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RESEARCH ARTICLE

Regulatory Feedback on Receptor and Non-receptor Synthesis for Robust Signaling

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Abstract

Elaborate feedback regulatory processes are thought to make biological development robust, i.e., resistant to changes induced by genetic or environmental perturbations. How this might be done is still not completely understood. Previous numerical simulations on reaction-diffusion models of Dpp gradients in Drosophila wing imaginal disc have showed that feedback (of the Hill function type) on (signaling) receptors and/or non-(signaling) receptors are of limited effectiveness in promoting robustness. Spatial nonuniformity of the feedback processes has also been shown theoretically to lead to serious shape distortion and a principal cause for ineffectiveness. Through mathematical modeling and analysis, the present paper shows that the spatially uniform nonlocal feedback mechanisms typically modify gradient shape through a shape parameter (that does not change with location). This in turn enables us to uncover new multi-feedback instrument for effective promotion of robust signaling gradients.

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1 Introduction

At some (early) stage of embryonic development of a biological organism, one or more proteins (known as morphogens or ligands) responsible for cell differentiation

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are synthesized and transported away from their sources to be bound to relevant cell receptors at different locations to form signaling morphogen-receptor complexes, known as a *signaling (spatial) gradients*. Such signaling gradients convey positional information for cells to adopt differential fates to result in tissue patterns. This process of cell differentiation is well established in developmental biology. For example, the morphogen *Decapentaplegic* (Dpp) involved in the development of the Drosophila wing imaginal disc is synthesized in a narrow region at the boundary between the anterior and posterior compartments of the disc. Dpp molecules produced are transported away from the localized source and flow out of the disc upon reaching its edge. Some Dpp molecules bind reversibly with the cell-surface signaling receptor *Thickvein* (Tkv) to form a spatial gradient of signaling morphogen concentration over the span of the wing imaginal disc. 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, 5]. Simple models of this process of gradient formation have been shown theoretically to produce a unique signaling gradient that is monotone and asymptotically stable with respect to small (one time) perturbations (see [6, 7] for example).

For normal biological development, it is important that signaling morphogen gradients not be easily altered by (sustained) genetic or epigenetic effects on the constitution of the biological organism [8]. Experiments (carried out by S. Zhou in A.D. Lander's lab (see also [9])) have shown that Dpp synthesis rate doubles when the ambient temperature is increased by $5.9°C$. With such an increase in Dpp synthesis rate, the simple models developed in [6, 7, 10] would predict an enhanced or (more commonly called) "aberrant" (or "abnormal") signaling gradient quantitatively and qualitatively different from that under the (lower) normal ambient temperature. Yet development of the wing imaginal disc generally does not change significantly with temperature changes of such magnitude. The insensitivity of system output to sustained alterations in input or system characteristics so necessary for normal development is often termed *robustness* of biological development. How this robustness requirement is met has been the subject of a number of recent studies [11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22].

Evidence exists that regulatory feedback processes play a role in rendering biological developments robust, i.e., a signaling gradient resistant to changes expected to be induced by genetic or environmental perturbations [23, 24]. How this might be accomplished is still not completely understood. Among the first attempt to determine mechanisms for attaining robust developments, a negative feedback on receptor synthesis rate was investigated in [25]. A Hill function type negative feedback was incorporated into the basic morphogen gradient model of [7] to reduce the synthesis rate of Tkv by an amount that depends on the aberrant signaling morphogen concentration. Some numerical simulations of the model show that robustness of the signaling gradient (and hence the corresponding development) with respect to a sustained genetic or epigenetic perturbation is not achieved for any of the 10^6 combinations of system

parameter values in a parameter space of 6-dimensions. A subsequent theoretical analysis delineated and confirmed the ineffectiveness of this negative feedback mechanism [26]. Briefly, a Hill type negative feedback reduces the receptor synthesis rate nonuniformly, disproportionately more so at locations of high signaling morphogen concentration. Such reduction generally leads to a modified gradient of different slope and convexity from the normal (wild-type) gradient. The theoretical results suggest that a spatially uniform negative feedback responding to some overall measure of abnormality or aberrancy (such as the average impact of the local changes on the system) may be more effective [26]. This suggestion has led to the initiation of a new general approach to attain robust development by way of feedback mechanisms that are spatially uniform [20]-[22],[27].

With a view that most feedback mechanisms have the ultimate effect of reducing the morphogen available for binding with signaling receptors, a proof-of-concept prototype model for a spatially uniform negative feedback on morphogen synthesis rate was first investigated in [27]. The findings in that preliminary effort provided the impetus to investigate in [22] the efficacy of spatial uniformity in other known feedback mechanisms. In this paper, we refine the models for modifying an aberrant signaling morphogen gradient (toward the original wild-type gradient) investigated in [22, 27] by modeling explicitly one of the processes known to reduce the availability of unbound morphogen molecules. Among the different ways to reduce Dpp concentration is their binding with other protein molecules to form morphogen complexes that do not signal for cell differentiation [28]. Such (non-signaling) companion proteins are known to exist for Dpp and other BMP family ligands. They include Notum [29], Nog (noggin) [30]-[32], Chd (chordin) [33, 34], Dally (division abnormally delayed) [35, 36], FST (follistatin) [37]-[40], Sog (short gastrulation) [41, 42], and various heparan sulfate proteoglycans [43]. Collectively, they are called *non-receptors* since they bind with morphogens such as Dpp but the resulting bound morphogen complexes have no role in cell differentiation.

Effects of non-receptors was modeled and analyzed in [44] where we extend the simple wing disc morphogen model of [6, 7] to include a fixed concentration of cell-surface non-receptor (induced instantaneously at the onset of the genetic or epigenetic perturbations at time t_e). This simplest model offered the first theoretical glimpse into the inhibiting effects of non-receptors on the formation and properties of steady state signaling morphogen-receptor gradients. Subsequently, large scale computational studies of non-receptors synthesized at a prescribed (perturbations-induced) fixed rate at time t_e to absorb excessive Dpp concentration were performed in [25]. Extensive numerical simulations spanning a 6-dimensional parameter space showed that less than 9% of gradients are of appropriate size and shape but with a mean *robustness index* (a numerical value to be defined in a later section as a measure of the deviation of the aberrant gradient from the wild type) more than doubling the threshold value defined to be acceptably close to the wild-type gradient prior to the perturbations. Most gradients generated are not biologically realistic for cell differ-

entiation (for reasons such as high receptor occupancy throughout the wing imaginal disc away from its distal edge). These observations have been validated theoretically in [17, 26, 45]. Adding negative feedback on receptor synthesis rate to *a modest concentration of non-receptors* was found in [25] to result in a slightly broader range of robust gradients that are biologically useful and robustness cannot be attained with higher non-receptor synthesis rates (than the receptor synthesis rate) with any level of negative feedback on receptor synthesis rate. Additional negative feedback on the prescribed non-receptor synthesis would only further degrade the aberrant gradient.

The theoretical results of [26] on a Hill function type feedback on receptor synthesis suggest that a spatially uniform feedback mechanism may avoid the effect of shape distortion associated with spatially nonuniform feedback. A (nonlocal) robustness-index based feedback mechanism has been developed in [22, 27] to provide a spatially uniform feedback process. More details and support (including known examples) for such feedback mechanisms will be deferred to a later section on our specific nonlocal spatially uniform feedback. We note here only that the improvements associated with the new feedback process applied to receptor synthesis rate (and a few other biological processes, such as the binding rate, and receptor-mediated degradation rate) were found insufficient for robust signaling [22]. However, the investigation enabled us to uncover unexpected benefits of appropriate multi-feedback mechanisms that are much more efficient in promoting robust signaling gradients. In relation to the numerical simulations of [25], we report in this paper one specific application of the multi-feedback combination to show how aberrancy induced positive feedback on non-receptor synthesis rate may be combined with a similar negative feedback on receptor synthesis for a very effective strategy for promoting robust signaling in biologically realistic ranges of system parameter values. We do this by examining a set of models that are the counterparts of those investigated in [25] but now with our new spatially uniform feedback instrument instead of the Hill function type previously employed. One significant feature of our approach is that the new models admit explicit exact solutions for biologically realistic gradients so that our results are theoretically conclusive and do not rely on numerical simulations. How non-receptors may or may not promote robust signaling can be seen explicitly from the mathematical expressions in terms of known functions for the signaling gradient concentration of the different models.

2 A Simple Extracellular Model of Dpp Gradient Formation

To understand better the results of numerical simulations of [25], we re-examine the same three approaches to robust signaling there but now by way of a spatially uniform feedback. For this purpose, we work with the normalized form of the one-dimensional extracellular model of the Dpp gradient formation of [7]. To simplify the analysis

without sacrificing any of the essential characteristics of the biological processes involved, we may take the wild-type Dpp synthesis rate $V_L(X,T)$ to be uniform in the direction along the boundary $X = -X_{\min}$ between the anterior and posterior compartments of the wing imaginal disc. Here, X is distance in direction perpendicular to the between-compartment boundary with X spanning $[-X_{\min}, X_{\max}]$, $X_{\max}$ being the edge of the posterior compartment. With Dpp synthesized at a uniform rate in a narrow strip $-X_{\min} < X < 0$, we idealize the synthesis rate by

$$V_L(X,T) = \bar{V}_L H(-x) \tag{1}$$

where $x = X/X_{\max}$. We also take the wild-type receptor synthesis rate $V_R(X,T)$ to be uniform throughout the posterior compartment with a steady state receptor concentration of

$$R_0 = \frac{V_R(X,T)}{k_R} = \frac{\bar{V}_R}{k_R} = \frac{\text{Uniform } Tkv \text{ Synthesis Rate}}{Tkv \text{ Degradation Rate Constant}} \tag{2}$$

prior to the onset of Dpp synthesis at $T = 0$. Just as (the normalized) distance x in direction normal to the compartment boundary measured in units of the maximum distal width $X_{\max}$ of the posterior compartment, we also normalize the physical time T by setting $t = DT/X_{\max}^2$ where D is the uniform diffusion coefficient.

Normal development of wing imaginal disc and other biological organisms may be altered by an enhanced morphogen synthesis rate stimulated by sustained genetic or epigenetic changes (in contrast to a one time perturbation of an existing steady state), starting at some time $t_e \geq 0$. For example, *Dpp* synthesis rate in Drosophila imaginal disc has been shown to double when the ambient temperature is increased by $5.9°C$ (shown by S. Zhou while in A.D. Lander's Lab). At a state of low receptor occupancy (LRO), basic models for signaling gradient formation would have the corresponding steady state *aberrant (abnormal)* signaling ligand concentration increasing proportionately (see (19)-(20) below) and its shape altered (and hence the cell fate at each spatial location as well) [7, 16]. Without the restriction of low receptor occupancy, these and other models also have also the steady state aberrant signaling gradient magnitude increased with synthesis rate, though not necessarily proportionately [7, 16, 17, 26].

Natural biological developments however are mostly unaffected by sustained environmental perturbations that these models would have altered them. To investigate possible mechanisms for managing (down-regulating) possible aberrant developments induced by genetic or epigenetic perturbations, we introduce an excess (amplification) factor $e \geq 1$ and work with a more general ligand synthesis rate $V_L(X,T) = e\bar{V}_L H(-x)$ instead of (1). The basic extracellular model for Dpp gradient formation of [7] then consists of the following three normalized differential equations for the normalized concentrations of free Dpp concentration $a_e(x,t)$, Dpp-Tkv complexes concentration (or *signaling Dpp gradient* for short) $b_e(x,t)$, and the unoccupied Tkv concentration $r_e(x,t)$, all measured in units of the steady state receptor

concentration R_0 introduced in (2):

$$\frac{\partial a_e}{\partial t} = \frac{\partial^2 a_e}{\partial x^2} - h_0 a_e r_e + f_0 b_e + v_e, \tag{3}$$

$$\frac{\partial b_e}{\partial t} = h_0 a_e r_e - (f_0 + g_0) b_e, \qquad \frac{\partial r_e}{\partial t} = v_R - h_0 a_e r_e + f_0 b_e - g_r r_e, \tag{4}$$

where the quantities h_0, g_0, and f_0 are (per unit morphogen concentration) binding rate, receptor-mediated degradation rate and dissociation rate, all normalized by $D/X_{\max}^2$. In the absence of feedback, the normalized Dpp and Tkv synthesis rates, v_e and v_R, are given by

$$v_e = \frac{eV_L/R_0}{D/X_{\max}^2} = e\bar{v}_L H(-x), \qquad v_R = \frac{\bar{V}_R/R_0}{D/X_{\max}^2} = \frac{k_R}{D/X_{\max}^2} \equiv g_r. \tag{5}$$

where $\bar{v}_L$ is a dimensionless morphogen synthesis rate (prior to exogenous perturbations) and e is the ligand synthesis amplification factor with $e = 1$ for the wild type.

The three differential equations are supplemented by the boundary conditions

$$x = -x_m\text{:} \quad \frac{\partial a_e}{\partial x} = 0, \qquad x = 1\text{:} \quad a_e = 0, \tag{6}$$

all for $t > 0$, and the initial conditions

$$t = 0\text{:} \quad a_e = b_e = 0, \quad r_e = 1. \tag{7}$$

The *initial-boundary value problem* (IBVP) defined by (3)-(7) and its modified forms have been analyzed as mathematical models for ligand activities and tissue pattern formation in several of the references cited (e.g., [7, 25, 26]). Some basic results from [7] are summarized below for comparison with the new results on the effects of non-receptors to be analyzed herein.

2.1 Time Independent Steady State

Given that both the ligand and receptor synthesis rates are time independent, it has been shown in [7] that the extracellular model system (3)-(7) has a unique steady state,

$$\{\bar{a}_e(x), \bar{b}_e(x), \bar{r}_e(x)\} = \lim_{t\to\infty} \{a_e(x,t), b_e(x,t), r_e(x,t)\}, \tag{8}$$

that is asymptotically stable with respect to small (one time) perturbations. With the three dependent variable not changing with time in steady state, the governing IBVP may be reduced to the following well-posed two-point boundary value problem (BVP) for $\bar{a}_e(x)$ [7]:

$$\bar{a}_e'' - \frac{g_0 \bar{a}_e}{\alpha_0 + \zeta_0 \bar{a}_e} + e\bar{v}_L H(-x) = 0, \tag{9}$$

$$\bar{a}_e'(-x_m) = 0, \qquad \bar{a}_e(1) = 0. \tag{10}$$

with

$$\bar{b}_e(x) = \frac{\bar{a}_e(x)}{\alpha_0 + \zeta_0 \bar{a}_e(x)}, \qquad \bar{r}_e(x) = \frac{\alpha_0}{\alpha_0 + \zeta_0 \bar{a}_e(x)} \tag{11}$$

where

$$\alpha_0 = \frac{f_0 + g_0}{h_0}, \qquad \zeta_0 = \frac{g_0}{g_r}. \tag{12}$$

We note again that the excess factor e is a constant for the level of abnormality in the ligand synthesis rate with $e = 1$ for the wild-type development.

For the signaling gradient to induce a distinct biological tissue pattern, it should *not* be nearly uniform over a significant spatial span of the solution domain $(-x_m, 1)$ as there would not be a pattern over that span. For this reason, the free morphogen concentration $\bar{a}_e(x)$ associated with a biologically realistic gradient system cannot be so large that

$$\zeta_0 \bar{a}_e \gg \alpha_0, \quad (x < 1) \tag{13}$$

or (with $f_0 \ll g_0$) $\bar{a}_e \gg g_r/h_0$ away from the edge $x = 1$. For the unlikely event that the condition (13) should be met, we would have

$$\bar{b}_e(x) = \frac{\bar{a}_e(x)}{\alpha_0 + \zeta_0 \bar{a}_e(x)} \simeq \frac{1}{\zeta_0} = \frac{g_r}{g_0}$$

uniform in x except for a boundary layer adjacent to the edge $x = 1$. While $\zeta_0 \bar{a}_e(x) = O(\alpha_0)$ is not the only requirement for a biologically realistic gradient system, we formulate it as a necessary condition for systems worthy of examination.

Criterion 1 *For a morphogen gradient system to induce a biological meaningful pattern, the free morphogen concentration $\bar{a}_e$ be $O(\alpha_0/\zeta_0) = O(g_r/h_0)$ or smaller.*

2.2 Low Receptor Occupancy

At the other extreme, when the free morphogen concentration $\bar{a}_e(x)$ is sufficiently low over the span of the solution interval $(0, 1)$ so that

$$\zeta_0 \bar{a}_e = g_0 \bar{a}_e / g_r \ll \alpha_0, \tag{14}$$

we may neglect terms involving $\zeta_0 \bar{a}_e$ in (9)-(11) to get an approximate set of solutions $\{A_e(x), B_e(x), R_e(x)\}$ determined by the initial value problem (IVP)

$$A_e'' - \mu_0^2 A_e + e\bar{v}_L H(-x) = 0, \tag{15}$$

$$A_e'(-x_m) = 0, \qquad A_e(1) = 0. \tag{16}$$

with

$$B_e(x) = \frac{A_e(x)}{\alpha_0}, \qquad R_e(x) = 1. \tag{17a}$$

and

$$\mu_0^2 = \frac{g_0}{\alpha_0} = \frac{g_0 h_0}{g_0 + f_0} \tag{18}$$

The exact solution for $A_e(x) \equiv eA_1(x)$ obtained previously in [7] is

$$A_1(x) = \begin{cases} \frac{\bar{v}_L}{\mu_0^2}\{1 - \frac{\cosh(\mu_0)}{\cosh(\mu_0(1+x_m))}\cosh(\mu_0(x+x_m))\} & (-x_m \le x \le 0) \\ \frac{\bar{v}_L}{\mu_0^2}\frac{\sinh(\mu_0 x_m)}{\cosh(\mu_0(1+x_m))}\sinh(\mu_0(1-x)) & (0 \le x \le 1) \end{cases}, \tag{19}$$

with

$$\bar{b}_e(x) \simeq B_e(x) = eB_1(x) = \frac{eA_1(x)}{\alpha_0}, \qquad \bar{r}_e(x) \simeq 1. \tag{20}$$

It would be natural to characterize a morphogen system with (14) to be in a state of *low receptor occupancy* (LRO) since there are few free ligand available to occupy the signaling receptors. However, if we have also $\mu_0 \gg 1$, the expression for $A_e(x)$ in the signaling range of $0 \le x < 1$ is effectively a boundary layer adjacent to the edge of the ligand production region, steep near $x = 0$ and dropping sharply to $e\bar{v}_L/\mu_0^2$ (which is rather small) away from that boundary. Generally, the bound (signaling) morphogen gradient $\bar{b}_e(x) \simeq B_e(x)$ should change gradually if it is to lead to a distinct biological pattern. To limit our discussion to these biologically meaningful gradient systems, we adopt the following definition for a gradient system in a steady state of LRO:

Definition 2 *A morphogen system is in a steady state of* ***low receptor occupancy*** *(LRO) if the condition (14) is satisfied and* $\mu_0 = O(1)$.

With the adoption of this definition, we may then restrict our attention mainly to LRO systems that give rise to distinctive biological tissue patterns. For such systems, the bound and free ligand concentrations change only gradually over their spatial span $[0, X_{\max}]$.

For gradient systems for which neither (14) nor (13) is met, the following condition provides a criterion for eliminating a group of gradient systems that is not biologically realistic and not of interest herein:

Criterion 3 *A gradient system is* ***not*** *biologically meaningful if* $\mu_0 \gg 1$.

While Criterion 1 eliminates signaling gradients that are pretty much flat and with signaling receptors saturated away from the edge $x = 1$, Criterion 3 eliminates signaling gradients that are also pretty much flat but with signaling receptors sparsely occupied away from ligand synthesis region.

2.3 Root-Mean-Square Signaling Differential

We wish to make use of the extracellular model summarized in the preceding subsections to gain more insight into the results from numerical simulations obtained in [25] and to investigate more effective feedback mechanisms to ensure robust development. We do this by working with a spatially uniform feedback to complement the conventional Hill function approach in modeling feedback processes. For this purpose, we need to have a quantitative measure of robustness that quantifies succinctly deviation from the wild-type development. One such measure,designated as the *root-mean-square signaling differential robustness index* (henceforth the R_b *robustness index* for short, or simply *robustness index* when there is no ambiguity) used in [16], is given below and applied to illustrate its effectiveness in measuring the aberrancy of a signaling gradient. This index is simpler to analyze compared to the *root-mean-square displacement differential robustness index* R_x (previously denoted by L in [25]) which was also used earlier in [16] and will be examined in later sections of this paper.

The (*signal*) *robustness index* R_b is the (normalized) root mean square of the deviation of the aberrant signaling gradient $b_e(x,t)$ from wild-type signaling gradient $b_1(x,t)$:

$$R_b(t) = \frac{1}{b_h - b_\ell}\sqrt{\frac{1}{(x_\ell - x_h)^2}\int_{x_h}^{x_\ell}[b_e(x,t) - b_1(x,t)]^2 dx} \tag{21}$$

where $0 \le b_\ell(t) < b_h(t) \le b_1(-x_m, t)$ and $-x_m \le x_h < x_\ell \le 1$. The quantities x_ℓ, x_h, b_ℓ and b_h may be chosen away from the extremities to minimize the exaggerated effects of outliers. For a system in steady state with

$$\bar{b}_1(x) = \lim_{t\to\infty} b_1(x,t), \qquad \bar{b}_e(x) = \lim_{t\to\infty} b_e(x,t), \tag{22}$$

$R_b(t)$ tends to a constant $\bar{R}_b$ with

$$\bar{R}_b = \lim_{t\to\infty} R_b(t) = \frac{1}{b_h - b_\ell}\sqrt{\frac{1}{(x_\ell - x_h)^2}\int_{x_h}^{x_\ell}[\bar{b}_e(x) - \bar{b}_1(x)]^2 dx}. \tag{23}$$

In subsequent developments, we set $x_h = 0$ since the region of ligand synthesis $[-x_m, 0)$ is not expected to contribute significantly to signaling. We also take $x_\ell = 1$ so that $b_\ell(1,t) = b_1(1,t) = 0$. For the case of low receptor occupancy, we take b_h to be $B_1(0)$, the explicit LRO approximation of the steady state wild-type signaling gradient concentration value $\bar{b}_1(0)$, known from (19) and (20) to be

$$b_h = B_1(0) = \frac{\bar{v}_L}{\alpha_0\mu_0^2}\frac{\sinh(\mu_0 x_m)\sinh(\mu_0)}{\cosh(\mu_0(1+x_m))} \simeq \bar{b}_1(0). \tag{24}$$

Having $\bar{b}_e(x)$ and $\bar{b}_1(x)$ (by any numerical software for BVP of ODE), it is straightforward to evaluate the integral (23) to obtain $\bar{R}_b$ to see whether or not the aberrancy of the signaling gradient (when distorted by exogenous perturbations) is still acceptable.

2.4 Approximate Robustness Index for LRO State

For a morphogen system in a state of LRO, we have from (19)-(20) the following approximate steady state solutions for the distorted signaling gradients, $B_e(x)$, of the (environmentally or genetically) perturbed system:

$$B_e(x) \sim eB_1(x) = \frac{e\bar{v}_L}{\alpha_0\mu_0^2}\frac{\sinh(\mu_0 x_m)\sinh(\mu_0(1-x))}{\cosh(\mu_0(1+x_m))}, \quad (0 \le x \le 1) \tag{25}$$

where $\mu_0^2 = g_0/\alpha_0 \simeq h_0$ since $f_0 \ll g_0$ for our model of the wing imaginal disc [**?**]. The parameter e is the excess (amplification) factor of the ligand synthesis rate. Then the LRO approximation of $\bar{R}_b$ (with $x_\ell = 1$, $x_h = 0$), denoted by ρ_e, is given by

$$\begin{aligned}\bar{R}_b &\sim \rho_e = \frac{e-1}{\sinh(\mu_0)}\sqrt{\int_0^1 [\sinh(\mu_0(1-x))]^2 dx} \\ &= \frac{e-1}{\sinh(\mu_0)}\sqrt{\frac{1}{2}\left(\frac{\sinh(2\mu_0)}{2\mu_0} - 1\right)} \equiv (e-1)\gamma(\mu_0).\end{aligned} \tag{26}$$

To be concrete and to make use of the finding of Zhou on the effect of a $5.9°C$ temperature change, we are mainly concerned with the *empirically observed case* of $e = 2$ in the subsequent development.

For a gradient system with $g_0 = 0.2$, $f_0 = 0.001$, $g_r = 1$, $h_0 = 10$, $x_m = 0.1$ and $\bar{v}_L = 0.05$ (corresponding to $\bar{V}_L = 0.002\ \mu M$, $\bar{V}_R = 0.04\ \mu M$, $D = 10^{-7} cm^2/\sec.$, $X_{\max} = 0.01cm$) with $\beta = \bar{v}_L/g_0 = 0.25$ in Table 2 of [7], the steady state is in LRO state. For this case, the approximate solution for $\bar{R}_b$ given by (26) is 0.3938... while the actual solution for $\bar{R}_b$ computed from an accurate numerical solution for the BVP for $\bar{a}_2(x)$ gives 0.3943... for a percentage error of less than 0.01%. If ligand synthesis rate is increased 20 times to $\bar{V}_L = 0.04\ \mu M$, the percentage error of the low receptor occupancy approximation is still less than 1%. These comparisons serve to validate the numerical simulation code developed for exact numerical solutions of our model. (Consistent with the subsequent ease of analysis for the LRO, we have adopted the simplifying approximation $\alpha_0 \simeq g_0/h_0$ in the computation for both $\bar{R}_b$ and ρ_e in this paper since $f_0 \ll g_0$.)

Our main interest however is in the use of the robustness index to induce an appropriate feedback mechanism for attaining robustness of signaling morphogen gradients. When an enhanced ligand system is not in a state of low receptor occupancy, the use of the approximate signaling robustness index based on the approximate solution (25) may not be sufficiently accurate. For these cases, numerical solutions of $\bar{b}_e(x)$ and the corresponding value for $\bar{R}_b$ should be used instead of $B_e(x)$ and ρ_0. If needed, their calculations by available mathematical software such Mathematica, Matlab or Maple should be straightforward.

3 Feedback on Receptor Synthesis Rate

Excessive ligand concentration is known to down-regulate its own signaling receptor synthesis. In particular, Decapentaplegic (Dpp) represses the synthesis of its own receptor Tkv [4]. Another example is Wingless (Wg) repressing its signaling receptor DFz2 [46]. The down-regulation of Tkv by aberrant signaling Dpp gradient was modeled by a negative feedback of the Hill function type in [25] and was found to be ineffective as a mechanism for promoting robust development of the wing imaginal disc. A theoretical analysis of the model in [26] confirms the results of the numerical experiment and shows that the spatially nonuniform feedback distorts the shape of the output gradient as the feedback mechanism works to reduce its aberrancy. The observation suggests that a spatially uniform feedback mechanism may be more effective for ensuring robustness. We consider in [22] the originally (normalized) spatially uniform receptor synthesis rate $\bar{v}_R$ being down-regulated to $v_R(t)$ by a negative feedback factor $\kappa^2(t)$ that is a function of the signaling robustness index $R_b(t)$ in the form

$$v_R(t) = \kappa^2(t)\bar{v}_R = \frac{\bar{v}_R}{1 + c\,[R_b(t-\tau)]^m} \tag{27}$$

where the parameter τ corresponds to a possible time delay and $\{c, m\}$ are two parameters to be chosen for appropriate feedback strength and sensitivity, respectively, similar to those for a Hill's function.

It should be noted that the feedback process in (27) at any location does not depend on the aberrancy of the signaling gradient at that location, only on an average measure $R_b(t)$ of the excess over the span of the wing disc. Since it is not sensitive to the local environment of individual cell, it is less likely to contribute to the shape distortion of the resulting gradients. Such a spatially uniform feedback mechanism obviously requires some disc-wide cooperation among cells. There are at least two possible examples of mechanisms that could create such spatial coordination, particularly in the wing disc.

One of these was described in the two papers by N. Barkai et al. [47, 48]. These papers describe an "expander-repressor scaling mechanism" in which Dpp represses an expander which then diffuses back into the wing disc and expands the Dpp gradient. In the model, the expander freely diffuses everywhere, which is why it is able to adjust Dpp decay uniformly across the disc. It is similar to (27) in that some aspect of Dpp gradient robustness is being controlled by Dpp through a mechanism that is spatially uniform and is an example of how non-spatial feedback might be triggered by changes to a morphogen gradient.

The other is the work of Yu et al. in [49] where the Fat/Dachsous pathway coordinates Dpp signaling over wide spatial ranges for cell growth. This is a completely different mechanism from the one developed in [47, 48]; but it has the same feature that cells end up cooperating over large spatial scales. The work shows another mechanism that allows an entire disc to respond to local perturbations.

Together with additional comments on such a non-local feedback mechanism in [22], the specific examples above provide concrete evidence supporting the possibility of spatially uniform non-local feedback processes such as (27) and those to be introduced in subsequent developments. For the investigation of the effects of our particular type of negative feedback on the receptor synthesis rate, we are interested in the modified signaling gradient (starting at $t = 0$) and the corresponding robustness index of the IBVP (3) - (7) but now for an enhanced ligand synthesis rate

$$v_L = e\bar{v}_L H(-x) \tag{28}$$

with an excess factor $e > 1$ and a down-regulated receptor synthesis rate given by (27). In the presence of the feedback, the three aberrant gradients of the new IBVP are to be denoted by $\{a_v(x,t), b_v(x,t), r_v(x,t)\} \equiv \{a_e(x,t;c), b_e(x,t;c), r_v(x,t;c)\}$ which reduce to $\{a_e(x,t), b_e(x,t), r_e(x,t)\}$ of the model without feedback (when $c = 0$). The corresponding robustness index is determined by

$$R_b(t) = \frac{1}{b_h}\sqrt{\int_0^1 [b_v(x,t) - b_1(x,t)]^2 dx}. \tag{29}$$

Unlike the situation in (21), $b_v(x,t) = b_e(x,t;c)$ now depends on $R_b(t)$.

As a consequence, (29) is an integral equation to be solved for the unknown $R_b(t)$ concurrently with the solution of the IBVP (3)-(7) with $\bar{v}_R$ replaced by $v_R(t) = \kappa^2(t)\bar{v}_R$. Such solutions have been obtained in [21, 20] and will not be pursued herein. Instead, we will focus on the corresponding steady state behavior with low receptor occupancy.

3.1 Time Independent Steady State with Feedback

It has been shown in [7] that the extracellular model system without feedback has a unique steady state. We are interested here also in the corresponding steady state for the case with the spatially uniform non-local feedback on the signaling receptor synthesis rate $\bar{V}_R$ of the type characterized by (27). We denote the corresponding time independent steady state aberrant gradients by $\{\bar{a}_v(x), \bar{b}_v(x), \bar{r}_v(x)\} \equiv \{\bar{a}_e(x;c), \bar{b}_e(x;c), \bar{r}_e(x;c)\}$ and the steady state robustness index $\bar{R}_b$ determined by

$$\bar{R}_b = \lim_{t\to\infty} R_b(t) = \frac{1}{b_h}\sqrt{\int_0^1 [\bar{b}_v(x) - \bar{b}_1(x)]^2 dx} \tag{30}$$

with b_h appropriately taken to be $B_1(0)$, the LRO approximation for $\left[\bar{b}_1(0;c)\right]_{c=0} = \left[\bar{b}_e(0;0)\right]_{e=1}$. (Note that $\bar{R}_b$ is positive unless $c = 0$ and $e = 1$.)

With $\partial(\ )/\partial t = 0$, the governing equations and boundary conditions for our extracellular model with feedback can again be reduced to a BVP for $\bar{a}_v$ alone:

$$\bar{a}_v'' - \frac{\bar{\kappa}^2 g_0 \bar{a}_v}{\alpha_0 + \zeta_0 \bar{a}_v} + e\bar{v}_L H(-x) = 0, \tag{31}$$

$$\bar{a}_v'(-x_m) = 0, \qquad \bar{a}_v(1) = 0. \tag{32}$$

where α_0 and ζ_0 as defined in (12) and

$$\bar{\kappa}^2(\bar{R}_b) = \frac{1}{1 + c\bar{R}_b^m}. \tag{33}$$

The corresponding signaling ligand and unoccupied receptor concentrations are given in terms of $\bar{a}_v$ by

$$\bar{b}_v(x) = \frac{\bar{\kappa}^2 \bar{a}_v(x)}{\alpha_0 + \zeta_0 \bar{a}_v(x)}, \qquad \bar{r}_v(x) = \frac{\bar{\kappa}^2 \alpha_0}{\alpha_0 + \zeta_0 \bar{a}_v(x)}, \tag{34}$$

respectively. The solution for $\bar{b}_v(x)$ with $\bar{R}_b$ as an unknown parameter is then used in (30) for the determination of $\bar{R}_b$.

Existence of a unique, non-negative, monotone decreasing solution for (31)-(32) can be proved by the method used in [7]. Sample solutions of the BVP and the signaling gradient $\bar{b}_v(x) = \bar{b}_e(x;c)$ for $c > 0$ (with $\left[\bar{b}_v(x)\right]_{c=0} = \bar{b}_e(x;0) = \bar{b}_e(x)$ without feedback) have been calculated and analyzed in [22]. Here, we complement these results by showing the existence and uniqueness of a positive robustness index $\bar{R}_b$ for the particular feedback problem. The method may be used to establish similar results for problems that include the effects of non-receptors in later sections.

To be concrete, we take $x_\ell = 1$, $x_h = 0$, and $b_\ell = 0$ henceforth so that

$$\bar{R}_b = \frac{1}{b_h}\sqrt{\int_0^1 [\bar{b}_e(x;c) - \bar{b}_1(x)]^2 dx} \equiv C(\bar{R}_b) \tag{35}$$

As indicated previously, we take b_h to be given by (24). We now work with (35) to show first that for a fixed positive c, $\bar{b}_e(x;c)$ is a decreasing function of $\bar{R}_b$ and then use the result in

$$\frac{dC}{d\bar{R}_b} = \frac{1}{b_h^2 C}\int_0^1 [\bar{b}_e(x;c) - \bar{b}_1(x)]\frac{\partial \bar{b}_e(x;c)}{\partial \bar{R}_b} dx, \tag{36}$$

to establish the existence, uniqueness and positivity of $\bar{R}_b$. For simplicity, we do this for the $m = 1$ case.

Upon differentiating all relations in the BVP for $\bar{a}_v(x) = \bar{a}_e(x;c)$ partially with respect to $\bar{R}_b$, we obtain

$$-w'' + \frac{\bar{\kappa}^2 g_0}{(\alpha_0 + \zeta_0 \bar{a}_v)^2} w - \frac{c\bar{\kappa}^4 g_0 \bar{a}_v}{\alpha_0 + \zeta_0 \bar{a}_v} = 0, \tag{37}$$

$$w'(-x_m;c) = 0, \qquad w(1;c) = 0 \tag{38}$$

where

$$w(x;c) = -\frac{\partial \bar{a}_e(x;c)}{\partial \bar{R}_b} = -\frac{\partial \bar{a}_v(x)}{\partial \bar{R}_b}.$$

Clearly, $w_\ell(x;c) \equiv 0$ is a lower solution of the BVP for $w(x;c)$ given

$$-w_\ell'' + \frac{\bar{\kappa}^2 g_0}{(\alpha_0 + \zeta_0 \bar{a}_v)^2} w_\ell - \frac{c\bar{\kappa}^4 g_0 \bar{a}_v}{\alpha_0 + \zeta_0 \bar{a}_v} = -\frac{c\bar{\kappa}^4 \bar{a}_v}{\alpha_0 + \zeta_0 \bar{a}_v} \leq 0$$

and by the conditions in (38) are also satisfied by $w_\ell(x;c) \equiv 0$. As an upper solution, we take

$$w_u(x;c) = \frac{\alpha_0 g_r}{g_0} e\bar{v}_L \left\{ \left(x_m + \frac{1}{2} \right) - x_m x - \frac{1}{2} x^2 \right\} \equiv \frac{\alpha_0 g_r}{g_0} a_u(x)$$

so that the boundary conditions for $w(x)$ are satisfied with $w_u(x;c) \geq 0$ for all x in $[-x_m, 1]$. The function $a_u(x)$, the re-scaled $w_u(x;c)$, was shown in [7] to be an upper solution for $\bar{a}_v(x)$ so that $a_u(x) \geq \bar{a}_v(x)$. With $\zeta_0 = O(10^{-1})$ while $c, 1 + c\bar{R}_b$ and $1 + \zeta_0 \bar{a}_v/\alpha_0$ are $O(1)$ quantities, we have

$$\begin{aligned} -w_u'' + \frac{\bar{\kappa}^2 g_0}{(\alpha_0 + \zeta_0 \bar{a}_v)^2} w_u - \frac{c\bar{\kappa}^4 g_0 a_v}{\alpha_0 + \zeta_0 \bar{a}_v} &= \\ \frac{\alpha_0 g_r}{g_0} e\bar{v}_L + \frac{\bar{\kappa}^2 g_r}{\alpha_0 + \zeta_0 \bar{a}_v} \left\{ \frac{a_u}{1 + \zeta_0 \bar{a}_v/\alpha_0} - \frac{c\zeta_0 \bar{a}_v}{1 + c\bar{R}_b} \right\} &\geq 0. \end{aligned}$$

Then the monotone method in [50, 51, 52] implies that $w(x;c)$ exists, is unique and non-negative so that

$$-w(x;c) \leq \frac{\partial \bar{a}_e(x;c)}{\partial \bar{R}_b} \left(= \frac{\partial \bar{a}_v(x)}{\partial \bar{R}_b} \right) \leq 0 \tag{39}$$

The development above leads to the following lemma on the non-positivity of the marginal value $\partial \tilde{b}_e(x;c)/\partial \bar{R}_b$:

Lemma 4 $\partial \bar{b}_e(x;c)/\partial \bar{R}_b \leq 0.$

Proof. Upon differentiating the expression for $\bar{b}_v(x) = \bar{b}_e(x;c)$ in (34) partially with respect to $\bar{R}_b$, we obtain

$$\frac{\partial \bar{b}_v(x)}{\partial \bar{R}_b} = \frac{\bar{\kappa}^2(R_b)\alpha_0}{(\alpha_0 + \zeta_0 \bar{a}_v)^2} \frac{\partial \bar{a}_v(x)}{\partial \bar{R}_b} - \frac{c\bar{\kappa}^4 \bar{a}_v}{\alpha_0 + \zeta_0 \bar{a}_v} \leq 0$$

in view of the second inequality of (39). ■

Proposition 5 *A positive solution of $\bar{R}_b = C(\bar{R}_b)$ exists and is unique.*

Proof. Together with $\bar{b}_e(x;c) > 0$, Lemma 4 implies $dC/d\bar{R}_b \leq 0$ as long as $\bar{b}_e(x;c) > \bar{b}_1(x)$ (see (36)). It follows that a solution exists for $\bar{R}_b = C(\bar{R}_b)$. It is unique since $C(\bar{R}_b)$ is monotone decreasing. It is positive since $C(\bar{R}_b)$ is nonnegative so that $\bar{R}_b$ is bounded below for $\bar{R}_b \geq 0$. ■

3.2 Low Receptor Occupancy

The LRO approximation for morphogen systems has been found useful for an understanding of the effects of various feedback mechanisms. The steady state approximation for the present feedback process has been obtained in [22]. The results are summarized below for reference later in the study of the effects of multi-feedback system involving non-receptors on robustness. In a state of *low receptor occupancy* prior to and after ligand synthesis enhancement so that

$$\zeta_0 \bar{a}_v \ll \alpha_0 \tag{40}$$

(including the special case where $c = 0$ and $e = 1$ so that $\bar{a}_v(x)$ reduces to $\bar{a}_1(x) = [\bar{a}_e(x,0)]_{e=1}$), $\bar{a}_v(x)$ may be approximated by $eA_v(x)$ with

$$A_v(x) = \begin{cases} \frac{\bar{v}_L}{\mu_c^2}\{1 - \frac{\cosh(\mu_c)}{\cosh(\mu_c(1+x_m))}\cosh(\mu_c(x+x_m))\} & (-x_m \le x \le 0) \\ \frac{\bar{v}_L}{\mu_c^2}\frac{\sinh(\mu_c x_m)}{\cosh(\mu_c(1+x_m))}\sinh(\mu_c(1-x)) & (0 \le x \le 1) \end{cases}, \tag{41}$$

where

$$\mu_c^2 = \bar{\kappa}^2(\rho_c)\frac{g_0}{\alpha_0}. \tag{42}$$

and ρ_c is the LRO approximation of $\bar{R}_b$ calculated from (30) using $eA_v(x)$ and $A_1(x,0)$ for $\bar{a}_v(x)$ and $\bar{a}_1(x) = [\bar{a}_e(x,0)]_{e=1}$, respectively (see [22]). The LOR solution $eA_v(x)$ is expected to be an accurate approximation of the exact solution $\bar{a}_v(x)$ and reduces to the LRO wild-type ligand concentration $A_1(x)$ when $c = 0$ and $e = 1$.

The corresponding LOR signaling gradient and free receptor concentration are given by

$$\bar{b}_v(x) = \bar{b}_e(x,c) \simeq eB_v(x) = \frac{eA_v(x)}{\alpha_0}, \qquad \bar{r}_v(x) \simeq \bar{\kappa}^2(\rho_c) \tag{43}$$

with

$$B_v(x) = \frac{\bar{v}_L}{g_0}\frac{\sinh(\mu_c x_m)}{\cosh(\mu_c(1+x_m))}\sinh(\mu_c(1-x)) \qquad (0 \le x \le 1). \tag{44}$$

It should be noted that the dissociation rate constant is usually much smaller than the degradation rate constant with $f_0/g_0 = O(10^{-2})$. To simplify our discussion, we have adopted the simplifying approximation $\alpha_0 = (f_0 + g_0)/h_0 \simeq g_0/h_0$ so that

$$\frac{g_0}{\alpha_0} \simeq h_0 \equiv \mu_0^2, \qquad \mu_c^2 = \frac{\mu_0^2}{1 + c\rho_c^m} \tag{45}$$

as in [22].

For $e > 1$, the yet unknown LOR robustness index ρ_c is to be determined by the LRO approximation of (30)

$$\bar{R}_b \sim \rho_c = \frac{1}{B_1(0)}\sqrt{\int_0^1 [eB_v(x) - B_1(x)]^2 dx} \equiv C_v(\rho_c). \tag{46}$$

Given (19), (20) and (44), the right hand side depends on ρ_c through $B_v(x)$; hence (46) is an equation for $\rho_c \left(\simeq \bar{R}_b\right)$:

$$f_c(\rho_c) = 0$$

where

$$\begin{aligned} f_c(\rho_c) \;\equiv\; & 2\sinh^2(\mu_0)\,\rho_c^2 - e_c^2\left(\frac{\sinh(2\mu_c)}{2\mu_c} - 1\right) - \left(\frac{\sinh(2\mu_0)}{2\mu_0} - 1\right) \\ & + 2e_c\left\{\frac{\sinh(\mu_c+\mu_0)}{\mu_c+\mu_0} - \frac{\sinh(\mu_c-\mu_0)}{\mu_c-\mu_0}\right\}, \end{aligned} \tag{47}$$

with

$$e_c = e\frac{\sinh(\mu_c x_m)\cosh(\mu_0(1+x_m))}{\sinh(\mu_0 x_m)\cosh(\mu_c(1+x_m))}, \qquad \mu_c^2 = \frac{h_0}{1+c\rho_c^m}. \tag{48}$$

Even without an explicit solution for $f_c(\rho_c) = 0$, we see from (46) that ρ_c is necessarily positive when $e > 1$ and therewith $0 < \bar{\kappa}^2(\rho_c) < 1$. It follows from

$$eB_v(0) = \frac{e\bar{v}_L}{g_0}\frac{\sinh(\mu_c x_m)}{\cosh(\mu_c(1+x_m))}\sinh(\mu_c)$$

that the order of magnitude of $B_e(0,c) = eB_v(0)$ is not changed in any significant way by the presence of the adopted feedback. With $\mu_0^2 \simeq h_0 = O(10)$, $\mu_c^2 = \bar{\kappa}^2(\rho_c)\mu_0^2$ and $0 < x_m \ll 1$, we can work with the approximate expression estimate

$$\begin{aligned} \frac{B_v(0)}{B_1(0)} &= \frac{\sinh(\mu_c)}{\sinh(\mu_0)}\,\frac{\sinh(\mu_c x_m)}{\sinh(\mu_0 x_m)}\,\frac{\cosh(\mu_0(1+x_m))}{\cosh(\mu_c(1+x_m))} \\ &= \frac{1-e^{-2\mu_c x_m}+o(e^{-2\mu_c x_m})}{1-e^{-2\mu_0 x_m}+o(e^{-2\mu_0 x_m})} \simeq \frac{1}{\sqrt{1+c\rho_c^m}} \end{aligned} \tag{49}$$

to get

$$\frac{eB_v(0)}{B_1(0)} = O\left(\frac{e}{\sqrt{1+c\rho_c^m}}\right).$$

with $\rho_c < 0.2$ for a robust gradient. Note that the ratio is smallest when the positive integer m is 1.

In addition, the negative feedback (27) also leads to a less convex modified ectopic signaling gradient since $\mu_c < \mu_0$ whenever $\rho_c > 0$. We summarize the development above by the following observation:

Conclusion 6 *When both the wild-type and ectopic gradients are in a state of LRO, the negative feedback mechanism (27) on receptor synthesis rate is not particularly effective for promoting robust development.*

3.3 Numerical Solution for the General Case

In order to confirm the usefulness of the LOR solution for the problem, we have reconfigured the integro-differential equation problem for $\bar{a}_v$ as a BVP for a system of ODE and solved it by any BVP solver (e.g., *bvp4c* in Matlab). Numerical results are reported below for a system characterized by the parameter values shown in Table I with $m = 1$.

Table I Some Sample Solutions

$$\begin{aligned} X_{\max} &= 0.01\ cm, \qquad X_{\min} = 0.001\ cm, \qquad k_{on}R_0 = 0.01/\sec./\mu M, \qquad m = 1 \\ k_{deg} &= 2\times 10^{-4}/\sec., \qquad k_R = 0.001/\sec., \qquad k_{off} = 10^{-6}/\sec., \qquad e = 2 \\ D &= 10^{-7}\ cm^2/\sec., \qquad \bar{V}_L = 0.002\ \mu M/\sec., \qquad \bar{V}_R = 0.04\ \mu M/\sec. \end{aligned}$$

c	$\bar{R}_b$	ρ_c	$\bar{b}_e(0;c)$	$eB_v(0)$	$\bar{b}_1(0)$	$B_1(0)$
0	0.3943	0.3938	0.1154	0.1169	0.0580	0.0584
0.5	0.3750	0.3736	0.1080	0.1096	0.0580	0.0584
1	0.3573	0.3565	0.1022	0.1062	0.0580	0.0584
2	0.3284	0.3284	0.0941	0.0959	0.0580	0.0584
4	0.2862	0.2872	0.0839	0.0857	0.0580	0.0584

The biological implication of the resulting robustness index is not particularly gratifying. The values of ρ_c and $\bar{R}_b$ shown in Table I differ by less than 1% for all five values of c. Their values for $c = 1$ are well above the acceptable level of 0.2 for a robust signaling gradient, a rather modest requirement set arbitrarily in [25]) for robustness. This is hardly surprising given the estimate for the amplitude of the explicit solution $eB_v(0)$ for the LRO approximation of $\bar{b}_v(0)$ in (49) being independent of feedback. Comparing the accurate numerical solution for $\bar{b}_1(0)$ and $\bar{b}_e(0,0)$ with $\bar{b}_v(0) = \bar{b}_e(0,c)$ reported in Table I shows that the latter is much closer to $\bar{b}_e(0)$ than $\bar{b}_1(0)$. More specifically, $\bar{b}_e(0)$ and $\bar{b}_v(0)$ are roughly double the magnitude of $\bar{b}_1(0)$, confirming the ineffectiveness of the feedback $\bar{\kappa}^2(R_b)$ for $c = 1$.

The LOR solution $eB_v(x)$ also shows that increasing the value of the parameter c to larger than 1 would further distort the shape of the gradient and ameliorate the amplitude reduction of the ectopic gradient through the terms involving hyperbolic functions with increasingly smaller μ_c. Accurate numerical solutions for the general case (not in a state of LRO) in Table I are qualitatively similar to the LRO results with the robustness index decreasing rather gradually as c increases. Figure 1 shows graphs of $\bar{b}_e(x,c)$ for $c = 0, 1, 2$ and 4 for comparison with $\bar{b}_1(x)$ to further illustrate these observations. Note that for all the graphs in the figure, the labels are generically $\bar{b}_e(x;c,Z,\eta,\sigma)$ with $Z = \eta = \sigma = 0$ for Figure 1 (since these parameters do not appear until later sections) and $\bar{b}_e(x;c,0,0,0) \equiv \bar{b}_e(x,c)$.

It is necessary then to look elsewhere for a more effective feedback instrument to attain robust development with respect to an aberrant Dpp synthesis rate. There are

a number of different such mechanisms available for this purpose [22]. In the next few sections, we will focus on only one of these, namely, the role of non-receptors on robust development to gain insight to the findings for model 3 in [25] and to help develop a more effective feedback strategy for promoting robust signaling gradients, hence robust biological development.

As we routinely work with normalized quantities, we give below for subsequent references the normalized parameters corresponding to the actual biological parameter values listed under Table I above to be used for computing various solutions:

$$g_0 = 0.2,\ g_r = 1.0,\ f_0 = 0.001,\ x_m = 0.1,\ v_L = 0.05 \tag{50}$$

The normalized value of receptor synthesis rate does not appear explicitly in the dimensionless BVP for the steady state solution, (31)-(34). It is involved in various normalized quantities such as v_L and will not be given here.

Figure 1: Spatially uniform negative feedback on receptor synthesis rate.

4 Effects of Non-receptors

The existence of inhibiting non-receptors and the associated feedback process are well documented for the BMP family ligands that includes the Dpp ligand of interest here (see [19, 31] and elsewhere). Known non-receptor type inhibitors include noggin, chordin, dally, follistatin, sog *and* various heparan sulfate proteoglycans. They are ubiquitous during wing imaginal disc and other biological developments (see [30],[32]-[33],[36]-[38],[40, 41] and references cited earlier). For the purpose of establishing robust signaling Dpp gradients, we are interested in the effects of relevant non-receptors as an inhibiting agent on such gradients. The impact of non-receptors on the (time independent) steady state of a signaling gradient has been investigated theoretically by analysis and numerical simulations in [17, 18, 25, 44, 45]. To the extent that inhibitors for promoting robust gradients are expected to be induced by sustained exogenous perturbations (as it would be a part of the wild-type development otherwise), the introduction of non-receptors in these models may be interpreted as a non-receptor synthesis rate of the form

$$V_N = \bar{V}_N H(t - t_e) \tag{51}$$

where $t_e > 0$ is the instant of the onset of genetic or epigenetic perturbations. In this section, we examine the consequences of non-receptors generated in this way principally to delineate similar results obtained in [25] for (51) in the presence of a negative feedback on receptor synthesis rate. The usefulness of non-receptors for promoting signaling gradient robustness will be investigated for a robustness index induced feedback mechanism on non-receptor synthesis rate in the next section.

To examine how the introduction of non-receptors affects the robustness of the signalling gradient as measured by the robustness index, we take as a reference level of non-receptor concentration $N_0 = \bar{V}_N/j_N$ with

$$Z = \frac{N_0}{R_0} = \frac{\bar{V}_N/j_N}{\bar{V}_R/k_R}, \qquad \bar{v}_N = \frac{X_{\max}^2}{D}\left\{\frac{\bar{V}_N}{N_0}\right\} = \frac{j_N}{D/X_{\max}^2} \tag{52}$$

where j_N is the degradation rate constant for the unoccupied non-receptors. Similar to signaling receptors, (normalized) free (cell surface bound) non-receptor concentration $n(x,t)$ is also bound reversibly to Dpp ligand (to form normalized bound non-receptors of concentration $c(x,t)$),

$$\{c, n\} = \frac{1}{N_0}\{[LN], [N]\}, \tag{53}$$

with a normalized binding rate constant $h_1 a$ (for binding between Dpp and non-receptors of concentration $[N]$), non-receptor-mediated degradation rate constant g_1 (for degradation of Dpp-non-receptor complexes $[LN]$), dissociation rate constant f_1 (for dissociation rate of Dpp-non-receptor complexes) and free non-receptor degradation rate constant g_n (for degradation of unoccupied non-receptors):

$$\{h_1, g_1, f_1, g_n\} = \frac{X_{\max}^2}{D}\{j_{on}R_0, j_{\deg}, j_{off}, j_N\}. \tag{54}$$

with $f_1 \ll g_1$ so that $\alpha_1 \simeq g_1/h_1$.

In terms of these normalized quantities, we have the following IBVP for the five normalized unknowns a, b, r, c and n [45]:

$$\frac{\partial a_e}{\partial t} = \frac{\partial^2 a_e}{\partial x^2} - h_0 a_e r_e + f_0 b_e - Z h_1 a_e n_e + Z f_1 c_e + \bar{v}_L H(-x)\left\{1 + (e-1)H(t-t_e)\right\}, \tag{55}$$

$$\frac{\partial b_e}{\partial t} = h_0 a_e r_e - (f_0 + g_0) b_e, \qquad \frac{\partial r_e}{\partial t} = \bar{v}_R - h_0 a_e r_e + f_0 b_e - g_r r_e, \tag{56}$$

$$\frac{\partial c_e}{\partial t} = h_1 a_e n_e - (f_1 + g_1) c_e, \qquad \frac{\partial n_e}{\partial t} = \bar{v}_N H(t - t_e) - h_1 a_e n_e + f_1 c_e - g_n n_e, \tag{57}$$

with the end conditions

$$x = -x_m: \quad \frac{\partial a_e}{\partial x} = 0, \qquad x = 1: \quad a_e = 0, \tag{58}$$

for $t > 0$, and (for $t_e > 0$) the initial conditions

$$t = 0: \quad a_e = b_e = c_e = n_e = 0, \qquad r_e = 1 \qquad (-x_m < x < 1). \tag{59}$$

As before, the parameter $e \geq 1$ is a measure of the excess (amplification) of the enhanced Dpp synthesis rate induced by a sustained exogenous perturbation initiated at $t_e > 0$. In this section, our first goal is to investigate whether the presence of a sufficiently high non-receptor synthesis rate may make the eventual signaling morphogen gradient $[LR]$ insensitive to an enhanced Dpp synthesis rate. Since we are interested in the steady state behavior for large t, the initial conditions (59) will not have a substantive role in our analysis.

4.1 Time-Independent Synthesis Rates

When all synthesis rates for ligands, receptors and non-receptors are time-invariant, the solution of the new five-component model is expected to tend to a steady state denoted by $\{\bar{a}_Z(x), \bar{b}_Z(x), \bar{c}_Z(x), \bar{r}_Z(x), \bar{n}_Z(x)\}$ (as abbreviations for $\{\bar{a}_e(x;Z), ..., \bar{n}_e(x;Z)\}$ when appropriate). For this steady state solution, we have $\partial(\ )/\partial t = 0$ so that the last four equations in (56) and (57) can be solved for $\bar{b}_Z(x), \bar{c}_Z(x), \bar{r}_Z(x)$ and $\bar{n}_Z(x)$ in terms of $\bar{a}_Z(x)$ to get (11) and

$$\bar{c}_Z(x) = \frac{\bar{a}_Z(x)}{\alpha_1 + \zeta_1 \bar{a}_Z(x)}, \qquad \bar{n}_Z(x) = \frac{\alpha_1}{\alpha_1 + \zeta_1 \bar{a}_Z(x)}, \tag{60}$$

with

$$\alpha_1 = \frac{f_1 + g_1}{h_1}, \qquad \{\zeta_0, \zeta_1\} = \left\{\frac{k_{\text{deg}}}{k_R}, \frac{j_{\text{deg}}}{j_N}\right\} = \left\{\frac{g_0}{g_r}, \frac{g_1}{g_n}\right\}. \tag{61}$$

where k_{deg} and j_{deg} are the receptor- and non-receptor-mediated degradation rate constant, respectively, of bound Dpp complexes. The results are then used to obtain from the steady state version of (55),

$$\bar{a}_Z'' - h_0 \bar{a}_Z \bar{r}_Z + f_0 \bar{b}_Z - h_1 Z \bar{a}_Z \bar{n}_Z + f_1 Z \bar{c}_Z + e\bar{v}_L H(-x) = 0, \tag{62}$$

and (58) a BVP for $\bar{a}_Z(x) = \bar{a}_e(x;Z)$ alone:

$$\bar{a}_Z'' - \frac{g_0 \bar{a}_Z}{\alpha_0 + \zeta_0 \bar{a}_Z} - \frac{Z g_1 \bar{a}_Z}{\alpha_1 + \zeta_1 \bar{a}_Z} + e\bar{v}_L H(-x) = 0, \tag{63}$$

$$\bar{a}_Z'(-x_m) = 0, \qquad \bar{a}_Z(1) = 0. \tag{64}$$

Evidently, $\bar{a}_Z(x)$ varies with Z (and occasionally written as $\bar{a}_e(x;Z)$) which reduces to $\bar{a}_e(x)$ of the basic model for $Z = 0$, i.e., $\bar{a}_e(x) = [\bar{a}_Z(x)]_{Z=0} = \bar{a}_e(x;0)$. Even if $e = 1$, $\bar{a}_1(x;Z)$ is different from the solution $\bar{a}_1(x) = \bar{a}_1(x;0)$, the free ligand concentration in the wild-type development without non-receptors (corresponding to $t_e = \infty$).

4.2 Low Receptor and Non-receptor Occupancy (LRNO)

For the signaling gradient to provide positional information that differentiates cell fates, the normalized concentration $b = [LR]/R_0$ should not be nearly uniform (with

a steep gradient adjacent to the absorbing edge or the ligand synthesis region). Positional indifference except for a steep gradient near the imaginal disc edge is not likely to occur if free ligand concentration is sufficiently low so that $\alpha_0 + \zeta_0 \bar{a}_Z \simeq \alpha_0$ and $\alpha_1 + \zeta_1 \bar{a}_Z \simeq \alpha_1$. A gradient system is said to be in a state of *low receptor and non-receptor occupancy* (LRNO) if

$$\alpha_0 + \zeta_0 \bar{a}_Z \simeq \alpha_0, \qquad \alpha_1 + \zeta_1 \bar{a}_Z \simeq \alpha_1 \ \text{ and } \ 1 < \mu_0 < \mu_Z = O(1). \tag{65}$$

The gradient system is *biologically differentiating* if it is in a state of LRNO.

When the system is in a state of LRNO, the ODE (63) can be linearized with the corresponding approximate solution denoted by $\{eA_Z(x), eB_Z(x), eC_Z(x), eN_Z(x), eR_Z(x)\}$. The relevant BVP has been reduced to a single ODE for $\bar{a}_Z(x) \simeq eA_Z(x)$:

$$A_Z'' - \mu_Z^2 A_Z + \bar{v}_L H(-x) = 0, \qquad A_Z'(-x_m) = A_Z(x) = 0 \tag{66}$$

where

$$\mu_Z^2 = \frac{g_0}{\alpha_0} + \frac{Z g_1}{\alpha_1} \simeq h_0 + Z h_1 \tag{67}$$

Its solution has been obtained in [25, 45] and elsewhere. With $f_0 \ll g_0$ and $f_1 \ll g_1$, we neglect (as in [22]) the effect of the dissociation rates and write $A_Z(x)$ as

$$A_Z(x) = \begin{cases} \frac{\bar{v}_L}{\mu_Z^2}\{1 - \frac{\cosh(\mu_Z)}{\cosh(\mu_Z(1+x_m))}\cosh(\mu_Z(x+x_m))\} & (-x_m \le x \le 0) \\ \frac{\bar{v}_L}{\mu_Z^2}\frac{\sinh(\mu_Z x_m)}{\cosh(\mu_Z(1+x_m))}\sinh(\mu_Z(1-x)) & (0 \le x \le 1) \end{cases}, \tag{68}$$

with $\mu_0^2 \simeq h_0$, $Z\mu_1^2 \simeq Z h_1$ and

$$\mu_Z^2 = \mu_0^2 + Z\mu_1^2 \simeq h_0 + h_1 Z. \tag{69}$$

The corresponding signaling gradient is $\bar{b}_Z(x) \simeq eB_Z(x)$ with

$$B_Z(x) = \frac{h_0 \bar{v}_L}{g_0 \mu_Z^2} \frac{\sinh(\mu_Z x_m)}{\cosh(\mu_Z(1+x_m)))} \sinh(\mu_Z(1-x)) \qquad (x \ge 0) \tag{70}$$

(keeping in mind $\alpha_0 = (g_0 + f_0)/h_0 \simeq g_0/h_0$) and

$$B_Z(0) = \frac{A_Z(0)}{\alpha_0} = \frac{h_0 \bar{v}_L}{g_0 \mu_Z^2} \frac{\sinh(\mu_Z x_m)\sinh(\mu_Z))}{\cosh(\mu_Z(1+x_m))} \tag{71}$$

Note that $B_Z(x)$ depends on Z through μ_Z.

For $h_1 \gtrsim h_0\,(> g_0)$ so that $\mu_Z^2 = \mu_0^2 > 1$, we have

$$\frac{\sinh(\mu_k x_m)\sinh(\mu_k))}{\cosh(\mu_k(1+x_m))} \sim \frac{1}{2}\left(1 - e^{-2\mu_k x_m}\right) \qquad (k = 0, Z)$$

and

$$\frac{eB_Z(0)}{B_1(0)} \simeq \frac{e}{1 + h_1 Z/h_0} \frac{1 - e^{-2\mu_Z x_m}}{1 - e^{-2\mu_0 x_m}}, \qquad \mu_Z x_m = \mu_0 x_m \sqrt{1 + h_1 Z/h_0}. \tag{72}$$

where $B_1(0) = [B_Z(0)]_{Z=0}$ ($\simeq \bar{b}_1(0;0)$). For $h_1 = h_0$ and $Z = O(1)$, we have (with $0 < x_m \ll 1$)

$$\bar{b}_Z(0) \simeq eB_Z(0) \simeq \frac{e}{1+Z}\frac{\bar{v}_L}{g_0}, \tag{73}$$

indicating that the amplitude of the aberrant signaling morphogen concentration (at $x = 0$) is reduced (approximately) to the wild-type concentration with $Z = 1$ for the empirically observable case of $e = 2$ (the amplification for Dpp synthesis rate associated with an increase of $5.9°C$ in ambient temperature). However, this does not mean that the aberrant gradient is reduced to the wild type since the shape parameter $\mu_Z^2 = h_0(1 + Z)$ would be $2h_0$ so that the slope and convexity of the aberrant signaling gradient would be changed substantially.

More generally, the aberrant concentration $\bar{b}_Z(0) \simeq eB_Z(0)$ may be kept to the wild-type level with a sufficiently high non-receptor synthesis rate so that $1+h_1Z/h_0 \geq e$. However, for a given h_1/h_0 ratio, the required level of Z increases with e resulting in at least two effects that cause a distortion of signaling gradient shape and thereby work against the desired reduction of aberrancy of the signaling gradient. First, the ratio $\left(1 - e^{-2\mu_Z x_m}\right) / \left(1 - e^{-2\mu_0 x_m}\right)$ is significantly larger than 1 for larger Z and a larger Z is needed to reduce the amplitude of the gradient (than that for $1+h_1Z/h_0 = e$). Second, the aberrant gradient shape would be distorted even more severely by a larger Z since the gradient shape parameter μ_Z^2 increases linearly with Z. Since the quantitative effects from a particular non-receptor synthesis rate can only be seen from the corresponding value of the (LRNO approximation of the) robustness index, denoted by ρ_Z, the dependence of ρ_Z on Z will be calculated in the next subsection. Here, we settle for the following relatively conservative observation:

Conclusion 7 *For gradient systems in a steady state of low receptor and non-receptor occupancy with $h_1/h_0 = O(1)$, the amplitude of their aberrant signaling gradient induced by the empirically observed synthesis rate amplification factor of $e = 2$ could be kept close to the wild-type amplitude (around $x = 0$) by a moderate non-receptor synthesis rate of $Z \simeq 1$ (as shown by the exact (numerical) solutions without the LRNO approximation in Figure 2).*

4.3 The LRNO Approximation of Robustness Index

As the presence of a fixed non-receptor concentration works both for and against robust development, whether or not the net effect is positive can only be seen from (the LRNO approximation ρ_Z of) our adopted measure of robustness $\bar{R}_b$. From (46), we have

$$\rho_Z = \frac{1}{B_1(0)}\sqrt{\int_0^1 [eB_Z(x) - B_1(x)]^2 dx} \tag{74}$$

or

$$f_Z(\rho_Z) = 0, \tag{75}$$

where $f_Z(\cdot)$ is $f_c(\cdot)$ as defined in (47) but with μ_c and e_c replaced by μ_Z and

$$e_Z = \frac{e}{1 + h_1 Z/h_0} \frac{\sinh(\mu_Z x_m)\cosh(\mu_0(1+x_m))}{\sinh(\mu_0 x_m)\cosh(\mu_Z(1+x_m))}, \tag{76}$$

respectively.

Note that unlike its counterpart (46), the right hand side of (74) is independent of ρ_Z so that it can be evaluated to get ρ_Z (without having to solve a nonlinear equation as we had to do for ρ_c). Upon writing (74) as

$$\sinh^2(\mu_0)\,\rho_Z^2 = \int_0^1 [I(x;Z)]^2 dx \equiv G(Z) \tag{77}$$

where

$$I(x;Z) = e_Z \sinh(\mu_Z(1-x)) - \sinh(\mu_0(1-x)),$$

it is straightforward to evaluate the integral in (77) to obtain

$$\begin{aligned} 2G(Z) &= e_Z^2\left(\frac{\sinh(2\mu_Z)}{2\mu_Z} - 1\right) + \left(\frac{\sinh(2\mu_0)}{2\mu_0} - 1\right) \\ &\quad -2e_Z\left\{\frac{\sinh(\mu_Z+\mu_0)}{\mu_Z+\mu_0} - \frac{\sinh(\mu_Z-\mu_0)}{\mu_Z-\mu_0}\right\}, \end{aligned} \tag{78}$$

Plotting $G(Z)$ shows that ρ_Z^2 is a convex function of Z with a positive minimum at some minimum point $Z_{\min} > 0$. Note that the convexity of ρ_Z can also be seen analytically from the following properties of $I(x;Z)$ for any x in $[0,1)$:

$$\begin{aligned} i)\quad I(x;0) &= (e-1)\sinh(\mu_0(1-x)) > 0 \qquad (0 \le x < 1) \\ ii)\quad \lim_{Z\to\infty}[I(x;Z)] &= -\sinh(\mu_0(1-x)) < 0 \qquad (0 \le x < 1). \end{aligned}$$

and, with $\mu_Z^2 \ge \mu_0^2 \gg 1$,

$$iii)\quad I(x;Z) \text{ monotone decreasing with increasing } Z.$$

As a consequence, we have the following negative result similar to the corresponding finding in [25]:

Proposition 8 *The robustness of a signaling gradient in a steady state of LRNO deteriorates with increasing Z for $Z > Z_{\min}$.*

For the illustrating example of Table I, the optimal ratio $Z_{\min}$ that gives the smallest ρ_Z is for $Z_{\min} \cong 1.092$ with $\rho_{\min} \cong 0.0670$ which is insignificantly below $\rho_Z = 0.0693...$ for $Z = 1$. However, both are significantly below $\rho_Z = 0.1365...$ for $Z = 2$ (even if the latter is still below robustness threshold). Beyond an optimal level of Z, continuing increase in non-receptor synthesis rate would reduce the aberrant gradient

concentration below the wild-type gradient $\bar{b}_1(x)$ and worsen the corresponding shape difference, eventually to an unacceptable level of robustness.

Superficially, this suggests that there is no need to consider Z values higher than $Z_{\min}$ for a given problem, with $Z_{\min} \simeq 1$ giving rise to an acceptable level of gradient shape distortion (as measured by our signal robustness index $\bar{R}_b$). However, $Z_{\min}$ may have to be larger for another problem for which there is a need for a larger amplitude reduction factor $1 + h_1 Z/h_0$. For example, Z would need to be 3 or larger for the same problem with the larger ligand synthesis amplification factor (aberrancy) $e = 4$ (see (72) or (73)). This suggests that the induced non-receptor synthesis should be an increasing function $Z(e)$ of the excess factor e. A larger Z value would induce a much more severe (and probably unacceptable) signaling gradient shape distortion as seen from (70) and (69). These observations strongly suggest the following conclusion consistent with the simulation results of [25]:

Conclusion 9 *A non-receptor synthesis rate in the form of (51) and (55) does not offer a biologically realistic mechanism for down-regulating aberrant signaling except for moderate aberrancy so that $Z_{\min} \simeq 1$. In the latter case, robustness is sensitive to any further increase in non-receptor concentration.*

Accurate numerical results for the exact solution (without the LRNO approximation) $\bar{b}_Z(x)$ and $\bar{R}_b$ have been obtained for different (uniform) non-receptor synthesis rates (as characterized by the parameter Z) with additional parameters associated with the non-receptors assigned the following values in the illustrating example (also examined in [45]):

$$j_{\deg} = k_{\deg}, \quad j_{on} = k_{on}, \quad j_{off} = 10k_{off}, \quad j_N = 10k_R$$

It is evident from the results reported in Table II that ρ_Z and $eB_Z(0) = B_e(0;Z) \simeq \bar{b}_e(0;Z)$ are quite accurate approximations of the corresponding numerical solutions for $\bar{R}_b(Z)$ and $\bar{b}_Z(0) = \bar{b}_e(0;Z)$ of the new model for (51). As such, the effects of non-receptors are pretty much delineated by the LRNO solution.

Table II

$(g_1 = 0.2, \quad h_1 = 10, \quad f_1 = 0.01, \quad g_n = 10, \quad v_L = 0.05, \quad e = 2)$

Z	$\bar{R}_b$	ρ_Z	$\bar{b}_2(0,Z)$	$eB_2(0;Z)$	$\bar{b}_1(0,0)$	$B_1(0,0)$
0	0.3943	0.3938	0.11517	0.1169	0.05790	0.0584
1	0.0714	0.0693	0.07398	0.0739	0.05790	0.0584
2	0.1298	0.1365	0.05597	0.0555	0.05790	0.0584

From either the computed LRNO solution or the numerical solutions for the original nonlinear BVP, we see that a moderate presence of non-receptors would generally bring $\bar{b}_Z(x) = \left[\bar{b}_e(x;c,Z)\right]_{c=0}$ closer to $\bar{b}_1(x)$ but generally renders $\bar{b}_Z(x)$ steeper and more convex than $\bar{b}_1(x)$ as shown in Figure 2. (Note that the notation $\bar{b}_e(x;0,Z,1,0)$

in Figure 2 corresponds to $\bar{b}_Z(x) = \bar{b}_e(x; Z)$.) The observations and conclusions above strongly suggest that the inhibiting mechanism (51) is not a realistic robustness promoting mechanism. It is examined here not only to elucidate the findings by numerical simulations in [25] but also, with the help of the explicit analytical solution, to eliminate it as a strategy for promoting signaling gradient robustness. This tentative conclusion will be further strengthened by the developments in the next two subsections.

Figure 2: Effects of Non-receptors

4.4 Addition of a Negative Feedback on Receptor Synthesis

The LRNO solution (70) shows that the distortion of the slope and convexity of aberrant signaling gradient by non-receptors is in the opposite direction relative to that induced by the feedback on signaling receptor synthesis rate given

$$\mu_Z^2 = \mu_0^2 \ + Z\mu_1^2 > \mu_0^2 \ > \frac{\mu_0^2}{1 + c\rho_c^m} = \mu_c^2.$$

It would seem that we may be able to take advantage of this observation through a two-inhibitor model with receptor and non-receptor synthesis rates of the form (27) and (51), respectively, to more effectively promote robust gradients. The LRNO solution for such a model, denoted by $\bar{a}_e(x; c, Z) \equiv \bar{a}_{RZ}(x) \simeq eA_{RZ}(x)$, is straightforward with

$$\begin{aligned} \bar{b}_e(x; c, Z) \ &\equiv \ \bar{b}_{RZ}(x) \sim eB_{RZ}(x) = \frac{e\bar{\kappa}^2(\rho_{RZ})}{\alpha_0} A_{RZ}(x) \\ &= \ \frac{e\bar{\nu}_L}{\alpha_0 \mu_{RZ}^2 \left(1 + c\rho_{RZ}^m\right)} \frac{\sinh(\mu_{RZ} x_m)}{\cosh(\mu_{RZ}(1 + x_m))} \sinh(\mu_{RZ}(1 - x)), \end{aligned} \tag{79}$$

for $0 \le x \le 1$, where ρ_{RZ} is the LRNO approximation for the robustness index $\bar{R}_b$ for the present model, and

$$\mu_{RZ}^2 = \frac{\mu_0^2}{1 + c\rho_{RZ}^m} + Z\mu_1^2 \simeq \frac{h_0}{1 + c\rho_{RZ}^m} + Zh_1 \tag{80}$$

It is evident from the expression for μ_{RZ}^2 that its numerical value for a given gradient system is not substantially different from $\mu_Z^2 \simeq h_0 + Zh_1$ for $c = O(1)$ since $\rho_{RZ} < \rho_Z = O\left(10^{-1}\right)$ for $Z = O(1)$. This observation is confirmed by accurate numerical solutions for the new model (with and without the LRNO approximation) reported in Table III for the same sample problem as in Table I and II. The results show that there is not an appreciable reduction in the robustness index with $c > 0$ for $Z = 1$. The minimal effect of the signaling gradient itself is illustrated in Figure 3 for $\bar{b}_e(x; c, Z)$. These observations are recorded as:

Conclusion 10 *In the presence of non-receptors (as an inhibiting agent for an aberrant signaling gradient) in the form of the synthesis rate (51) and (55), the (steady state limit of a) spatially uniform non-local negative feedback (27) on receptor synthesis rate reduces the signaling gradient concentration and ameliorates the distortion of signaling gradient shape but not appreciably and only for $Z \leq 1$. For $Z \geq 2$, the robustness index $\bar{R}_b \simeq \rho_Z$ actually deteriorates with the addition of the negative feedback on receptor synthesis rate.*

Similar to the case without the negative feedback on receptor synthesis, the results in Table III also show that too high a non-receptor-to-receptor synthesis rate ratio ($Z \geq 2$ in our example) would cause an excessive reduction of signal morphogen concentration and too severe a shape distortion to result in an unacceptable robustness index. For $Z \geq 2$ (needed for $e > 2$), the addition of feedback on receptor synthesis rate is actually deleterious to robust development for the illustrating example. The slight reduction of the shape distortion parameter μ_{RZ}^2 for a positive c, does not compensate for the considerably larger reduction of the amplitude reduction factor (that drives the amplitude of the signaling gradient at $x = 0$ to well below the concentration of the wild-type gradient at the same location). The corresponding graphs of $\bar{b}_{RZ}(x) = \bar{b}_e(x; c, Z)$ for different Z and c in Figure 3 clearly show why the robustness index $\bar{R}_b$ may eventually deteriorate with increasing Z.

To confirm, we have from

$$\rho_{RZ} = \frac{1}{B_1(0)} \sqrt{\int_0^1 [eB_{RZ}(x) - B_1(x)]^2 dx} \tag{81}$$

the equation for determining ρ_{RZ},

$$f_{RZ}(\rho_{RZ}) = 0, \tag{82}$$

where $f_{RZ}(\cdot)$ is $f_c(\cdot)$ as defined in (47) but with μ_c and e_c replaced by μ_{RZ} (with m taken to be 1 in (27) as before) and

$$e_{RZ} = \frac{e}{1 + (1 + c\rho_{RZ})\, h_1 Z / h_0} \frac{\sinh(\mu_{RZ} x_m) \cosh(\mu_0 (1 + x_m))}{\sinh(\mu_0 x_m) \cosh(\mu_{RZ}(1 + x_m))}, \tag{83}$$

respectively. Solutions for some typical ρ_{RZ} calculated from (82) are shown in Table III to confirm the observations made above and re-affirm Conclusion 9.

Table III

($g_0 = g_1 = 0.2$, $h_0 = h_1 = 10$, $g_r = 1$, $g_n = 10$; $f_0 = 0.001$, $f_1 = 0.01$, $v_L = 0.05$, $e = 2$)

Z	c	$\bar{R}_b$	ρ_{RZ}	$\bar{b}_{RZ}(0)$	$B_{RZ}(0)$	$\bar{b}_1(0)$
0	0	0.3943	0.3938	0.11517	0.1169	0.0579
0	1	0.3557	0.3565	0.10222	0.1062	0.0579
0	2	0.3269	0.3284	0.09407	0.0959	0.0579
1	0	0.0714	0.0693	0.07398	0.0739	0.0579
1	1	0.0619	0.0612	0.07550	0.0710	0.0579
1	2	0.0560	0.0566	0.06894	0.0688	0.0579
2	0	0.1298	0.1365	0.05597	0.0555	0.0579
2	1	0.1471	0.1547	0.05778	0.0497	0.0579
2	2	0.1683	0.1766	0.04463	0.0449	0.0579

Figure 3: Effects of Negative Feedback on Receptor Synthesis for $Z > 0$

4.5 Root-Mean-Square Displacement Differential

With μ_{RZ} and e_{RZ} both depending on ρ_{RZ}, the relation (82) is now a highly nonlinear equation for ρ_{RZ}. Still, the expression for the new shape distortion factor μ_{RZ} in (80) shows clearly that it is not possible for the negative feedback on receptor synthesis rate to reduce the shape distortion to an acceptable level if the non-receptor to receptor ratio Z should be much higher than 1. That this is not reflected in the computed values of ρ_{RZ} in Table III suggests that the signaling robustness index R_b is by itself not always an adequate measure of robustness. For this reason, we have also introduced in [25, 16] its companion robust index R_x (denoted by L in [25]) that measures the root-mean-square displacement differential of the ectopic signaling gradient.

Let $x_e(b)$ and $x_1(b)$ be the location where the aberrant and wild-type gradients attains the value b, respectively, i.e., $\bar{b}_c(x_e) = \bar{b}_1(x_1) = b$. In steady state, the *displacement robustness index* $\bar{R}_x$ is defined by

$$\bar{R}_x = \frac{1}{x_\ell - x_h}\sqrt{\frac{1}{b_h - b_\ell}\int_{b_\ell}^{b_h}[x_e(b) - x_1(b)]^2 db}. \tag{84}$$

To minimize the effects of outliers, we may limit the range of b to be the interval (b_ℓ, b_h) with $0 \le b_\ell < b_h \le \bar{b}(0)$. (We may take $b_\ell = \bar{b}(0)/10$ and $b_h = 9\bar{b}(0)/10$ for instance.)

For gradients in a steady state of LRNO, the dependence of displacement $\Delta x = x_e(b) - x_1(b)$ on any feedback for a non-negative range of x_e and x_1 is through the expression

$$x_e = 1 - \frac{1}{\mu_x}\sinh^{-1}\left(\frac{b}{\beta_x}\right), \qquad x_1 = 1 - \frac{1}{\mu_0}\sinh^{-1}\left(\frac{b}{\beta_0}\right)$$

where μ_x^2 is μ_{RZ}^2 with ρ_{RZ} replaced by ρ_x (the LRNO approximation for $\bar{R}_x$) and

$$\beta_x = \frac{e\bar{v}_L\kappa^2}{\alpha_0\mu_x^2}\frac{\sinh(\mu_x x_m)}{\cosh(\mu_x(1+x_m))}, \qquad \beta_0 = \frac{\bar{v}_L}{\alpha_0\mu_0^2}\frac{\sinh(\mu_0 x_m)}{\cosh(\mu_0(1+x_m))}.$$

The LRNO approximation of $\bar{R}_x$ is then

$$\bar{R}_x \simeq \rho_x = \frac{1}{x_\ell - x_h}\sqrt{\frac{1}{b_h - b_\ell}\int_{b_\ell}^{b_h}\left[\frac{1}{\mu_0}\sinh^{-1}\left(\frac{b}{\beta_0}\right) - \frac{1}{\mu_x}\sinh^{-1}\left(\frac{b}{\beta_x}\right)\right]^2 db}. \quad (85)$$

With $\mu_0^2 \gg 1$, we may, to a good first approximation, work with the asymptotic values of these expressions to get

$$\Delta x = c_0 + c_1 \ln(b) \quad (86)$$

where

$$c_0 = \frac{1}{\mu_{RZ}}\ln\left(\frac{\beta_{RZ}}{2}\right) - \frac{1}{\mu_0}\ln\left(\frac{\beta_0}{2}\right), \qquad c_1 = \left(\frac{1}{\mu_0} - \frac{1}{\mu_{RZ}}\right). \quad (87)$$

It follows that

$$\begin{aligned}\bar{R}_x \;&\simeq\; \rho_x \simeq \rho_0 = \frac{1}{x_\ell - x_h}\sqrt{\frac{1}{b_h - b_\ell}\int_{b_\ell}^{b_h}\left[c_0 + c_1\ln(b)\right]^2 db} \\ &= \frac{1}{x_\ell - x_h}\sqrt{\frac{1}{b_h - b_\ell}\left[c_0^2 b + 2c_0c_1 b\{\ln(b) - 1\} + c_1^2\left(b\{\ln(b)\}^2 - 2(b\{\ln(b) - 1\})\right]_{b_\ell}^{b_h}}\end{aligned}$$

For sample calculations, we take $b_h = b(0)$ and $b_\ell = b(1) \approx 0$ so that

$$\rho_0 = \sqrt{c_0^2 + 2c_0c_1\{\ln(b_h) - 1\} + c_1^2\left(\{\ln(b_h)\}^2 - 2\{\ln(b_h) - 1\}\right)} \quad (88)$$

For more moderate values of $h_0 = h_1$ (with $\mu_0 = \sqrt{h_0} = O(3)$), we would work with the exact inverse functions

$$x_e(b) = 1 - \frac{1}{\mu_x}\ln(q_x(b)), \qquad x_1(b) = 1 - \frac{1}{\mu_0}\ln(q_0(b)) \quad (89)$$

in (85) where

$$q_x(b) = \frac{b}{\beta_x} + \sqrt{\left(\frac{b}{\beta_x}\right)^2 + 1}$$

and $q_0(b)$ is $q_x(b)$ with β_x replaced by β_0. Table IV reports some typical results for the illustrating example of Tables I - III by the exact inverses (89):

Table IV

($g_0 = g_1 = 0.2$, $h_0 = h_1 = 10$, $g_r = 1$, $g_n = 10$, $f_0 = 0.001$, $f_1 = 0.01$, $v_L = 0.05$, $e = 2$)

Z	0	0	0	1	1	1	2	2	2
c	0	1	2	0	1	2	0	1	2
ρ_{RZ}	0.3938	0.3565	0.3284	0.0693	0.0612	0.0566	0.1365	0.1547	0.1766
ρ_x	0.1987	0.2095	0.2159	0.0576	0.0575	0.0581	0.1454	0.1579	0.1737

With robustness measured by the new robustness index, the results for the illustrative example computed from the LRNO solution in Table IV now show clearly how ρ_x deteriorates with increasing c even for typical values of Z around $Z_{\min}$ (while ρ_{RZ} deteriorates with increasing c only for $Z > Z_{\min}$). They further strengthen Conclusion 9 and complement it with the following (instead of Conclusion 10):

Conclusion 11 *In the presence of the non-receptor inhibiting agent of the form (51) and (55), the (steady state limit of a) negative spatially uniform non-local feedback (27) on receptor synthesis rate more readily exacerbates the aberrancy of the signaling gradient concentration when measured by the displacement robustness index ρ_x.*

Given the different (and sometimes opposite) ways how the negative feedback on receptor synthesis rate impacts the two robustness indices of an aberrant gradient for $Z \geq 0$, we should generally measure robustness by calculating both robustness indices before reaching any conclusion about the robustness of the signaling gradient.

5 Feedback on Non-receptor Synthesis Rates

5.1 The Model

Non-receptors as an inhibiting agent for down-regulating an aberrant gradient in the form (51) led to Conclusions 9 - 11 because the required synthesis rate has the consequence of increasing the shape distortion parameter severely for $Z > 1$ (which is needed for aberrancy factor $e > 2$). This would not be the case if the level of feedback depends on deviation from the wild-type gradient as measured by (one of the robustness indices. For this purpose, we consider a positive feedback on non-receptor synthesis rate V_N that also depends on the signaling robustness index of the form

$$V_N = \bar{V}_N \left[\eta + \sigma R_b^s(t-\tau)\right] H(t-t_e) \tag{90}$$

where the parameter σ and s characterize the strength and sensitivity of the feedback and the parameter τ pertains to a possible time delay on the effect of feedback (and $t_e > 0$ is again the instant of onset of the genetic or epigenetic perturbations). The model in the previous section corresponds to $\eta = 1$ and $\sigma = 0$ while the case $\eta = 0$ and $\sigma > 0$ offers a positive feedback for stimulating a non-receptor synthesis rate commensurate with the aberrancy measured by the robustness index R_b. The subsequent development of the theory for this new feedback process is similar if the signaling robustness index R_b is replaced by the corresponding displacement robustness index R_x as defined in (84).

Anticipating a limiting time-independent steady state of signaling gradient, we expect

$$V_N \to \bar{V}_N \left[\eta + \sigma \bar{R}_b^s\right] = \bar{V}_N \bar{\kappa}_N^2(\eta, \sigma) \qquad (s > 0) \tag{91}$$

as the system approaches a time independent steady state. To maximize the effect of $\bar{R}_b$ in the feedback (91), the parameter s will be set to 1 thereafter (similar to setting $m = 1$ in the feedback on receptor synthesis rate (27)). For that reason, the parameter s will not be displayed explicitly as a parameter of $\bar{\kappa}_N^2$ in (91) and elsewhere. Given Conclusion 9 and the fact that an instantaneously induced fixed synthesis rate is rather unrealistic, we limit our discussion for the $\eta = 0$ case. An aberrancy dependent *positive* feedback on non-receptor synthesis is also more consistent with experimental observations reported in references cited in the last section.

Upon modifying the model of Sec. 3 with the feedback on the receptor synthesis rate (27) to include the feedback (90), we reduce the new steady state problem to the following BVP for $\bar{a}_{RN}(x) = \bar{a}_e(x; c, Z, \eta, \sigma)$:

$$\bar{a}''_{RN} - \frac{\bar{\kappa}^2 g_0 \bar{a}_{RN}}{\alpha_0 + \zeta_0 \bar{a}_{RN}} - Z \frac{\bar{\kappa}_N^2 g_1 \bar{a}_{RN}}{\alpha_1 + \zeta_1 \bar{a}_{RN}} + e\bar{\nu}_L H(-x) = 0, \tag{92}$$

$$\bar{a}'_{RN}(-x_m) = 0, \qquad \bar{a}_{RN}(1) = 0. \tag{93}$$

Correspondingly, the various remaining gradients are given by

$$\bar{b}_{RN}(x) = \frac{\bar{\kappa}^2 \bar{a}_{RN}}{\alpha_0 + \zeta_0 \bar{a}_{RN}}, \qquad \bar{r}_{RN}(x) = \frac{\bar{\kappa}^2 \alpha_0}{\alpha_0 + \zeta_0 \bar{a}_{RN}} \tag{94}$$

$$\bar{c}_{RN}(x) = \frac{\bar{\kappa}_N^2 \bar{a}_{RN}}{\alpha_1 + \zeta_1 \bar{a}_{RN}}, \qquad \bar{n}_{RN}(x) = \frac{\bar{\kappa}_N^2 \alpha_1}{\alpha_1 + \zeta_1 \bar{a}_{RN}} \tag{95}$$

with $\bar{b}_{RN}(x) = \bar{b}_e(x; c, Z, \eta, \sigma)$, etc. For a particular wing imaginal disc, it is clear from the model that the robustness of its development depends on the strength of the feedback on receptor synthesis rate characterized by the parameter c and the strength of the non-receptor synthesis rate characterized by the parameters η and σ.

5.2 Low Receptor and Non-receptor Occupancy

When both receptors and non-receptors are in a state of low occupancy, we may linearize the ODE (92) to get for $\bar{a}_{RN}(x) \simeq eA_{RN}(x)$

$$A''_{RN} - \mu_{RN}^2 A_{RN} + \bar{\nu}_L H(-x) = 0, \tag{96}$$

subject to the two end conditions (93) with

$$\begin{aligned} \mu_{RN}^2 &= \mu_R^2 + Z\mu_N^2 = \bar{\kappa}^2(\rho_{RN})\mu_0^2 + Z\bar{\kappa}_N^2(\rho_{RN})\mu_1^2 \\ &\simeq \frac{h_0}{1 + c\rho_{RN}} + Zh_1\left(\eta + \sigma\rho_{RN}\right), \end{aligned} \tag{97}$$

where ρ_{RN} is the LRNO approximation of $\bar{R}_b$ for the present model and we have made the highly accurate approximations $\mu_0^2 = h_0$ and $\mu_1^2 = h_1$ consistent with the simplification made in the earlier sections and in [22].

The exact solution for $A_{RN}(x) \equiv A_e(x; c, Z, \eta, \sigma)$ is given by

$$A_{RN}(x) = \begin{cases} \frac{\bar{v}_L}{\mu_{RN}^2}\{1 - \frac{\cosh(\mu_{RN})}{\cosh(\mu_{RN}(1+x_m))}\cosh(\mu_{RN}(x+x_m))\} & (-x_m \le x \le 0) \\ \frac{\bar{v}_L}{\mu_{RN}^2}\frac{\sinh(\mu_{RN}x_m)}{\cosh(\mu_{RN}(1+x_m))}\sinh(\mu_{RN}(1-x)) & (0 \le x \le 1) \end{cases} \tag{98}$$

In the range $0 \le x \le 1$, we have

$$\begin{aligned} \bar{b}_{RN}(x) &\simeq eB_{RN}(x) = \frac{e\bar{\kappa}^2(\rho_{RN})}{\alpha_0}A_{RN}(x) \\ &= \frac{e\bar{\kappa}^2(\rho_{RN})\bar{v}_L}{\alpha_0\mu_{RN}^2}\frac{\sinh(\mu_{RN}x_m)}{\cosh(\mu_{RN}(1+x_m))}\sinh(\mu_{RN}(1-x)) \end{aligned} \tag{99}$$

where μ_{RN}^2 is as given in (97). From the expression

$$\begin{aligned} \alpha_0\bar{b}_{RN}(0) &\simeq e\bar{\kappa}^2(\rho_{RN})A_{RN}(0) \\ &= \frac{e\bar{v}_L}{\mu_{RN}^2}\frac{\bar{\kappa}^2(\rho_{RN})\sinh(\mu_{RN}x_m)}{\cosh(\mu_{RN}(1+x_m)))}\sinh(\mu_{RN}). \end{aligned}$$

we obtain

$$\begin{aligned} \bar{b}_{RN}(0) &\simeq eB_{RN}(0) \\ &\simeq \frac{e\bar{v}_L/g_0\,(1-e^{-2\mu_{RN}x_m})}{1+Z\,(1+c\rho_{RN})\,(\eta+\sigma\rho_{RN})\,(h_1/h_0)} \end{aligned} \tag{100}$$

given $f_k \ll g_k$ for both $k = 0$ and 1. Correspondingly, we have

$$\frac{\bar{b}_{RN}(0)}{\bar{b}_1(0)} \simeq \frac{eB_{RN}(0)}{B_1(0)} \sim \frac{e(1-e^{-2\mu_{RN}x_m})/(1-e^{-2\mu_0 x_m})}{1+Z\,(1+c\rho_{RN})\,(\eta+\sigma\rho_{RN})\,(h_1/h_0)}O(1) \tag{101}$$

It is evident from (100)-(101) that, in the presence of non-receptors, a spatially uniform non-local positive feedback of the type (90) with $\eta > 0$ or $\sigma > 0$ (with $Z = 1$) reduces the magnitude of the signaling gradient (toward the wild type gradient) but also induces a shape change relative to that without any feedback. While this effect on the gradient shape counters that of the negative feedback on receptor synthesis, it may over-compensate the latter if the magnitude of η should be too large relative to what is needed to restore the signaling gradient to the corresponding wild-type gradient. With $\eta = 0$, the non-receptor synthesis rate needed to achieve robustness can be adjusted by the level of aberrancy of the signaling gradient through the feedback strength parameter σ, hence more likely to be successful in keeping the aberrant signaling gradient close to the wild-type gradient prior to the exogenous perturbations.

The net results of the different effects due to a positive feedback on non-receptor synthesis rate with $\eta = 0$ and $\sigma > 0$ is reflected in the LRNO robustness index

$$\rho_{RN} = \frac{1}{B_1(0)}\sqrt{\int_0^1 [eB_{RN}(x) - B_1(x)]^2 dx} \tag{102}$$

or

$$f_{RN}(\rho_{RN}) = 0, \tag{103}$$

where $f_{RN}(\cdot)$ is $f_c(\cdot)$ as defined in (47) but with μ_c and e_c replaced by μ_{RN} (with m and s both taken to be 1) and

$$e_{RN} = \frac{e}{1 + (1 + c\rho_{RN})(\eta + \sigma\rho_{RN}) h_1 Z/h_0} \frac{\sinh(\mu_{RN} x_m)\cosh(\mu_0(1+x_m))}{\sinh(\mu_0 x_m)\cosh(\mu_{RN}(1+x_m))}, \tag{104}$$

respectively. With μ_{RN} and e_{RN} both depending on ρ_{RN}, the relation (103) is now a highly nonlinear equation for ρ_{RN}.

5.3 Robustness Dependent Positive Feedback on Non-receptor Synthesis

Given Conclusion 9, we are interested here in a positive feedback on non-receptor synthesis that is robustness index dependent. The LRNO results for such a feedback alone is obtained from those of the previous section by setting $\eta = 0$ and $c = 0$. The signaling morphogen concentration $\left[\bar{b}_{RN}(0)\right]_{c=\eta=0}$, denoted by $\bar{b}_N(x) \simeq eB_N(x)$, is given by

$$\begin{aligned} \bar{b}_N(x) &\simeq eB_N(x) = \frac{e}{\alpha_0} A_N(x) \qquad (x \geq 0) \\ &= \frac{e\bar{\nu}_L/g_0}{1 + Z\sigma\rho_N h_1/h_0} \frac{\sinh(\mu_N x_m)}{\cosh(\mu_N(1+x_m))} \sinh(\mu_N(1-x)) \end{aligned} \tag{105}$$

where $\rho_N = [\rho_{RN}]_{c=\eta=0}$ is the LRNO approximation of the robustness index and

$$\mu_N^2 = \mu_0^2 + Z\mu_N^2 = h_0 + Zh_1\sigma\rho_N. \tag{106}$$

The signaling ligand concentration at $x = 0$ is

$$\bar{b}_N(0) \simeq eB_N(0) = \frac{e\bar{\nu}_L}{g_0} \frac{\lambda_N}{1 + Z\sigma\rho_N h_1/h_0}$$

where

$$\lambda_N = \frac{\sinh(\mu_N x_m)\sinh(\mu_N)}{\cosh(\mu_N(1+x_m))}.$$

For the example used throughout this paper, we have $h_1/h_0 = 1$ and therewith

$$\mu_N^2 = h_0(1 + Z\sigma\rho_N). \tag{107}$$

For $h_0 \gg 1$ (but $\mu_0 = O(1)$ as in our illustrative example)

$$\frac{\bar{b}_N(0)}{\bar{b}_1(0)} \simeq \frac{eB_N(0)}{B_1(0)} \simeq \frac{e\,(1 - e^{-2\mu_N x_m})}{(1 + Z\sigma\rho_N)\,(1 - e^{-2\mu_0 x_m})}. \tag{108}$$

The LRNO approximation of the relative magnitude $\bar{b}_N(0)/\bar{b}_1(0)$ depends on the robustness index through the shape distortion parameter μ_N and the amplitude reduction factor $1 + Z\sigma\rho_N h_1/h_0$. The LRNO approximation ρ_N of the (signaling) robustness index is determined by

$$f_N(\rho_N) = 0, \tag{109}$$

where $f_N(\cdot)$ is $f_c(\cdot)$ as defined in (47) but with μ_c and e_c replaced by μ_N (with m and s both taken to be 1) and

$$\begin{aligned} e_N &= \frac{e}{1 + Z\sigma\rho_N} \frac{\sinh(\mu_N x_m)\cosh(\mu_0(1 + x_m))}{\sinh(\mu_0 x_m)\cosh(\mu_N(1 + x_m))} \\ &\simeq \frac{e}{1 + Z\sigma\rho_N} \frac{1 - e^{-2\mu_N x_m}}{1 - e^{-2\mu_0 x_m}} \qquad (\mu_N \geq \mu_0 \gg 1), \end{aligned} \tag{110}$$

respectively (having specialized to the case $h_1 = h_0$).

The following observations are immediate from (110):

- The amplitude reduction factor $1 + Z\sigma\rho_N$ is considerably smaller than the corresponding factor for the model with the non-receptor synthesis rate (51) when $Z\sigma$ is $O(Z)$ for the latter model (or $O(Z\eta)$ in the feedback (91)) since ρ_N is expected to be considerably less than unity.

- The shape distortion parameter μ_N is considerably smaller than the corresponding parameter μ_Z so that the shape of $\bar{b}_N(x) \simeq eB_N(x)$ is less steep and less convex than $\bar{b}_Z(x) \simeq eB_Z(x)$.

By the first observation, the reduction of the signaling differential robustness index $\bar{R}_b \simeq \rho_N$ for $Z\eta = 0$ (and $Z\sigma = 1$) is expected to be considerably more modest than the corresponding reduction of $\bar{R}_b \simeq \rho_Z$ for $Z\eta = 1$ (and $Z\sigma = 0$). In particular, it barely meets the conservative threshold of $\rho_N \leq 0.2$ with $\rho_N = 0.1972$ for $Z\sigma = 2$ for our illustrative example. This is understandable given the amplitude reduction factor is now $(1 + Z\sigma\rho_{RN})^{-1}$ (for $\eta = 0$) instead of $(1 + Z)^{-1}$ (for $\eta = 1$ and $\sigma = 0$) with $e/(1 + Z) = 1$ for $e = 2$ and $Z = 1$. We need $\sigma\rho_N = O(1)$, i.e., $\sigma = O(5)$, for the reduction to be comparable to the $\eta = 1$ (and $\sigma = 0$) case. On the other hand, the shape distortion (for $\eta = 0$ and $\sigma = 1$) is now less severe with $\mu_N^2 = h_0\,(1 + Z\sigma\rho_N h_1/h_0) < h_0\,(1 + Zh_1/h_0)$ since $\rho_N < 1$ for some degree of robustness. The actual benefit (or cost) for a $\eta = 0$ and $\sigma = 1$ feedback on non-receptor synthesis rest on the net effect from the two opposite bulleted impacts above on the two robustness indices.

While this net effect may be case specific, it should be evident from the expression (107) that the impact of any increase in the synthesis rate ratio $Z\sigma$ is much less than full (as it would be for the synthesis rate (51)) as only a fraction ρ_N of $Z\sigma$ is felt by the gradient system. Moreover, that fraction would be further reduced by the impact

of the increase in $Z\sigma$ on reducing the robustness index. In other words, the impact of any increase in $Z\sigma$ is doubly palliated (as long as the robustness index is less than unity), thereby reducing its effect on the shape distortion parameter μ_N. In this sense, non-receptors as an inhibiting agent introduced through the feedback (91) is stable, at least more so than through (51). This enables us to assert the following conclusion:

Conclusion 12 *The feedback (91) with $\eta = 0$ is more stable and biologically realistic than $\eta > 0$ (and $\sigma = 0$) for down-regulating the aberrant signaling gradient.*

Remark 13 *For $\sigma > 0$, the addition of $\eta > 0$ would further decrease the amplitude reduction factor to $(1 + Z(\eta + \sigma\rho_{RN}))^{-1}$. However, the gain is offset by a more severe shape distortion resulting from $\mu_{RN}^2 = h_0 \{1 + Z(\eta + \sigma\rho_{RN})\} > h_0 \{1 + Z\sigma\rho_{RN}\}$.*

5.4 An Effective Multi-Feedback Instrument for Robust Development

With $\mu_Z > \mu_N \geq \mu_0 > 1 \geq \mu_c$, the shape distortion induced by the feedback (91) with $\eta = 0$ is opposite to that by the negative feedback (33) on the receptor synthesis rate. Acting alone, the latter feedback has no impact on the amplitude reduction factor. Administering concurrently with the positive feedback on non-receptor, the two mitigating effects on shape distortion should help to lower the robustness index of the aberrant signaling gradient due to each feedback acting alone. To take advantage of this observation, we specialize the general results of Subsection 5.2 to the case $\eta = 0$ so that the positive feedback on non-receptor is robustness dependent. The signaling morphogen concentration $\left[\bar{b}_{RN}(0)\right]_{\eta=0}$, to be denoted by $\bar{b}_{RS}(x) \simeq eB_{RS}(x)$, is given by

$$\begin{aligned} \bar{b}_{RS}(x) &\simeq eB_{RS}(x) = \frac{e\bar{\kappa}^2(\rho_{RS})}{\alpha_0} A_{RS}(x) \qquad (x \geq 0) \qquad (111) \\ &= \frac{e\bar{\nu}_L/g_0}{1 + Z\sigma\rho_{RS}\,(1 + c\rho_{RS})\,h_1/h_0} \frac{\sinh(\mu_{RS}x_m)}{\cosh(\mu_{RS}(1 + x_m))} \sinh(\mu_{RS}(1 - x)) \end{aligned}$$

where $\rho_{RS} = [\rho_{RN}]_{\eta=0}$ is the LRNO approximation of the robustness index and

$$\mu_{RS}^2 = \mu_c^2 + Z\mu_S^2 = \frac{h_0}{1 + c\rho_{RS}} + Zh_1\sigma\rho_{RS} \qquad (112)$$

5.4.1 The LRNO signaling morphogen concentration $eB_{RS}(0)$

The LRNO signaling morphogen concentration at $x = 0$ is then given by

$$eB_{RS}(0) = \frac{e\bar{\nu}_L/g_0}{1 + Z\sigma\rho_{RS}\,(1 + c\rho_{RS})\,h_1/h_0}\lambda_{RS} \qquad (113)$$

where

$$\lambda_{RS} = \frac{\sinh(\mu_{RS} x_m)\sinh(\mu_{RS})}{\cosh(\mu_{RS}(1+x_m))}. \tag{114}$$

For the example used throughout this paper, we have $h_1/h_0 = 1$ and therewith

$$\left[\mu_{RN}^2\right]_{\eta=0} = h_0\left(\frac{1}{1+c\rho_{RS}} + Z\sigma\rho_{RS}\frac{h_1}{h_0}\right) \equiv \mu_{RS}^2 \tag{115}$$

and

$$\frac{\bar{b}_{RS}(0)}{\bar{b}_1(0)} \sim \frac{e\left(1-e^{-2\mu_{RS}x_m}\right)}{\left(1+Z\sigma\rho_{RS}\left(1+c\rho_{RS}\right)h_1/h_0\right)\left(1-e^{-2\mu_0 x_m}\right)}. \tag{116}$$

It is evident from (116) that the amplitude reduction factor is now larger than that for $c = 0$ so that it has the effect of reducing $\bar{b}_N(0) < \bar{b}_e(0)$. At the same time, we have $\mu_{RS}^2 < \mu_N^2$ which reduces the shape distortion caused by $Z\sigma > 0$. Together, they enable us to conclude:

Conclusion 14 *Concurrent applications of the positive feedback on non-receptor synthesis (91) with $\eta = 0$ and a (moderate strength) negative feedback on receptor synthesis rate $\bar{\kappa}^2(\bar{R}_b)\bar{v}_R$ promote more effectively robustness of a signaling gradient than either feedback acting alone.*

For a sufficiently large value of $c > 0$, μ_{RS}^2 may be reduced to nearly μ_0^2 so that the resulting aberrant gradient shape would approach that of the wild type. With $\mu_{RS}^2 \cong \mu_0^2$ for c in the range that renders

$$\left(1+c\rho_{RS}\right)\left(1-Z\sigma\rho_{RS}\frac{h_1}{h_0}\right) \cong 1, \tag{117}$$

we have

$$\begin{aligned} eB_{RS}(0) &\simeq \frac{e\bar{\nu}_L}{g_0}\left(1-Z\sigma\rho_{RS}\frac{h_1}{h_0}\right)\frac{\sinh(\mu_0 x_m)\sinh(\mu_0)}{\cosh(\mu_0(1+x_m))} \\ &= \frac{e\bar{\nu}_L\lambda_{RS}}{g_0\left(1+c\rho_{RS}\right)}\frac{\sinh(\mu_0 x_m)\sinh(\mu_0)}{\cosh(\mu_0(1+x_m))}. \end{aligned} \tag{118}$$

Whether the resulting aberrant signaling gradient is sufficiently close to the wild type can only be read from the corresponding two robustness indices.

5.4.2 The LRNO Robustness Index

The LRNO approximation of the *differential signaling robustness index* ρ_{RS} is determined by

$$f_{RS}(\rho_{RS}) = 0, \tag{119}$$

where $f_{RS}(\cdot)$ is $f_c(\cdot)$ as defined in (47) but with μ_c and e_c replaced by μ_{RS} (with m and s both taken to be 1) and

$$e_{RS} = \frac{e}{1+Z\sigma\rho_{RS}\,(1+c\rho_{RS})}\frac{\sinh(\mu_{RS}x_m)\cosh(\mu_0(1+x_m))}{\sinh(\mu_0 x_m)\cosh(\mu_{RS}(1+x_m))} \tag{120}$$

$$\simeq \frac{e}{1+Z\sigma\rho_{RS}\,(1+c\rho_{RS})}\frac{1-e^{-2\mu_{RS}x_m}}{1-e^{-2\mu_0 x_m}}, \tag{121}$$

respectively (having specialized to the case $h_1 = h_0$). The relations (119) and (120) also apply to the corresponding *differential displacement robustness index* ρ_x if we replace ρ_{RS} in $\bar{\kappa}^2$, $\bar{\kappa}_N^2$, μ_{RS}^2, e_{RS} and f_{RS} by ρ_x.

One effect from the addition of a negative feedback on signaling receptor synthesis rate is to increase the amplitude reduction factor to $1+Z\sigma\rho_{RS}\,(1+c\rho_{RS})\,h_1/h_0$ and thereby decreases the "amplitude" of $eB_{RS}(x)$ (see (113). Generally, this effect should reduce ρ_{RS}. However, it is not difficult to see that

Lemma 15 *ρ_{RS} does not tend to 0 as $c \to \infty$.*

Proof. Assuming the opposite so that $\rho_{RS} \to 0$ as $c \to \infty$, there are three possibilities pertaining to the magnitude of $c\rho_{RS}$:

i) $c\rho_{RS} \to 0$ with

$$eB_{RS}(x) \to eB_1(x).$$

It follows that $\rho_{RS} \to \rho_0 > 0$ as given in (26) contradicting the assertion to the contrary.

ii) There exists a positive ϖ such that $\rho_{RS} \to \varpi > 0$ and

$$eB_{RS}(x) = \frac{e\bar{v}_L}{g_0}\frac{\sinh(\mu_{RS}x_m)}{\cosh(\mu_{RS}(1+x_m))}\sinh(\mu_{RS}(1-x))$$

with

$$\mu_{RS}^2 = \frac{\mu_0^2}{1+\varpi} < \mu_0^2.$$

It follows that $eB_{RS}(x)$ is generally not $B_1(x)$ and $\rho_{RS} > 0$ as $c \to \infty$, contradicting the assertion to the contrary.

iii) $c\rho_{RS} \to \infty$ but $c\rho_{RS}^2 \to \varpi$, then we have $1+Z\sigma\rho_{RS}\,(1+c\rho_{RS})\,h_1/h_0 \to 1+Z\sigma\varpi h_1/h > 1$ and $\mu_{RS}^2 \to 0 < \mu_0^2$ so that ρ_{RS} must again be positive in the limit as $c \to \infty$ and we again arrive at a contradiction. ■

As a consequence of the lemma above, we have the following observations on the robustness index:

- For a fixed value of $Z\sigma$ (and $\eta = 0$), ρ_{RS} must eventually reach a minimum and begin to increase with further increase in c tending to a finite limit ρ_∞. Since the limit ρ_∞ depends on the value of $Z\sigma$, the specific two-feedback mechanism is said to be *stable* (but not asymptotically stable).

- For any moderate value of $Z\sigma$ (and $\eta = 0$) to result in a corresponding ρ_N, the following inequalities on the shape distortion parameter and the amplitude reduction factor hold

$$\begin{aligned} i) \quad & \mu_0^2 \left(\frac{1}{1 + c\rho_N} + Z\sigma\rho_N \right) < \mu_0^2 \left(1 + Z\sigma\rho_N\right) = \mu_N^2, \\ ii) \quad & 1 + Z\sigma\rho_N \left(1 + c\rho_N\right) \frac{h_1}{h_0} > 1 + Z\sigma\rho_N h_1/h_0 \ . \end{aligned}$$

  Hence, the addition of a moderate strength negative feedback on receptor synthesis rate should bring the aberrant signaling gradient closer to the wild type so that $\rho_{RS} < \rho_N$.

- For a fixed moderate value of $Z\sigma$ so that $Z\sigma\rho_N < 1$, a sufficiently large value of c, say c_1, would render

$$\frac{1}{1 + c\rho_N} + Z\sigma\rho_N \approx 1,$$

  and keep the gradient shape close to the wild-type gradient shape. Increasing c well beyond c_1 would reduce the shape parameter of the modified gradient well below unity, thereby would distort the gradient unduly in the opposite direction and work against robustness.

These results provide a specific realization of how an appropriate two-feedback combination of receptor and non-receptor synthesis rates may enhance both aberrant signaling ligand concentration reduction and shape-change amelioration:

Conclusion 16 *A multi-feedback instrument of the type (27) and (91) with $\eta = 0$ is both effective and stable for down-regulating the aberrant signaling gradient without distorting unacceptably the slope and convexity of the wild-type signaling gradient.*

The development above applies also to the displacement differential robustness index ρ_x. As such the same qualitative conclusion may also be said about both indices. And both should be examined for robust signaling since they measure different features of the signaling gradient. Table V gives the two indices for the illustrative example for a range of c and $Z\sigma$ showing the benefits of an appropriate combination of the multi-feedback instrument.

Table V
$g_0 = g_1 = 0.2, \quad h_0 = h_1 = 10, \quad g_r = 1, \quad g_n = 10$
$f_0 = 0.001, \quad f_1 = 0.01, \quad v_L = 0.05, \ e = 2, \ \eta = 0$

$c\backslash Z\sigma$	0	1	2	4	
0	0.3938	0.2539	0.1972	0.1421	ρ_{RZ}
	0.1987	0.1514	0.1249	0.0949	ρ_x
1	0.3565	0.2263	0.1769	0.1289	ρ_{RZ}
	0.2095	0.1526	0.1239	0.0930	ρ_x
2	0.3284	0.2073	0.1626	0.1194	ρ_{RZ}
	0.2159	0.1521	0.1223	0.0911	ρ_x
4	0.2872	0.1815	0.1431	0.1059	ρ_{RZ}
	0.2187	0.1483	0.1180	0.0872	ρ_x

Remark 17 *Similar to the $c = 0$ case, the addition of $\eta > 0$ to the present two-feedback system (of $c > 0$ and $\sigma > 0$) would be a two edge sword: a further reduction of the amplitude reduction factor on the one hand, and an increase of the shape distortion factor μ_{RN}^2 the other hand. The former does not always promote robustness as it may down-regulate the signaling gradient to a level substantially below the wild-type gradient.*

Figure 4: Positive Feedback on Non-receptor Synthesis Only ($c = \eta = 0$)

Figure 5: Feedback on Receptor and Non-receptor Synthesis ($c > 0,\ \eta = 0$)

6 Concluding Remarks

When an abnormal genetic or epigenetic perturbation interrupts an ongoing biological development, one or more agents that counteract the unwelcome effects of the induced signaling distortion need to be activated by the onset of abnormal development to down-regulate the aberrancy. This means the existence of some kind of feedback process in order to promote robust signaling. Feedback has long been seen as a mechanism for attaining robust biological development and specific feedback loops have been identified in the morphogen literature such as [13, 23, 29, 19, 24] and others. Though the conventional Hill function type (theoretical) negative feedback on receptor synthesis rate proves to be ineffective for this purpose [17, 26, 25], we have shown in [22] that a multi-feedback strategies involving direct (robustness index induced) reduction of morphogen synthesis rates are capable of promoting robust signaling. To the extent that feedback mechanisms may not down-regulate morphogen synthesis rate directly, we need to determine a more realistic multi-feedback mechanism for robust signaling.

Among the possible mechanisms for achieving robust signaling that are biologically meaningful and realistic, the potential of non-receptors down-regulating signaling (and hence promoting robust development) has already been established theoretically in [17, 18]. While numerical simulations in [25] show that Hill function type feedback involving non-receptors often results in gradient systems that are either still

unacceptably aberrant or biologically unrealistic, we show in this paper that a biologically realistic multi-feedback strategy involving a positive feedback on non-receptors and another known feedback process (such as a negative feedback on receptor synthesis rate) exists and is effective in promoting signaling gradient robustness. The result also suggests that other combinations of known feedback processes should be explored.

Understanding how robust signaling can be attained by multi-feedback mechanisms is important not only to shed light on the reliability of developing signaling gradient systems, but also to help explain the ubiquitous presence of the many elaborate regulatory schemes in morphogen systems.

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(University of California, Irvine)

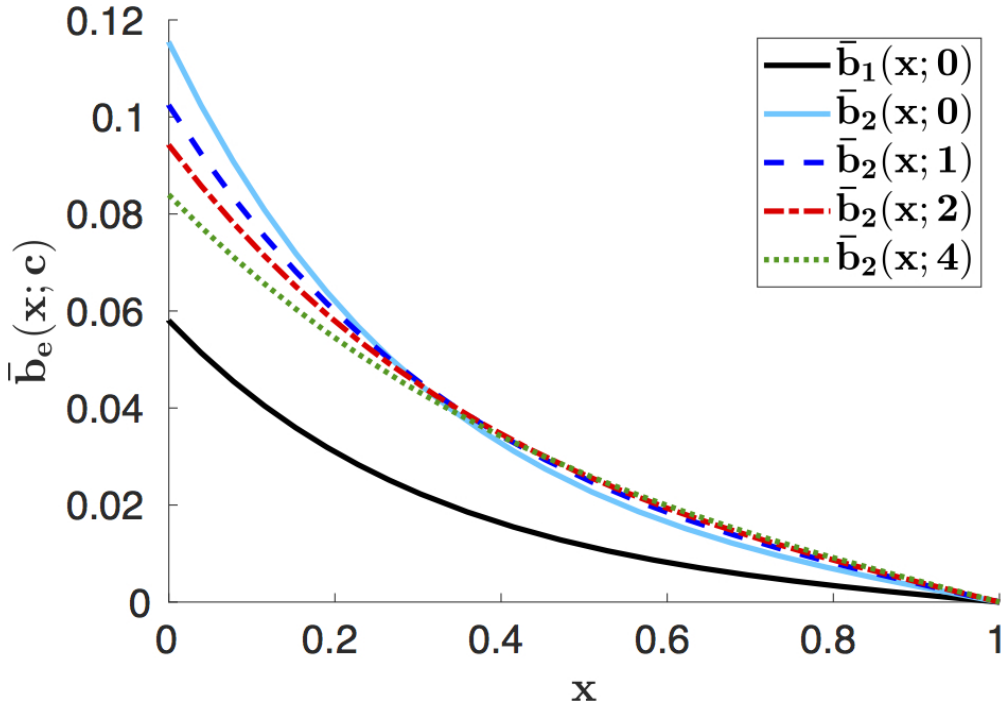

Figure 1: Spatially uniform negative feedback on receptor synthesis rate.

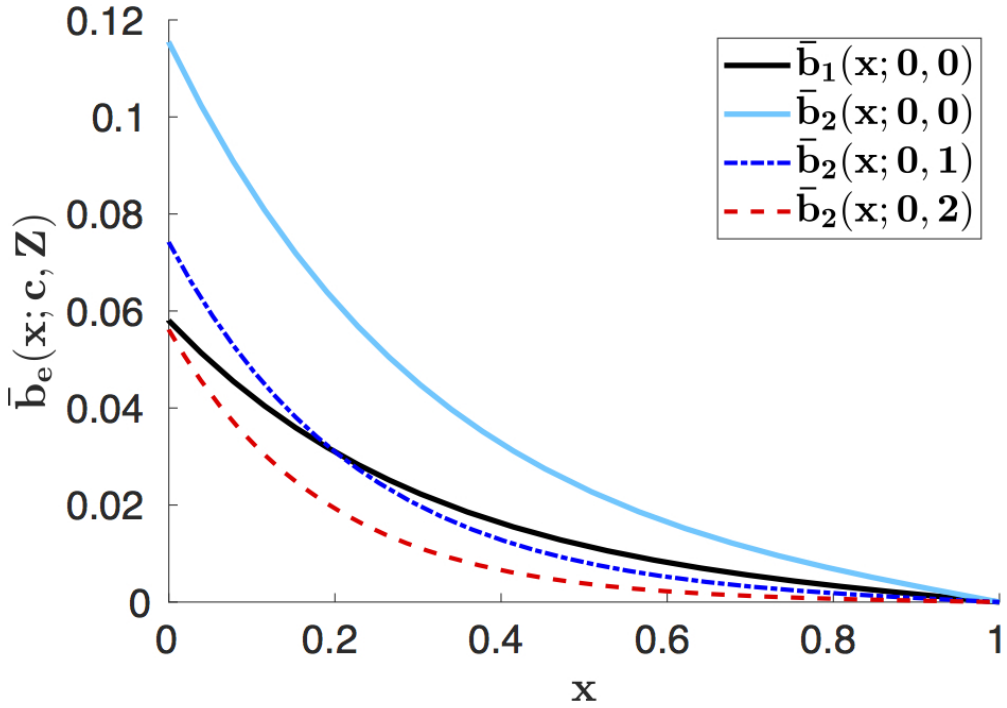

Figure 2: Effects of non-receptors

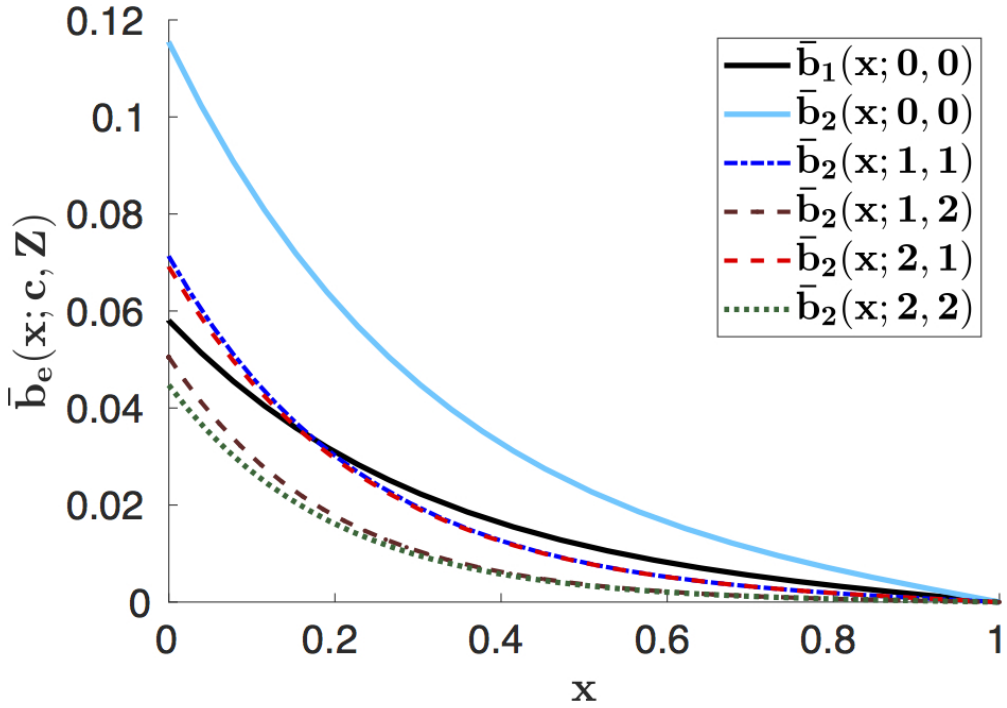

Figure 3: Effects of negative feedback on receptor synthesis rate for Z > 0.

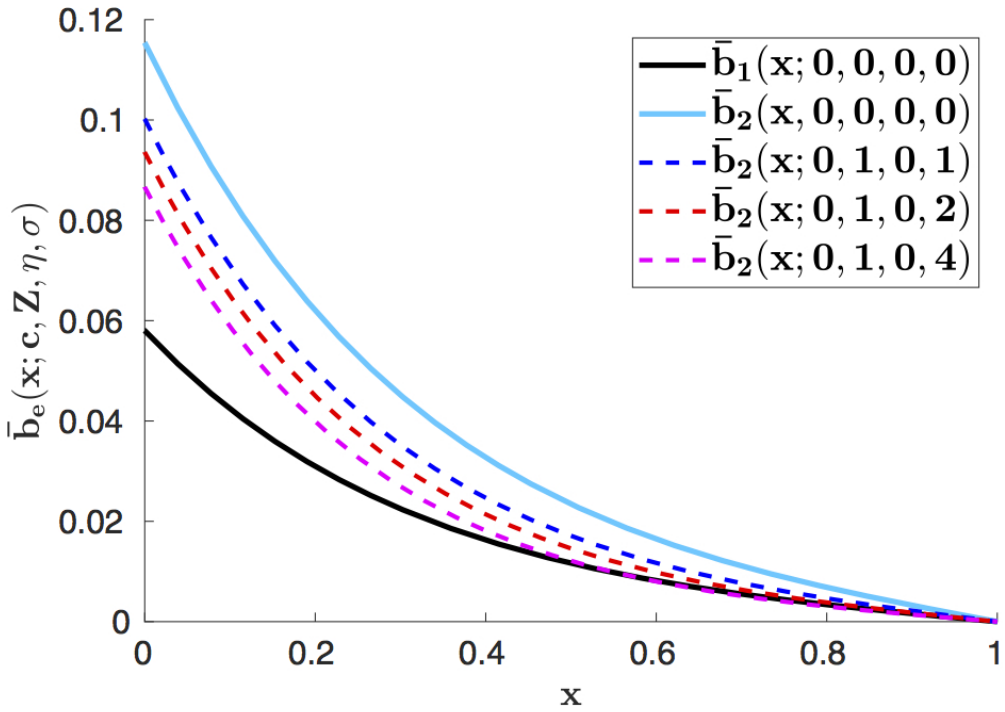

Figure 4: Positive feedback on non-receptor synthesis rate only (c = η = 0).

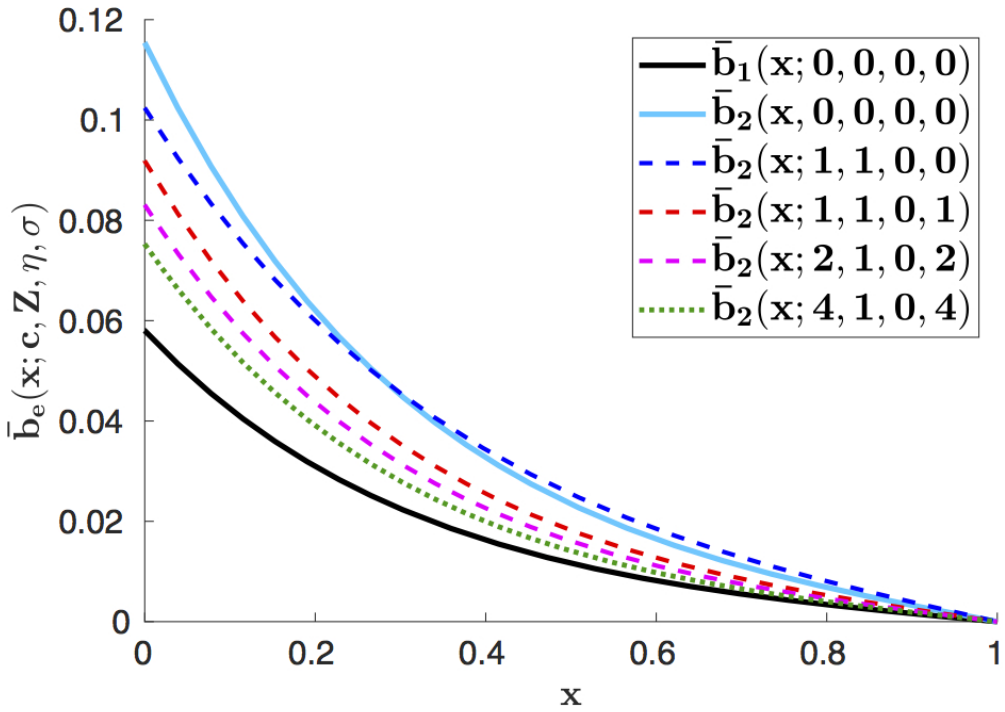

Figure 5: Feedback on receptor and non-receptor synthesis rates (c > 0, η = 0).