for the ectopic IBVP in this time interval,

$$v_L = v_e^{(1)}(x,t) = \frac{e\bar{v}_L H(-x)}{1 + cR_b^{(1)}(t_1)} = e\kappa_1 \bar{v}_L H(-x) \qquad (t_1 \le t < t_2), \tag{44}$$

is a known function. The solution of the IBVP in $[t_1, t_2)$, denoted by $\left\{a_e^{(1)}, b_e^{(1)}, r_e^{(1)}\right\}$, can be obtained by numerical methods with the continuity condition

$$\left\{a_e^{(1)}(x,t_1),\ b_e^{(1)}(x,t_1),\ r_e^{(1)}(x,t_1)\right\} = \left\{a_e^{(0)}(x,t_1),\ b_e^{(0)}(x,t_1),\ r_e^{(0)}(x,t_1)\right\}$$

as the initial conditions and with the quantities on the right already known from the solution for the previous interval.

Note that feedback on the synthesis rate is now effective for the interval $[t_1, t_2)$. As such the solution of the IBVP (1), (2), (8) and (9) for the ectopic problem in this time interval is generally different from the corresponding solution without feedback, i.e., the special case $c = 0$. Anticipating the subsequent development, we adopt the notation $\kappa_0 = 1$ so that we may write

$$\kappa_1 = \frac{\kappa_0}{1 + cR_b^{(1)}(t_1)}.$$

**5.3. The Interval $[t_2, t_3)$.** In the next interval $2\tau = t_2 \le t < t_3 = 3\tau$ corresponding to $k = 2$, the enhanced ligand synthesis rate with feedback is

$$v_L = \frac{v_e^{(1)}(x,t)}{1 + cR_b(t_2)} \equiv v_e^{(2)}(x,t).$$

Similar to the previous interval, $R_b(t_2)$ for the new interval is completely determined from the solution of the previous interval at the junction $t_2$ between the two adjacent intervals:

$$R_b(t_2) = \frac{1}{\bar{b}_h} \int_0^1 [b_e^{(1)}(x,t_2) - b_1(x,t_2)]^2 dx \equiv R_b^{(2)}(t_2).$$

The corresponding solution of the IBVP (1), (2), (8) and (9), to be denoted by $\left\{a_e^{(2)},\ b_e^{(2)},\ r_e^{(2)}\right\}$, may be obtained by numerical methods starting from the continuity conditions at $t_2$:

$$\{a_e^{(2)}(x,t_2),\ b_e^{(2)}(x,t_2), r_e^{(2)}(x,t_2)\} = \{a_e^{(1)}(x,t_2),\ b_e^{(1)}(x,t_2), r_e^{(1)}(x,t_2)\}. \tag{45}$$

with the quantities on the right already obtained in the discussion of the previous interval. The synthesis rate for the ectopic IBVP with feedback in this time interval may be written as

$$v_L = v_e^{(2)}(x,t) = \ e\bar{v}_L \kappa_2 H(-x) \qquad (t_2 \le t < t_3) \tag{46}$$

with

$$\kappa_2 = \frac{\kappa_1}{1 + cR_b^{(2)}(t_2)} = \frac{\kappa_0}{\left[1 + cR_b^{(2)}(t_2)\right]\left[1 + cR_b^{(1)}(t_1)\right]}. \tag{47}$$

**5.4. The Interval** $[t_k, t_{k+1})$. $k \geq 3$ **:.** In a general interval $k\tau = t_k \leq t < t_{k+1} = (k+1)\tau$ for $k \geq 3$, the enhanced ligand synthesis rate with feedback is

(48)
$$v_L = \frac{v_e^{(k-1)}(x,t)}{1 + cR_b(t_k)} = \frac{e\bar{v}_L \kappa_{k-1} H(-x)}{\left[1 + cR_b^{(k)}(t_k)\right]} = e\bar{v}_L \kappa_k H(-x) \equiv v_e^{(k)}(x,t) \quad (t_{k-1} \leq t - \tau < t_k)$$

with

$$R_b^{(k)}(t_k) = \frac{1}{\bar{b}_h} \int_0^1 [b_e^{(k-1)}(x,t_k) - b_1(x,t_k)]^2 dx. \tag{49}$$

Hence, the feedback adjusted synthesis rate of the ectopic IBVP in the interval $t_k \leq t < t_{k+1}$ is

$$v_L = v_e^{(k)}(x,t) = \quad e\bar{v}_L \kappa_k H(-x) \quad (t_k \leq t < t_{k+1}) \tag{50}$$

with

$$\kappa_k(t_1, \ ...., \ t_k) = \frac{1}{\prod\limits_{j=1}^{k} \left[1 + cR_b^{(j)}(t_j)\right]} \tag{51}$$

given $\kappa_0 = 1$. In the expression (51), the robustness indices $\{R_b^{(j)}(t_j)\}$ are calculated from the formula (49) (with $k$ replaced by $j$ ) sequentially with increasing $j$ starting from $j = 1$.

The corresponding solution of the IBVP (1), (2), (8) and (9), denoted by $\left\{a_e^{(k)}, \ b_e^{(k)}, \ r_e^{(k)}\right\}$, may be obtained by numerical methods starting from the continuity conditions at $t_k$:

$$\{a_e^{(k)}(x,t_k), \ b_e^{(k)}(x,t_k), r_e^{(k)}(x,t_k)\} = \{a_e^{(k-1)}(x,t_k), \ b_e^{(k-1)}(x,t_k), r_e^{(k-1)}(x,t_k)\}.$$

**5.5. Numerical Results.** With $\kappa_k$ decreasing with $k$, $R_b^{(k)}$ also decreases with $k$; the iterative algorithm above is expected to converge. To compare the effectiveness of cumulative repeated feedback adjustments during the transient phase with that of steady state theory obtained in [12] (see also Table 1 of this paper) we apply the present approach to Example 1 examined in [12]. With the system parameter values given in Table 1 earlier, we take $c = 1$ (along with $n = 1$ as specified earlier) and $\tau = 4$ in units of $(D/X_{\max}^2)^{-1}$ with the system reaching steady state around $5\tau = 20$ .

Note that the top curve in the Figure 1 is essentially the quasi-steady state $b_e$ (for $e = 2$) without feedback and with the signaling gradient reaching steady state around $t = 20$. The expected role of any feedback adjustment is to bring $b_e^{(5)}(x, 20)$ as close as possible to the the bottom steady state wild-type signaling gradient curve in that figure ($e = 1$ and no feedback). After the feedback adjustments become effective (starting with $k = 2$), the cumulative effect of the repeated adjustments continues to reduce the signaling gradient in successive time intervals, leading to a gradient $b_e^{(5)}(x, 20)$ very close to the wild-type gradient prior to synthesis rate being enhanced from $e = 1$ to $e = 2$.

Even more important is the shape of the wild-type gradient being maintained by the feedback adjusted ectopic gradient. More specifically, the spatially uniform nonlocal feedback mechanism does not suffer the same fate besetting the Hill's function type feedback mechanism applied to the signaling receptor synthesis rate first found in [19].

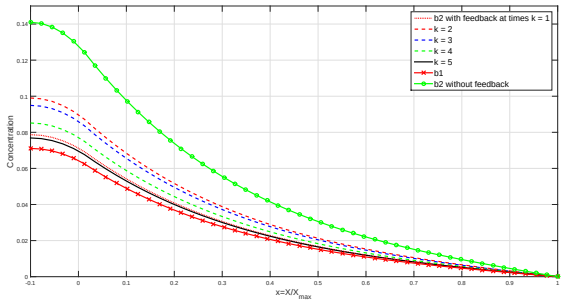


FIGURE 1. Various Signaling Gradients (some with feedback adjustment). (1) Wild-type $b_1(x, 20)$ with ($e = 1$ and $c = 0$) at the bottom. (2) Ectopic $b_e(x, 20)$(with $e = 2$ and $c = 0$) at the top. (3) In between from top down are $b_e(x, t_k)$ at $t = t_2, t_3, t_4, t_1$ and $t_5 = 20$.

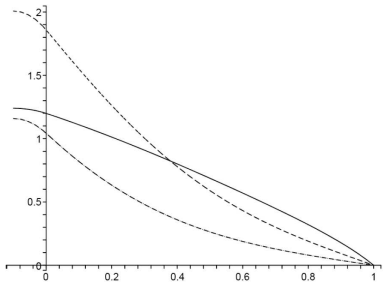

FIGURE 2. Negative feedback of Hills function type on receptor synthesis rate. (1) Wild-type signaling gradient (e = 1, no feedback) - bottom dashed curve. (2) Ectopic signaling gradient (e = 2 without feedback) - top dashed curve. (3) Ectopic signaling gradient (e = 2) with negative feedback on receptor synthesis.

TABLE 2. Comparison of $R_b(x, t_k)$.

| Transient (c=1) | | Steady State |
|---|---|---|
| No Delay (k) | With Delay (k) | From [12] (c) |
| 0.24937 (1) | 0.24937 (1) | 0.39379 (0) |
| 0.26524 (2) | 0.36501 (2) | 0.24051 (1) |
| 0.18738 (3) | 0.31731 (3) | 0.18296 (2) |
| 0.10145 (4) | 0.18769 (4) | ..... |
| 0.03769 (5) | 0.05747 (5) | 0.11163 (4) |

In the first column of Table 2, we report robustness index values at different instants in time for the feedback mechanism described above. Except for an increase in $R_b^{(2)}(x, t_2)$ over $R_b^{(1)}(x, t_1)$ (because there is no feedback adjustment for the interval $[0, 4)$), modifications of the synthesis stimulated by feedback repeatedly reduce the robustness index to below the acceptable robustness threshold value of 0.2 by $t = 12$ and well below that threshold by $t = 20$ (effectively in steady state). We contrast these results to the steady state approach taken in [12] where the same nonlocal spatially uniform feedback mechanism becomes effective when the system

reaches its steady state. The results using that approach obtained in [12] and reproduced in the third column of Table 2 show that $R_b(x,\infty)$ is well above 0.2 for $c = 1$ (the same value of $c$ used for all cases in the first column).

## 6. Transient Feedback with Delay

In this section, we investigate the *second model* when the effect of feedback through the robustness index $R_b(\bar{t})$ is recorded at the start of the time interval $t_k$ but reacting with a delayed time $\tau$ so that $\bar{t}_k = t_k - \tau = t_{k-1}$:

$$v_L(x,t) \equiv v_e^{(k)}(x,t) = \frac{v_e^{(k-1)}(x,t)}{1 + cR_b(t_{k-1})} \equiv e\bar{v}_L d_k H(-x)$$

We now examine the evolution of the signaling gradient with time in successive time intervals $[t_k, t_{k+1})$.

**6.1. The Interval $[0,t_1)$.** In the initial interval $(0 = t_0 \le t < t_1 = \tau)$ corresponding to $k = 0$, the enhanced ligand synthesis rate with (our spatially uniform) feedback is

$$v_L = \frac{e\bar{v}_L H(-x)}{1 + cR_b(t_0 - \tau)} = \frac{e\bar{v}_L H(-x)}{1 + cR_b(-\tau)} = e\bar{v}_L H(-x) \equiv v_e^{(0)}(x,t) \tag{52}$$

(again with the subscript $e$ corresponding the magnitude of ectopicity) since $b_e(x,t) = b_1(x,t) = 0$ for $t$ prior to the onset of morphogen synthesis so that

$$R_b(-\tau) = \frac{1}{\bar{b}_h}\int_0^1 [b_e(x,-\tau) - b_1(x,-\tau)]^2 dx = 0.$$

With

$$R_b(t_0 - \tau) \equiv R_b^{(0)}(-\tau) = 0$$

so that feedback is not effective given the delay, the synthesis rate of the ectopic IBVP (1), (2), (8) and (9) in this time interval is the same as that without feedback. The solution, to be denoted by $a_e^{(0)}(x,t)$, $b_e^{(0)}(x,t)$ and $r_e^{(0)}(x,t)$ (including the wild type corresponding to $e = 1$ in (43)) can be obtained by numerical methods with the initial conditions (9) at $t = 0$.

**6.2. The Interval $[t_1,t_2)$.** In this next interval $\tau = t_1 \le t < t_2 = 2\tau$ corresponding to $k = 1$, the enhanced ligand synthesis rate with feedback is

$$v_L = \frac{v_e^{(1)}(x,t)}{1 + cR_b(t_1 - \tau)} = \frac{e\bar{v}_L H(-x)}{1 + cR_b(0)} \equiv v_e^{(1)}(x,t) \qquad (0 \le t - \tau < \tau).$$

With

$$\begin{aligned} R_b(t_1 - \tau) &= \frac{1}{\bar{b}_h}\int_0^1 [b_e(x,0) - b_1(x,0)]^2 dx \\ &= \frac{1}{\bar{b}_h}\int_0^1 [b_e^{(0)}(x,0) - b_1(x,0)]^2 dx = 0, \end{aligned}$$

given the initial conditions $b_e(x,0) = b_1(x,0) = 0$, we have also

$$R_b(t_1 - \tau) \equiv R_b^{(1)}(0) = 0$$

in the interval $t_1 \le t < t_2$ . The synthesis rate for the ectopic IBVP in this time interval is

$$v_L = v_e^{(1)}(x,t) = \frac{e\bar{v}_L H(-x)}{1 + cR_b^{(1)}(0)} = e\bar{v}_L H(-x) \qquad (t_1 \le t < t_2).$$

The solution, denoted by $\left\{a_e^{(1)}(x,t),\ b_e^{(1)}(x,t),\ r_e^{(1)}(x,t)\right\}$ is the same as one without feedback and can be obtained by the usual numerical methods with the continuity condition

$$\left\{a_e^{(1)}(x,t_1),\ b_e^{(1)}(x,t_1),\ r_e^{(1)}(x,t_1)\right\} = \left\{a_e^{(0)}(x,t_1),\ b_e^{(0)}(x,t_1),\ r_e^{(0)}(x,t_1)\right\}$$

as initial conditions. Note that the quantities on the right have already been obtained for the previous interval.

With feedback on the synthesis rate not yet effective for the combined interval $[0,t_2)$, the solution of the IBVP (1), (2), (8) and (9) for the ectopic problem in the time interval $t_1 \le t < t_2$ is merely a continuation of that for the previous interval.

**6.3. The Interval $[t_2,t_3)$.** In the next interval $2\tau = t_2 \le t < t_3 = 3\tau$ corresponding to $k=2$, the enhanced ligand synthesis rate with feedback is

$$v_L = \frac{v_e^{(1)}(x,t)}{1+cR_b(t_2-\tau)} = \frac{e\bar{v}_L H(-x)}{1+cR_b(\tau)} \equiv v_e^{(2)}(x,t)$$

with

$$\begin{aligned} R_b(t_2-\tau) &= \frac{1}{\bar{b}_h}\int_0^1 [b_e(x,\tau)-b_1(x,\tau)]^2 dx \\ &= \frac{1}{\bar{b}_h}\int_0^1 [b_e^{(1)}(x,\tau)-b_1(x,\tau)]^2 dx = R_b^{(2)}(t_1) \end{aligned}$$

given $b_e^{(2)}(x,t_1) = b_e^{(1)}(x,t_1)$ by continuity. For the interval $t_1 \le \bar{t} = t-\tau < t_2$ and feedback adjustment effective only at $t_1$, we have

$$R_b(t_2-\tau) = R_b^{(2)}(t_1)$$

and the feedback modified synthesis rate for the ectopic IBVP in this time interval is

$$v_L = v_e^{(2)}(x,t) = \frac{e\bar{v}_L H(-x)}{1+cR_b^{(2)}(t_1)} \equiv e\bar{v}_L d_2 H(-x) \qquad (t_2 \le t < t_3) \tag{53}$$

with

$$d_2 = \frac{1}{1+cR_b^{(2)}(t_1)}. \tag{54}$$

With the feedback adjusted synthesis rate completely known for the interval $t_2 \le t < t_3$, the solution of the IBVP (1), (2), (8) and (9), denoted by $\left\{a_e^{(2)},\ b_e^{(2)},\ r_e^{(2)}\right\}$, can be obtained by numerical methods starting from the continuity conditions at $t_2$:

$$\{a_e^{(2)}(x,t_2),\ b_e^{(2)}(x,t_2), r_e^{(2)}(x,t_2)\} = \{a_e^{(1)}(x,t_2),\ b_e^{(1)}(x,t_2), r_e^{(1)}(x,t_2)\}. \tag{55}$$

with the quantities on the right already obtained for the previous interval.

Given $v_L = v_e^{(2)}(x,t) = e\bar{v}_L d_2(t_1) H(-x)$ which is known but different from $v_e^{(1)}(x,t) = v_e^{(0)}(x,t) = e\bar{v}_L H(-x)$, feedback on the synthesis rate is effective in the interval $[t_2,t_3)$. As such the solution of the IBVP for the transient ligand concentration for the ectopic problem is expected to be different from the corresponding solution without feedback in this interval.

**6.4. The Interval $[t_k, t_{k+1})$. $k \geq 3$.** In period $k$, where $k\tau = t_k \leq t < t_{k+1} = (k+1)\tau$ for $k \geq 3$, the enhanced ligand synthesis rate with feedback is

$$v_L = \frac{v_e^{(k-1)}(x,t)}{1 + cR_b(t_k - \tau)} = \frac{e\bar{v}_L d_{k-1} H(-x)}{[1 + cR_b(t_{k-1})]} \equiv v_e^{(k)}(x,t) \qquad (t_{k-1} \leq t - \tau < t_k) \tag{56}$$

with

$$\begin{aligned} R_b(t_k - \tau) &= \frac{1}{\bar{b}_h} \int_0^1 [b_e(x, t_{k-1}) - b_1(x, t_{k-1})]^2 dx \\ &= \frac{1}{\bar{b}_h} \int_0^1 [b_e^{(k-1)}(x, t_{k-1}) - b_1(x, t_{k-1})]^2 dx = R_b^{(k)}(t_{k-1}) \end{aligned}$$

Hence, the feedback adjusted synthesis rate of the ectopic IBVP in the interval $t_k \leq t < t_{k+1}$ is

$$v_L = v_e^{(k)}(x,t) = \quad ed_k \bar{v}_L H(-x) \tag{57}$$

where $d_0 = d_1 = 1$ and

$$d_k(t_1, \ ...., \ t_{k-1}) = \frac{1}{\prod_{j=2}^{k} \left[1 + cR_b^{(j)}(t_{j-1})\right]} \qquad (k \geq 2).$$

The solution of the IBVP, denoted by $\left\{a_e^{(k)}, \ b_e^{(k)}, r_e^{(k)}\right\}$, can be obtained by numerical methods starting with the continuity condition:

$$\{a_e^{(k)}(x,t_k), b_e^{(k)}(x,t_k), r_e^{(k)}(x,t_k)\} = \{a_e^{(k-1)}(x,t_k), b_e^{(k-1)}(x,t_k), r_e^{(k-1)}(x,t_k)\}.$$

**6.5. Numerical Results.** To compare the cumulative effects from repeated (delayed) feedback adjustments during the transient phase with that of steady state theory obtained in [12], we apply the present approach to the Example 1 examined in [12]. With the system parameter values given in Table 1 earlier, we take $c = 1$ and $\tau = 4$ in units of $(D/X_{\max}^2)^{-1}$ to obtain a similar set of solutions for delayed feedback modified $b_e(x,t)$ at $t_k = \tau, 2\tau, 3\tau, 4\tau$ and $5\tau = 20$ as well as $b_e(x,t)$ without feedback for $e = 1$ (wild-type) and $e = 2$ (ectopic gradient). The results are shown in Figure 3.

As in Figure 1, the top curve in the figure is essentially the quasi-steady state $b_e$ (for $e = 2$) without feedback with the signaling gradient reaching steady state around $t = 20$, with the onset of morphogen synthesis at $t = 0$. The expected role of any feedback adjustment is to bring $b_e^{(5)}(x, 20)$ as close as possible to the the bottom steady state wild-type signaling gradient curve in that figure ($e = 1$ and no feedback). After the feedback adjustments become effective (starting with $k = 2$), the cumulative effect of the repeated adjustments in this second model continues to reduce the signaling gradient in successive time intervals, leading to a gradient $b_e^{(5)}(x, 20)$ very close to the wild-type gradient prior to synthesis rate being enhanced from $e = 1$ to $e = 2$.

However, there are significant differences between the same nonlocal spatially uniform feedback mechanism without and with delay. For example, the feedback adjusted ectopic signaling gradient is seen to be slightly higher than the ectopic gradient during the early transient phase of $k = 1$; but this relative position is reversed for the same feedback mechanism without delay. The reason for the difference is associated with the unmodified increase in the deviation of the ectopic gradient from the wild-type for a second period so that feedback adjustment of the development actually occurs one period later when the feedback implementation is delayed by one period.

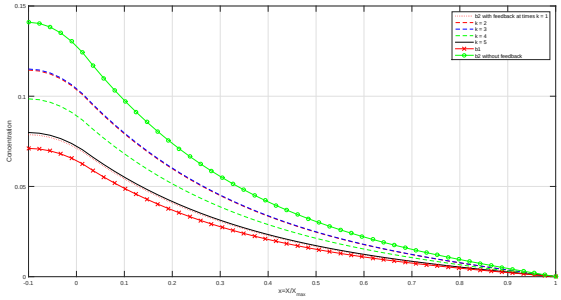

FIGURE 3. Various Signaling Gradients (some with delayed feedback): (1) Wild-type with ($e = 1$ and $c = 0$) at the bottom. (2) Ectopic (with $e = 2$ and $c = 0$) at the top. (3) In between from top down are $b_e^{(k)}(x,t)$ at $t_k = 2\tau, 3\tau, 4\tau, 5\tau = 20$ and $\tau$.

In the second column of Table 2, we report robustness index values at different instants in time for the same feedback mechanism as in the previous section but now with a delay in sensing the need for, and in implementing feedback adjustments. Given that modification of the synthesis rate stimulated by feedback only begins to be effective starting with period 2. There is then no effect of feedback in period 0 and period 1 leading to a larger $R_b$ after the initial period. To reach the same robustness index with the delayed feedback would require additional adjustments well into the steady state phase or an increase in the value for $c$ to 2 or higher.

## 7. Time Dependent LRO Problem

**7.1. A Perturbation for the Transient Phase of a LRO State.** From the results for our illustrating example, we see that both the wild-type and ectopic steady state behavior meet the requirements for a LRO state. For such cases, we may take during the transient phase the following perturbation expansions in terms of a small dimensionless parameter $\varepsilon$:

$$\{a_e, b_e, r_e\} = \{0, 0, 1\} + \sum_{i=1} \{A_e^{(i)}(x,t), B_e^{(i)}(x,t), R_e^{(i)}(x,t)\}\varepsilon^i$$

with $0 < \varepsilon \ll 1$. (One possible choice of the small parameter $\varepsilon$ is the dimensionless ligand synthesis rate $\bar{v}_L$ which is necessarily small for a state of low receptor occupancy as delineated in a previous section.) Upon substituting these expansions in to the IBVP (1), (2), (8) and (9), the leading terms of the expansions $\{A_e^{(1)}(x,t), B_e^{(1)}(x,t), 1\}$ and the first order correction term $R_e^{(1)}(x,t)$ for receptor concentration are found to be determined by the simpler IBVP:

$$\frac{\partial A_e^{(1)}}{\partial t} = \frac{\partial^2 A_e^{(1)}}{\partial x^2} - (h_0 + g_L)A_e^{(1)} + f_0 B_e^{(1)} + v_L(x,t), \tag{58}$$

$$\frac{\partial B_e^{(1)}}{\partial t} = h_0 A_e^{(1)} - (f_0 + g_0)B_e^{(1)}, \qquad \frac{\partial R_e^{(1)}}{\partial t} = -h_0 A_e^{(1)} + f_0 B_e^{(1)} - g_R R_e^{(1)}, \tag{59}$$

with

$$x = -x_m : \quad \frac{\partial A_e^{(1)}}{\partial x} = 0, \qquad x = 1 : \quad A_e^{(1)} = 0, \tag{60}$$

all for $t > 0$, and

$$t = 0: \quad A_e^{(1)} = B_e^{(1)} = R_e^{(1)} = 0 \qquad (0 \le x \le 1). \tag{61}$$

The two unknowns $A_e^{(1)}(x,t)$ and $B_e^{(1)}(x,t)$ may be solved separate with the solution used in the second ODE of (59) and the initial condition $R_e^{(1)}(x,0) = 0$ for the determination of $R_e^{(1)}(x,t)$. The first approximation solution $\left\{A_e^{(1)}(x,t), B_e^{(1)}(x,t), 1\right\}$ is designated as the *low receptor occupancy solution* for the problem.

**7.2. Eigenfunction Expansions.** For $t$ in the interval $[t_k, t_{k+1})$, we have for the model with delay

$$v_L(x,t) = e\kappa_k \bar{v}_L H(-x) \equiv v_e^{(k)}(x,t), \qquad \kappa_k = \frac{1}{\prod\limits_{j=2}^{k}\left[1 + cR_b^{(j)}(t_{j-1})\right]}, \tag{62}$$

which is independent of time except for the constant $\kappa_k$ characterizing the cumulative effect of prior feedback. A particular solution of the linear IBVP for $A_e^{(1)}(x,t)$ and $B_e^{(1)}(x,t)$ for that interval is the steady state LRO solution $\bar{A}_{ek}(x)$ given in (18) and

$$\bar{B}_{ek}(x) = \frac{\bar{A}_{ek}(x)}{\alpha_0}, \tag{63}$$

with $e\bar{v}_L$ replaced by $e\kappa_k\bar{v}_L$. It is worth pointing out that the particular solution pair $\{\bar{A}_{ek}(x), \bar{B}_{ek}(x)\}$, though time independent for at least the initial period, generally changes from period to period since the synthesis rate $v_L$ is being adjusted with a new robustness index from period to period. For that reason, we take the LRO solution $A_e^{(1)}(x,t)$ and $B_e^{(1)}(x,t)$ in period $k$ as a sum of the corresponding particular solution and a transient counterpart

$$A_e^{(1)}(x,t) = \bar{A}_{ek}(x) + \hat{A}_{ek}(x,t), \qquad B_e^{(1)}(x,t) = \bar{B}_{ek}(x) + \hat{B}_{ek}(x,t). \tag{64}$$

The complementary solutions $\hat{A}_e(x,t)$ and $\hat{B}_e(x,t)$ are determined by the IBVP

$$\frac{\partial \hat{A}_{ek}}{\partial t} = \frac{\partial^2 \hat{A}_{ek}}{\partial x^2} - (h_0 + g_L)\,\hat{A}_{ek} + f_0 \hat{B}_{ek}, \tag{65}$$

$$\frac{\partial \hat{B}_{ek}}{\partial t} = h_0 \hat{A}_{ek} - (f_0 + g_0)\hat{B}_{ek}, \tag{66}$$

subject to the boundary conditions

$$x = -x_m: \quad \frac{\partial \hat{A}_{ek}}{\partial x} = 0, \qquad x = 1: \quad \hat{A}_{ek} = 0, \tag{67}$$

for $t_k \le t < t_{k+1}$ and initial conditions

$$\left\{ \begin{array}{c} \hat{A}_{ek}(x,t_k) + \bar{A}_{ek}(x) \\ \hat{B}_{ek}(x,t_k) + \bar{B}_{ek}(x) \end{array} \right\} = \left\{ \begin{array}{c} \hat{A}_{ek-1}(x,t_k) + \bar{A}_{e(k-1)}(x) \\ \hat{B}_{e(k-1)}(x,t_k) + \bar{B}_{e(k-1)}(x) \end{array} \right\} \tag{68}$$

for $0 \le x \le 1$.

Given the linearity of the governing PDE and auxiliary conditions, the complementary solution of this IBVP may be obtained by eigenfunction expansions

$$\left\{ \begin{array}{c} \hat{A}_{ek}(x,t) \\ \hat{B}_{ek}(x,t) \end{array} \right\} = \sum_{j=0}^{\infty} \left\{ \begin{array}{c} a_{kj}(t) \\ b_{kj}(t) \end{array} \right\} \phi_j(x) \tag{69}$$

with

$$\phi_j(x) = \sin\left(\lambda_j \frac{1-x}{1+x_m}\right), \qquad \lambda_j = \left(j + \frac{1}{2}\right)\pi. \tag{70}$$

Upon substitution of (69) into (65)-(66), we obtain

$$\frac{d}{dt}\begin{pmatrix} a_{kj}(t) \\ b_{kj}(t) \end{pmatrix} = -C(\lambda_j^2)\begin{pmatrix} a_{kj}(t) \\ b_{kj}(t) \end{pmatrix} \qquad (j = 0, 1, 2, \ ....)$$

where

$$C(\lambda_j^2) = \begin{bmatrix} c_{11} & c_{12} \\ c_{21} & c_{22} \end{bmatrix} = \begin{bmatrix} \lambda_j^2 + h_0 + g_L & -f_0 \\ -h_0 & f_0 + g_0 \end{bmatrix}.$$

The solution of the first order system for $a_{kj}(t)$ and $b_{kj}(t)$ is

$$\begin{pmatrix} a_{kj}(t) \\ b_{kj}(t) \end{pmatrix} = \begin{pmatrix} a_{kj}^{(1)} e^{-\omega_{kj}^{(1)} t} + a_{kj}^{(2)} e^{-\omega_{kj}^{(2)} t} \\ b_{kj}^{(1)} e^{-\omega_{kj}^{(1)} t} + b_{kj}^{(2)} e^{-\omega_{kj}^{(2)} t} \end{pmatrix} \tag{71}$$

where $\omega_{kj}^{(1)}$ and $\omega_{kj}^{(2)}$ are the two roots

$$\begin{pmatrix} \omega_{kj}^{(1)} \\ \omega_{kj}^{(2)} \end{pmatrix} = \frac{1}{2}\left\{ Tr[C] \pm \sqrt{(Tr[C])^2 - 4Det[C]} \right\}$$

of the quadratic equation for $\omega_{kj}$

$$\det\left[\omega_{kj} I - C(\lambda_j^2)\right] = \omega_{kj}^2 + Tr[C]\omega_{kj} + Det[C] = 0.$$

The coefficients $\{a_{kj}^{(1)}, a_{kj}^{(2)}, b_{kj}^{(1)}, b_{kj}^{(2)}\}$ are related by

$$b_{kj}^{(m)} = \frac{h_0 a_{kj}^{(m)}}{\omega_{kj}^{(m)} + f_0 + g_0}$$

with the two remaining unknowns $a_{kj}^{(1)}$ and $a_{kj}^{(2)}$ found from the two continuity conditions (68) in the form

$$\begin{aligned} &\begin{Bmatrix} a_{kj}^{(1)} e^{-\omega_{kj}^{(1)} t_k} + a_{kj}^{(2)} e^{-\omega_{kj}^{(2)} t_k} \\ b_{kj}^{(1)} e^{-\omega_{kj}^{(1)} t_k} + b_{kj}^{(2)} e^{-\omega_{kj}^{(2)} t_k} \end{Bmatrix} + \begin{Bmatrix} \bar{a}_{kj} \\ \bar{b}_{kj} \end{Bmatrix} \\ = &\begin{Bmatrix} a_{(k-1)j}^{(1)} e^{-\omega_{kj}^{(1)} t_k} + a_{(k-1)j}^{(2)} e^{-\omega_{kj}^{(2)} t_k} \\ b_{(k-1)j}^{(1)} e^{-\omega_{kj}^{(1)} t_k} + b_{(k-1)j}^{(2)} e^{-\omega_{kj}^{(2)} t_k} \end{Bmatrix} + \begin{Bmatrix} \bar{a}_{(k-1)j} \\ \bar{b}_{(k-1)j} \end{Bmatrix} \end{aligned}$$

where $\{\bar{a}_{nj}, \bar{b}_{nj}\}$ are the coefficients of the eigenfunction expansions for the known particular solutions $\bar{A}_{ek}(x)$ and $\bar{B}_{ek}(x)$, respectively,

$$\int_{-x_m}^{1} [\phi_j(x)]^2 \, dx \begin{Bmatrix} \bar{a}_{nj} \\ \bar{b}_{nj} \end{Bmatrix} = \int_{-x_m}^{1} \phi_j(x) \begin{Bmatrix} \bar{A}_{en}(x) \\ \bar{B}_{en}(x) \end{Bmatrix} dx.$$

and $\{a_{(k-1)j}^{(1)}, \ ...., \ b_{(k-1)j}^{(2)}\}$ are known from the solution of period $(k-1)$, i.e., $[t_{k-1}, t_k)$.

Given

$$c_{11}c_{22} - c_{12}c_{21} = g_0(\lambda_j^2 + h_0 + g_L) + f_0\left(\lambda_j^2 + g_L\right) > 0 \tag{72}$$

and

$$\begin{aligned} Tr[C])^2 - 4Det[C] &= (c_{11} + c_{22})^2 - 4(c_{11}c_{22} - c_{12}c_{21}) \\ &= (c_{11} - c_{22})^2 + 4c_{12}c_{21} = (c_{11} - c_{22})^2 + f_0 h_0 > 0, \end{aligned} \tag{73}$$

both $\omega_{kj}^{(1)}$and $\omega_{kj}^{(2)}$ are real (by (73)) and positive (by (72)). It follows from (71) that the transient components are dissipative and decay with time.

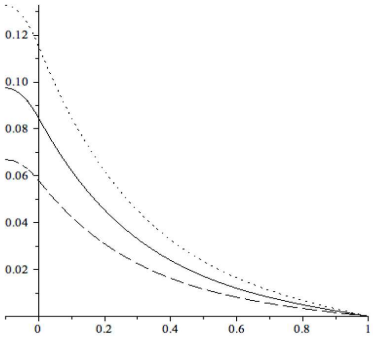


FIGURE 4. Steady state signaling gradients from [12].

**7.3. Numerical Results.** The solution process above has been implemented to obtain an approximate LRO solution for our problem in [25] where we have also simplified the calculations by taking advantage of the fact that $f_0 \ll g_0$. Given that the example in Table 1 is of the LRO type, the eigenfunction solution of this section provides a mean to validate the numerical solutions discussed in the previous sections (but with the parameters in robustness index

$$R_b(t) = \frac{1}{b_h - b_\ell}\sqrt{\frac{1}{x_\ell - x_h}\int_{x_h}^{x_\ell}[b_e(x,t) - b_1(x,t)]^2 dx} \tag{74}$$

(as defined in (20)) re-set to take advantage of the orthogonality of the eigenfunctions). More specifically, we now take $x_h = -x_m$ instead of 0 but keep $x_\ell = 1$ to get

$$R_b(t) = \frac{1}{\bar{b}_h}\sqrt{\frac{1}{1 + x_m}\int_{-x_m}^{1}[b_e(x,t) - b_1(x,t)]^2 dx}. \tag{75}$$

While we may make use of the correct value $\tilde{b}_e(-x_m)$ for $\bar{b}_h$, it is just as appropriate to continue to use $\bar{b}_h = \bar{b}_1(0)$ as we have done herein.

## 8. Concluding Remarks

Robustness with respect to an ectopic signaling gradient resulting from genetic or epigenetic perturbations requires one or more signaling inhibiting agents to be stimulated (by the enhanced signaling morphogen concentration) and up-regulated above their normal level. This means the existence of some kind of feedback process in order to promote robustness. Feedback has long been seen as a mechanism for maintaining stable developments and specific feedback loops have been identified in the morphogen literature such as [13, 14, 15, 16, 18]. While the conventional Hill function type negative feedback on receptor synthesis rate proves to be ineffective for this purpose [19, 20, 21], we have shown in [12] that a nonlocal spatially uniform feedback process based on a spanwise average of excess signaling can play such a role.

In the proof of concept investigation [12], the feedback loop employed by-passed the actual agent responsible for down-regulating the ectopic signaling and implemented a negative feedback directly on the ligand synthesis rate (given the ultimate effect of the inhibition is equivalent to a reduction of ligand for binding with signaling receptors). In addition, the feedback is taken to be effective as the development reaches a (quasi-) steady state. The present investigation complements the work

in [12] by examining the same feedback mechanism but now allowing it to be effective during the transient phase of the development, possibly repeated until a quasi-steady state of development is reached.

The transient phase feedback mechanism applied to the same example investigated in [12] shows some significant differences from the feedback in steady state process. The most obvious difference is that the new mechanism attains robustness for $c = 1$ (and $n = 1$) with $R_b$ well below 0.1 when the development reaches quasi-steady state whether or not there is a delay in the feedback process. In fact, $R_b$ is already below the robustness threshold of 0.2 even during the transient phase (see Table 2). In contrast, for the feedback in steady state approach with $c = 1$ and $n = 1$, the robustness index is $> 0.24$ which is well above the 0.2 threshold. With $n$ kept at 1, the latter mechanism would require $c = 2$ for $\bar{R}_b$ to decrease to 0.183... but still $> 0.1$ with $c = 4$. This advantage of the transient feedback is not surprising since the process allows for several adjustments before reaching steady state while the feedback in steady state effectively allows for only one feedback modification.

Another difference in comparing the graphs for $\bar{b}_2(x)$ by feedback in steady state with $b_e(x,t)$ with nearly the same robustness index value by feedback in transient shows that the latter more closely resembls the wild-type gradient. Below from bottom to top in Figure 4 are the graphs of $\bar{b}_1(x)$ (without feedback), steady state feedback adjusted $\bar{b}_2(x)$ and $\bar{b}_2(x)$ without feedback obtained for [12]. While the robustness index value $\bar{R}_b = 0.18293...$ for the $c = 2$ case is below the acceptable threshold, the middle curve in Figure 4 is only half way between $\bar{b}_1(x)$ and $\bar{b}_2(x)$. In contrast, the corresponding $b_e^{(3)}(x,t_3)$ in Figure 1 with a robustness index value of $R_b(t_3) = 0.18738...$ is considerably closer to the wild-type gradient. The difference persists in a few other cases computed.

From these and other results, it is reasonable to conclude that feedback in transient is more favorable (than feedback in steady state) to robust development of a biological organism that initiates (a spatially uniform nonlocal) feedback for adjusting the level of inhibition as it experiences environmental or genetic perturbations. Hence, it should be of primary interest as we begin to investigate feedback on the actual inhibitors/activators for regulating ectopic signaling gradients. For an enhancement in ligand synthesis rate for example, down-regulation of the resulting signaling gradient may be accomplished by a positive feedback to enhance synthesis of non-receptors or receptor mediated degradation. It may also be accomplished by a negative feedback to inhibit binding between ligands and their signaling receptors. While the effects of feedback in transient for these and other regulating mechanisms are being examined in [25] and elsewhere, feedback in steady state will also be investigated to provide a lower bound on what may be accomplished by the particular inhibiting/activating agent (see [26] for example).

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## Appendix A. Feedback on Receptor Synthesis Rate[4]

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[3]Research supported by NSF MCBU Summer Research Program
[4]Jeanness Cochran also participated in this research project.

**A.1. Nonlocal Uniform Feedback on Receptor Synthesis.** In Section 5, we pointed out a number of important differences between the feedback adjusted ectopic signaling gradients resulting from the conventional Hill's function approach and our nonlocal uniform feedback mechanism. As one important difference, the present approach keeps the shape (slope and curvature) of the adjusted ectopic gradient similar and close to shape of the wild-type gradient while the Hill's function approach typically changes the gradient from convex to concave shape downstream from the local source as shown in Figure 2. However, the feedback that modified the ectopic gradient in Figure 2 was applied to the receptor synthesis rate (and not the ligand synthesis rate). For a more appropriate comparison, we should investigate the shape of the corresponding gradient when the present nonlocal uniform feedback mechanism is applied to the receptor synthesis rate as was done in [19] to obtain Figure 2.

To this end, we consider here a related negative feedback of the spatially uniform nonlocal type investigated herein on signaling receptor synthesis rate with $v_R(x,t)$ in (2) given by

$$v_R = \frac{\bar{v}_R}{1 + c\left[R_b(t-\tau)\right]^n}, \tag{A.1}$$

(instead of $\bar{v}_R$). We limit discussion here to the case of the effect of such a feedback on the steady state behavior. With (10), (21) and (22), we have from (1), (2) and (8) the following equations for the steady state behavior:

$$\bar{a}_e'' - h_0\bar{a}_e\bar{r}_e + f_0\bar{b}_e - g_L\bar{a}_e + e\bar{v}_L H(-x) = 0, \tag{A.2}$$

$$0 = h_0\bar{a}_e\bar{r}_e - (f_0+g_0)\bar{b}_e, \qquad 0 = \bar{\kappa}\bar{v}_R - h_0\bar{a}_e\bar{r}_e + f_0\bar{b}_e - g_R\bar{r}_e, \tag{A.3}$$

where a bar on top of a quantity designates steady state behavior. The subscript "$e$" indicates the level of ectopicity corresponding to the values of the parameter $e$ in the morphogen synthesis rate term, and

$$\bar{\kappa} = \frac{1}{1 + c\left[\bar{R}_b\right]^n}, \tag{A.4}$$

where $\bar{R}_b$ is the robustness index in steady state. Note that the wild-type behavior corresponds to $e = 1$ and $\bar{R}_b = 0$ (which can be achieved by setting $c = 0$).

The two equations in (A.3) may be solved for $\bar{b}$ and $\bar{r}$ in terms of $\bar{a}$ to get

$$\bar{b} = \frac{\bar{\kappa}\bar{a}}{\alpha_0 + \zeta_0\bar{a}}, \qquad \bar{r} = \frac{\bar{\kappa}\alpha_0}{\alpha_0 + \zeta_0\bar{a}}. \tag{A.5}$$

These expressions enable us to eliminate $\bar{b}$ and $\bar{r}$ from (1) to get a single second order ODE for $\bar{a}$:

$$\bar{a}'' - \frac{\bar{\kappa}\bar{a}}{\alpha_0 + \zeta_0\bar{a}} - g_L\bar{a} + e\bar{v}_L H(-x) = 0 \tag{A.6}$$

The ODE (A.6) is augmented by the boundary conditions (8),

$$\bar{a}'(-x_m) = \bar{a}(1) = 0, \tag{A.7}$$

to form a two point BVP for $\bar{a}$.

**A.2. Method of Solution for $\bar{a}$.** The notations used for the BVP for $\bar{a}(x)$ is deceiving. The parameter $\bar{\kappa}$ that appears in the second term of (A.6) involves an integral of some function of the unknown $\bar{a}(x)$. As such the BVP is for an integro-differential equation (rather than an ODE) for the unknown $\bar{a}(x)$. While a single pass algorithm is possible for this problem (see [12]), it is less complicated to use the following rather natural iterative scheme:

(1) Make an initial guess $\bar{R}_{b0}$ for $\bar{R}_b$ and solve the BVP problem for the resulting ODE for $\bar{a}(x)$ to get $\bar{a}_0(x)$ for that initial iterate for the robustness index.
(2) Use (32) in the form

$$\bar{R}_{b(k+1)} = \frac{1}{\bar{b}_h}\sqrt{\int_0^1 [\bar{b}_e(x;\bar{R}_{bk}) - \bar{b}_1(x)]^2 dx}. \tag{A.8}$$

to determine the robustness index for the solution $\bar{a}_0(x)$. Denote the result by $\bar{R}_{b1}$. In (A.8), $\bar{b}_e(x;\bar{R}_{bk})$ is given by (A.5) in terms of $\bar{a}_e(x;\bar{R}_{bk})$ which is the solution of the BVP for the available robustness index $\bar{R}_{bk}$ and $\bar{b}_1(x)$ the wild-type signaling gradient (corresponding to $e = 1$ and $c = 0$).
(3) Obtain the solution of the BVP for the new iterate $\bar{R}_{b1}$ to get a new solution $\bar{a}_1(x)$ and repeat the process.
(4) In general, having $\bar{a}_k(x)$, from $\bar{R}_{bk}$, use (A.8) to calculate a new iterate for $\bar{R}_b$ until the solution converges (with the issue of convergence discussed previously in [12]).

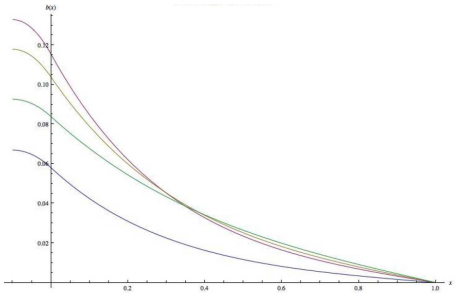

FIGURE 5. Signaling Gradients with Nonlocal Uniform Feedback on Receptor Synthesis: From top down, the curves are 1) $\bar{b}_e(x)$ for $e = 2$ without feedback ($c = 0$), 2) steady state feedback adjusted LRO approximation (with $e = 2$, $c = 4$); 3) steady state feedback adjusted numerical solution (with $e = 2$, $c = 4$), and 4) wild-type signaling gradient $\bar{b}_1(x)$ (with $e = 1$ and $c = 0$).

**A.3. Numerical Results.** For the example with parameter values given in Table 1, we have for the ectopic case with $e = 2$ and no feedback, a robustness index value of 0.39380 (identical to that given earlier in Table 2 and considerably higher than the acceptable threshold of 0.2). The corresponding robustness indices for feedback adjusted ectopic gradients for several values of $c$ are given in Table 3 below:

While it seems that robustness may be attained with sufficiently large $c$, more relevant to the issue of feedback adjusted signaling gradient shape change of interest here is seen from the graphs shown in Figure 5 of the corresponding signaling gradients for $c = 4$. The third graph from the top in this figure is the numerical solution for $\bar{b}_e(x)$ corresponding to $\bar{R}_b$ after 10 iterations. It remains convex while

TABLE 3. $\bar{R}_{bk}(x)$ for Steady State Feedback on Receptor Synthesis Rate.

| c | $\bar{R}_{bk}$ | k |
|---|---|---|
| 0 | 0.39380 | - |
| 1 | 0.35733 | 5 |
| 2 | 0.32837 | 7 |
| 4 | 0.28615 | 10 |

the corresponding graph by the Hill's function type feedback given in Figure 3 is concave downstream from the local source.

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