Long Signaling Cascades Tend to Attenuate Retroactivity

Hamid R. Ossareh
Department of Electrical Engineering and Computer Science
University of Michigan, Ann Arbor, MI

Alejandra C. Ventura
Department of Biology
University of Buenos Aires, Buenos Aires, Argentina
Department of Internal Medicine
University of Michigan, Ann Arbor, MI

Sofia D. Merajver
Department of Internal Medicine
University of Michigan, Ann Arbor, MI

Domitilla Del Vecchio
Department of Mechanical Engineering
MIT, Cambridge, MA[1]

[1]Address: Department of Mechanical Engineering, MIT, Cambridge, MA 02139, ddv@mit.edu

1 Supplementary Information

1.1 Proof of $sign(w_i^*) = -sign(w_{i+1}^*)$ for all $i \in \{1, ..., n-1\}$

Substituting x_{i+1} from equation (8) into equation (9), we obtain that

$$w_i^* = T_i(\overline{W}_i w_{i-1}^* - \overline{W}_{i-1}^* \widetilde{E}_{i+1} \frac{\overline{k}_{i+1}}{k_{i+1}} w_{i+1}^*), \text{ for } i \in \{1, ..., n-1\}. \quad \text{(SI-1)}$$

From the first of equations (3), setting the time derivatives to zero we obtain that $0 = -\delta w_0^*$. Thus, from equation (SI-1) with $i = 1$ we obtain that

$$w_1^* = -T_1 \overline{W}_0^* \frac{\overline{k}_2 \widetilde{E}_2}{k_2} w_2^*. \quad \text{(SI-2)}$$

Base case: We prove that the property holds for $i = 1$. Since $T_1 \overline{W}_0^* \frac{\overline{k}_2 \widetilde{E}_2}{k_2} \geq 0$, equation (SI-2) proves the base case.

Induction Step: Assume that $sign(w_{i-1}^*) = -sign(w_i^*)$. We prove that $sign(w_i^*) = -sign(w_{i+1}^*)$. Employing equation (SI-1), we have that

$$w_i^* = T_i \overline{W}_i w_{i-1}^* - T_i \overline{W}_{i-1}^* \frac{\overline{k}_{i+1} \widetilde{E}_{(i+1)}}{k_{i+1}} w_{i+1}^*.$$

To simplify notation, let $G_1 \triangleq T_i \overline{W}_i$ and $G_2 \triangleq T_i \overline{W}_{i-1}^* \frac{\overline{k}_{i+1} \widetilde{E}_{(i+1)}}{k_{i+1}}$. Then, we have that $w_i^* = G_1 w_{i-1}^* - G_2 w_{i+1}^*$ from which we obtain that

$$w_{i+1}^* = \frac{G_1}{G_2} w_{i-1}^* - \frac{1}{G_2} w_i^*. \quad \text{(SI-3)}$$

In order to proceed, we consider two cases: $w_{i-1}^* \geq 0$ and $w_{i-1}^* \leq 0$ and employ the fact that $G_1 \geq 0$ and $G_2 \geq 0$. (Case 1) If $w_{i-1}^* \geq 0$, then by the induction assumption we have that $w_i^* \leq 0$; hence by (SI-3) we obtain that

$$w_{i+1}^* = \frac{G_1}{G_2} |w_{i-1}^*| + \frac{1}{G_2} |w_i^*| \geq 0.$$

(Case 2) If $w_{i-1}^* \leq 0$, then by the induction assumption we have that $w_i^* \geq 0$; hence by (SI-3) we obtain that

$$w_{i+1}^* = -\frac{G_1}{G_2} |w_{i-1}^*| - \frac{1}{G_2} |w_i^*| \leq 0.$$

This proves that $sign(w_i^*) = -sign(w_{i+1}^*)$ for all $i \in \{1, ..., n-1\}$.

1.2 Derivation of the expressions Ψ_i for $i \in \{1, ..., n-1\}$

Here, we seek to show that for $i \in \{1, ..., n-1\}$ we have that

$$\Psi_i = \frac{\frac{\overline{K}_{i+1} E_{(i+1)T}}{(\overline{W}^*_{i+1} + \overline{K}_{i+1})^2} \frac{\overline{k}_{i+1}}{k_{i+1}}}{1 + \frac{\overline{K}_i E_{iT}}{(\overline{W}^*_i + \overline{K}_i)^2} \left[1 + \frac{\overline{k}_i}{k_i} \left(1 + \frac{K_i}{\overline{W}^*_{i-1}} \left(1 + \left(1 + \frac{\overline{W}^*_i}{\overline{K}_i} \right) \frac{\overline{W}^*_i}{\overline{W}^*_{i-1}} \Psi_{i-1} \right) \right) \right]} \tag{SI-4}$$

and

$$\Psi_0 = 0.$$

We have that $\Psi_0 = 0$ because $w^*_0 = 0$. We show identity (SI-4) by induction on the cascade stage number i.

Base case: From equation (SI-2), we have that $w^*_1 = -T_1 \overline{W}^*_0 \frac{\overline{k}_2 \widetilde{E}_2}{k_2} w^*_2$ from which we obtain that $|w^*_1| = T_1 \overline{W}^*_0 \frac{\overline{k}_2 \widetilde{E}_2}{k_2} |w^*_2|$. Employing equation (11) with $i = 1$ and simplifying yields

$$|w^*_1| = \frac{\frac{\overline{k}_2 \widetilde{E}_2}{k_2}}{1 + \widetilde{E}_1 \left(1 + \frac{\overline{k}_1}{k_1} \left(1 + \frac{K_1}{\overline{W}^*_0} \right) \right)} |w^*_2|.$$

Thus $\Psi_1 = \frac{\frac{\overline{k}_2 \widetilde{E}_2}{k_2}}{1 + \widetilde{E}_1 \left(1 + \frac{\overline{k}_1}{k_1} \left(1 + \frac{K_1}{\overline{W}^*_0} \right) \right)}$ and the base case is proven.

Induction step: Define $\psi_i := \frac{\frac{\overline{K}_{i+1} E_{(i+1)T}}{(\overline{W}^*_{i+1} + \overline{K}_{i+1})^2} \frac{\overline{k}_{i+1}}{k_{i+1}}}{1 + \frac{\overline{K}_i E_{iT}}{(\overline{W}^*_i + \overline{K}_i)^2} \left[1 + \frac{\overline{k}_i}{k_i} \left(1 + \frac{K_i}{\overline{W}^*_{i-1}} \left(1 + \left(1 + \frac{\overline{W}^*_i}{\overline{K}_i} \right) \frac{\overline{W}^*_i}{\overline{W}^*_{i-1}} \psi_{i-1} \right) \right) \right]}$ and assume $|w^*_{i-1}| = \psi_{i-1} |w^*_i|$. We prove that $|w^*_i| = \psi_i |w^*_{i+1}|$.

Since we have that $sign(w^*_i) = -sign(w^*_{i-1})$ and $\psi_{i-1} > 0$, we can rewrite the induction assumption as $w^*_{i-1} = -\psi_{i-1} w^*_i$. Employing equation (SI-1) along with the induction assumption, we obtain that

$$w^*_i = -T_i \overline{W}_i \psi_{i-1} w^*_i - T_i \overline{W}^*_{i-1} \frac{\overline{k}_{i+1} \widetilde{E}_{i+1}}{k_{i+1}} w^*_{i+1} .$$

Solving this equation for w^*_i, we obtain that

$$w^*_i = -\frac{T_i \overline{W}^*_{i-1} \frac{\overline{k}_{i+1} \widetilde{E}_{i+1}}{k_{i+1}}}{1 + T_i \overline{W}_i \psi_{i-1}} w^*_{i+1} = -\frac{\frac{\overline{k}_{i+1} \widetilde{E}_{i+1}}{k_{i+1}}}{\frac{1}{T_i \overline{W}^*_{i-1}} + \frac{\overline{W}_i}{\overline{W}^*_{i-1}} \psi_{i-1}} w^*_{i+1},$$

from which , employing the expression of T_i given in equation (11) and the expression of $\overline{W}_i$ from equation (5), we obtain that

$$w_i^* = -\frac{\frac{\overline{k}_{i+1}\widetilde{E}_{i+1}}{k_{i+1}}}{1+\widetilde{E}_i\left[1+\frac{\overline{k}_i}{k_i}\left(1+\frac{K_i}{\overline{W}_{i-1}^*}\left(1+\left(1+\frac{\overline{W}_i^*}{\overline{K}_i}\right)\frac{\overline{W}_i^*}{\overline{W}_{i-1}^*}\psi_{i-1}\right)\right)\right]}w_{i+1}^*$$

Since

$$\frac{\frac{\overline{k}_{i+1}\widetilde{E}_{i+1}}{k_{i+1}}}{1+\widetilde{E}_i\left[1+\frac{\overline{k}_i}{k_i}\left(1+\frac{K_i}{\overline{W}_{i-1}^*}\left(1+\left(1+\frac{\overline{W}_i^*}{\overline{K}_i}\right)\frac{\overline{W}_i^*}{\overline{W}_{i-1}^*}\psi_{i-1}\right)\right)\right]} = \psi_i,$$

it follows that $|w_i^*| = \psi_i|w_{i+1}^*|$ and hence that $\Psi_i = \psi_i$ for all $i \in \{1, ..., n-1\}$.

1.3 Derivation of the upper and lower bounds on Ψ_i for $i \in \{1, ..., n-1\}$

The next result shows an upper bound for the equilibrium values $\overline{W}_i^*$.

Lemma 1. *Define*

$$B_i \triangleq \begin{cases} \frac{\overline{k}}{\delta} & i = 0 \\ \min\left(W_{iT}, \frac{\overline{K}_i}{\left(\frac{\overline{k}_i}{k_i}+1\right)\frac{E_{iT}}{W_{iT}}-1}\right) & i \in \{1, ..., n\} \quad \textit{and} \quad \left(\frac{\overline{k}_i}{k_i}+1\right)\frac{E_{iT}}{W_{iT}} - 1 \geq 0 \\ W_{iT} & i \in \{1, ..., n\} \quad \textit{and} \quad \left(\frac{\overline{k}_i}{k_i}+1\right)\frac{E_{iT}}{W_{iT}} - 1 < 0. \end{cases} \tag{SI-5}$$

Then $\overline{W}_i^* \leq B_i, \qquad i \in \{0, ..., n\}$.

Proof. The value of $\overline{W}_0^*$ is given by equation (4), so that the first case follows immediately. The third case follows trivially from the conservation equation (1).

To prove the second case, consider that $\overline{X}_i + \overline{Y}_i \leq W_{iT}$, which along with equations (7) and (6) lead to

$$\frac{\overline{k}_i}{k_i}\frac{E_{iT}}{1+\frac{\overline{K}_i}{\overline{W}_i^*}} + \frac{E_{iT}}{1+\frac{\overline{K}_i}{\overline{W}_i^*}} \leq W_{iT}.$$

Since we also have that $\left(\frac{\overline{k}_i}{k_i}+1\right)\frac{E_{iT}}{W_{iT}} - 1 \geq 0$, we obtain that

$$\overline{W}_i^* \leq \frac{\overline{K}_i}{\left(\frac{\overline{k}_i}{k_i}+1\right)\frac{E_{iT}}{W_{iT}} - 1}. \tag{SI-6}$$

Since also $\overline{W}_i^* \leq W_{iT}$, the second case is shown. ∎

The next result shows an upper bound and a lower bound for the values of $\widetilde{E}_i$.

Lemma 2. *The value of $\widetilde{E}_i$ satisfies*

$$\frac{E_{iT}}{\left(\overline{K}_i + B_i\right)\left(1 + \frac{B_i}{\overline{K}_i}\right)} \leq \widetilde{E}_i \leq \frac{E_{iT}}{\overline{K}_i}.$$

with B_i as defined in Lemma 1.

Proof. Employing equation (6), we obtain that

$$\overline{Y}_i = \frac{1}{1 + \frac{\overline{K}_i}{\overline{W}_i^*}} E_{iT}.$$

Therefore,

$$\overline{E}_i = E_{iT} - \overline{Y}_i = E_{iT} - \frac{1}{1 + \frac{\overline{K}_i}{\overline{W}_i^*}} E_{iT} = \frac{1}{1 + \frac{\overline{W}_i^*}{\overline{K}_i}} E_{iT}.$$

Substituting the above into equation (10) results in

$$\widetilde{E}_i = \left(\frac{1}{\overline{W}_i^* + \overline{K}_i}\right)\left(\frac{1}{1 + \frac{\overline{W}_i^*}{\overline{K}_i}}\right) E_{iT}.$$

This is a monotonically decreasing function of $\overline{W}_i^*$. Since $0 \leq \overline{W}_i^* \leq B_i$ by Lemma 1, the result follows. ■

We can thus obtain an upper bound on the gain Ψ_i at every stage i, which is not recursive nor depends on the equilibrium value. Specifically, Employing Lemma 2 and Lemma 1 we obtain that

$$\Psi_i \leq \frac{\frac{\overline{k}_{i+1}}{k_{i+1}} \frac{E_{(i+1)T}}{K_{i+1}}}{1 + \frac{E_{iT}}{\left(\overline{K}_i + B_i\right)\left(1 + \frac{B_i}{\overline{K}_i}\right)} \left(1 + \frac{\overline{k}_i}{k_i}\left(1 + \frac{K_i}{\overline{W}_{i-1}^*}\right)\right)} \leq \frac{\frac{\overline{k}_{i+1}}{k_{i+1}} \frac{E_{(i+1)T}}{K_{i+1}}}{1 + \frac{E_{iT}}{\left(\overline{K}_i + B_i\right)\left(1 + \frac{B_i}{\overline{K}_i}\right)} \left(1 + \frac{\overline{k}_i}{k_i}\left(1 + \frac{K_i}{B_{i-1}}\right)\right)}. \tag{SI-7}$$

As a consequence, having $\frac{\frac{\overline{k}_{i+1}}{k_{i+1}} \frac{E_{(i+1)T}}{K_{i+1}}}{1 + \frac{E_{iT}}{\left(\overline{K}_i + B_i\right)\left(1 + \frac{B_i}{\overline{K}_i}\right)} \left(1 + \frac{\overline{k}_i}{k_i}\left(1 + \frac{K_i}{B_{i-1}}\right)\right)} < 1$ is a sufficient condition for attenuation at stage i, which is not iterative and does not depend on the equilibrium value.

From the expressions of Ψ_i and the lower bound on $\widetilde{E}_i$ from Lemma 2, we can also obtain a necessary condition for attenuation at every stage as follows.

Specifically, to have attenuation at stage i, we must have that $\Psi_{i-1} < 1$, so that by Lemma 2 we obtain that

$$\Psi_i = \frac{\frac{\overline{k}_{i+1}\widetilde{E}_{i+1}}{k_{i+1}}}{1+\widetilde{E}_i\left[1+\frac{\overline{k}_i}{k_i}\left(1+\frac{K_i}{\overline{W}^*_{i-1}}\left(1+\left(1+\frac{\overline{W}^*_i}{\overline{K}_i}\right)\frac{\overline{W}^*_i}{\overline{W}^*_{i-1}}\Psi_{i-1}\right)\right)\right]}$$
$$> \frac{\frac{\overline{k}_{i+1}\widetilde{E}_{i+1}}{k_{i+1}}}{1+\widetilde{E}_i\left[1+\frac{\overline{k}_i}{k_i}\left(1+\frac{K_i}{\overline{W}^*_{i-1}}\left(1+\left(1+\frac{\overline{W}^*_i}{\overline{K}_i}\right)\frac{\overline{W}^*_i}{\overline{W}^*_{i-1}}\right)\right)\right]}.$$

Since $\Psi_i < 1$, we have that necessarily the right-hand side must be smaller than 1. If the necessary condition is violated at stage i, then either stage $i-1$ or stage i amplify a perturbation as it propagates from downstream to upstream.

1.4 Proof that $sign(z_i) = -sign(z_{i+1})$ and derivation of Φ_i for all $i \in \{1, ..., n-1\}$

To simplify notation, define for all $i \in \{1, ..., n\}$

$$F_i := \widetilde{E}_i\frac{\overline{k}_i}{k_i}\frac{K_i}{\overline{W}^*_{i-1}}\left(1+\left(1+\frac{\overline{W}^*_i}{\overline{K}_i}\right)\frac{\overline{W}^*_i}{\overline{W}^*_{i-1}}\Psi_{i-1}\right), \tag{SI-8}$$

so that Ψ_i can be re-written as

$$\Psi_i = \frac{\frac{\overline{k}_{i+1}\widetilde{E}_{i+1}}{k_{i+1}}}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\overline{k}_i}{k_i}+F_i}. \tag{SI-9}$$

Here, we seek to demonstrate that $sign(z_i) = -sign(z_{i+1})$ for all $i \in \{1, ..., n-1\}$ and that

$$\Phi_i = \left(\frac{\widetilde{E}_i\frac{\overline{k}_i}{k_i}+F_i}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\overline{k}_i}{k_i}+F_i}\right)\left(\frac{\frac{\overline{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}}{\widetilde{E}_{i+1}\frac{\overline{k}_{i+1}}{k_{i+1}}+F_{i+1}}\right),$$

where F_i is given by Equation (SI-8).

We write z_i in terms of w^*_{i+1}. From equations (8), we have that $x_i = \frac{\overline{k}_i}{k_i}\widetilde{E}_i w^*_i$, so that

$$z_i = w^*_i + y_i + x_{i+1} = w^*_i(1+\widetilde{E}_i) + \frac{\overline{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}w^*_{i+1},$$

for all $i \in \{1, ..., n-1\}$. Since, $w^*_i = -\Psi_i w^*_{i+1}$, we have that

$$z_i = -\Psi_i w^*_{i+1}(1+\widetilde{E}_i) + \frac{\overline{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}w^*_{i+1} = \left(-\Psi_i(1+\widetilde{E}_i) + \frac{\overline{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}\right)w^*_{i+1}.$$

Substituting the expression of Ψ_i from equation (SI-9) and rearranging the terms, we obtain that

$$z_i = \left(\frac{\overline{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}\right)\left(\frac{\widetilde{E}_i\frac{\overline{k}_i}{k_i} + F_i}{1 + \widetilde{E}_i + \widetilde{E}_i\frac{\overline{k}_i}{k_i} + F_i}\right)w^*_{i+1}. \qquad \text{(SI-10)}$$

Since $\frac{\overline{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1} \geq 0$ and $\left(\frac{\widetilde{E}_i\frac{\overline{k}_i}{k_i}+F_i}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\overline{k}_i}{k_i}+F_i}\right) \geq 0$, it follows that $sign(z_i) = sign(w^*_{i+1})$ and therefore also that $sign(z_{i+1}) = sign(w^*_{i+2})$. Since $sign(w^*_{i+1}) = -sign(w^*_{i+2})$, we also have $sign(z_i) = -sign(z_{i+1})$ for all $i \in \{1, ..., n-2\}$.

To prove that $sign(z_{n-1}) = -sign(z_n)$, we write x_{n+1} as a function of w^*_n. Employing equation (SI-1) and the fact that $w^*_{n-1} = -\Psi_{n-1}w^*_n$, we obtain that

$$w^*_n = -T_n\overline{W}_n\Psi_{n-1}w^*_n - T_n\overline{W}^*_{n-1}x_{n+1}.$$

Solving for w^*_n and simplifying finally yields to

$$w^*_n = -\frac{1}{1 + \widetilde{E}_n + \widetilde{E}_n\frac{\overline{k_n}}{k_n} + F_n}x_{n+1},$$

so that $x_{n+1} = -(1+\widetilde{E}_n+\widetilde{E}_n\frac{\overline{k_n}}{k_n}+F_n)w^*_n$. This equation, in turn, leads to (considering that $z_n = w^*_n + y_n + x_{n+1}$)

$$z_n = -(\widetilde{E}_n\frac{\overline{k_n}}{k_n} + F_n)w^*_n, \qquad \text{(SI-11)}$$

which shows that $sign(z_n) = -sign(w^*_n)$. Since, from the first part of the proof, $sign(z_{n-1}) = sign(w^*_n)$, we have that $sign(z_{n-1}) = -sign(z_n)$. Therefore, $sign(z_i) = -sign(z_{i+1})$ for all $i \in \{1, ..., n-1\}$.

We next show that $\Phi_i = \left(\frac{\widetilde{E}_i\frac{\overline{k}_i}{k_i}+F_i}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\overline{k}_i}{k_i}+F_i}\right)\left(\frac{\frac{\overline{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}}{\widetilde{E}_{i+1}\frac{\overline{k}_{i+1}}{k_{i+1}}+F_{i+1}}\right)$. Employing equation (SI-

10) in the definition of Φ_i for $i \in \{1, ..., n-2\}$, we obtain that

$$
\begin{aligned}
\Phi_i = \left|\frac{z_i}{z_{i+1}}\right| &= \left| \frac{\left(\frac{\bar{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}\right)\left(\frac{\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}\right)w^*_{i+1}}{\left(\frac{\bar{k}_{i+2}}{k_{i+2}}\widetilde{E}_{i+2}\right)\left(\frac{\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}}{1+\widetilde{E}_{i+1}+\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}}\right)w^*_{i+2}} \right| \\
&= \frac{\left(\frac{\bar{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}\right)\left(\frac{\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}\right)}{\left(\frac{\bar{k}_{i+2}}{k_{i+2}}\widetilde{E}_{i+2}\right)\left(\frac{\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}}{1+\widetilde{E}_{i+1}+\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}}\right)}\Psi_{i+1} \\
&= \frac{\left(\frac{\bar{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}\right)\left(\frac{\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}\right)}{\left(\frac{\bar{k}_{i+2}}{k_{i+2}}\widetilde{E}_{i+2}\right)\left(\frac{\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}}{1+\widetilde{E}_{i+1}+\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}}\right)}\left(\frac{\frac{\bar{k}_{i+2}\widetilde{E}_{i+2}}{k_{i+2}}}{1+\widetilde{E}_{i+1}+\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}}\right) \\
&= \left(\frac{\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}\right)\left(\frac{\frac{\bar{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}}{\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}}\right).
\end{aligned}
$$

Since $\frac{\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i}{1+\widetilde{E}_i+\widetilde{E}_i\frac{\bar{k}_i}{k_i}+F_i} < 1$ and $\frac{\frac{\bar{k}_{i+1}}{k_{i+1}}\widetilde{E}_{i+1}}{\widetilde{E}_{i+1}\frac{\bar{k}_{i+1}}{k_{i+1}}+F_{i+1}} < 1$, we have that $\Phi_i < 1$. To determine Φ_{n-1}, we recall from equation (SI-11) that $|z_n| = (\widetilde{E}_n\frac{\bar{k}_n}{k_n} + F_n)|w^*_n|$, so that

$$
\begin{aligned}
\Phi_{n-1} = \left|\frac{z_{n-1}}{z_n}\right| &= \frac{\left(\frac{\bar{k}_n}{k_n}\widetilde{E}_n\right)\left(\frac{\widetilde{E}_{n-1}\frac{\bar{k}_{n-1}}{k_{n-1}}+F_{n-1}}{1+\widetilde{E}_{n-1}+\widetilde{E}_{n-1}\frac{\bar{k}_{n-1}}{k_{n-1}}+F_{n-1}}\right)w^*_n}{(\widetilde{E}_n\frac{\bar{k}_n}{k_n}+F_n)w^*_n} \\
&= \left(\frac{\frac{\bar{k}_n}{k_n}\widetilde{E}_n}{\widetilde{E}_n\frac{\bar{k}_n}{k_n}+F_n}\right)\left(\frac{\widetilde{E}_{n-1}\frac{\bar{k}_{n-1}}{k_{n-1}}+F_{n-1}}{1+\widetilde{E}_{n-1}+\widetilde{E}_{n-1}\frac{\bar{k}_{n-1}}{k_{n-1}}+F_{n-1}}\right).
\end{aligned}
$$

Since $\frac{\frac{\bar{k}_n}{k_n}\widetilde{E}_n}{\widetilde{E}_n\frac{\bar{k}_n}{k_n}+F_n} < 1$ and $\frac{\widetilde{E}_{n-1}\frac{\bar{k}_{n-1}}{k_{n-1}}+F_{n-1}}{1+\widetilde{E}_{n-1}+\widetilde{E}_{n-1}\frac{\bar{k}_{n-1}}{k_{n-1}}+F_{n-1}} < 1$, we also have that $\Phi_{n-1} < 1$.

1.5 Proof that $|w^*_n| < |d_T|$

We find an explicit expression for $\frac{|w^*_n|}{|d_T|}$ and show that it is less than 1.

By setting the time derivative equal to zero in the last of equations (3) and solving for x_{n+1}, we obtain that

$$x_{n+1} = \frac{1}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}} \left(\overline{D} w_n^* + \overline{W}_n^* d_T \right). \tag{SI-12}$$

From equation (9) we have that

$$w_n^* = T_n \left(\overline{W}_n w_{n-1}^* - \overline{W}_{n-1}^* x_{n+1} \right). \tag{SI-13}$$

Substituting equation (SI-12) into equation (SI-13) and also noting that $w_{n-1}^* = -\Psi_{n-1} w_n^*$, we obtain that

$$w_n^* = T_n \left(-\overline{W}_n \Psi_{n-1} w_n^* - \overline{W}_{n-1}^* \frac{1}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}} \left(\overline{D} w_n^* + \overline{W}_n^* d_T \right) \right).$$

So we have that

$$w_n^* + T_n \left(\overline{W}_n \Psi_{n-1} w_n^* + \overline{W}_{n-1}^* \frac{1}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}} \overline{D} w_n^* \right) = T_n \left(-\overline{W}_{n-1}^* \frac{1}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}} \overline{W}_n^* d_T \right).$$

To simplify notation, let

$$P_1 \triangleq \frac{1}{1 + T_n \left(\overline{W}_n \Psi_{n-1} + \overline{W}_{n-1}^* \frac{1}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}} \overline{D} \right)} \tag{SI-14}$$

$$P_2 \triangleq T_n \overline{W}_{n-1}^* = \frac{1}{1 + \widetilde{E}_n (1 + \frac{\overline{k}_n}{k_n} (1 + \frac{K_n}{\overline{W}_{n-1}^*}))} \tag{SI-15}$$

$$P_3 \triangleq \frac{\overline{W}_n^*}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}}. \tag{SI-16}$$

Therefore, we have that $w_n^* = -P_1 P_2 P_3 d_T$, i.e., $\frac{|w_n^*|}{|d_T|} = P_1 P_2 P_3$. From the expressions of P_i and of T_n from equation (11) we can see that $P_i < 1$ for $i \in \{1, 2, 3\}$. Therefore, $\frac{|w_n^*|}{|d_T|} < 1$.

This result implies that regardless of the number of stages or the parameters of the cascade, we always have attenuation from d_T, the disturbance downstream, to w_n^*, the active protein at the last stage.

1.6 Proof that $|z_n| < |d_T|$

In the previous section we obtained that $w_n^* = -P_1P_2P_3d_T$ with P_i given by equations (SI-14)-(SI-16). From equations (SI-12) we obtain that

$$\begin{aligned}
z_n &= w_n^* + y_n + x_{n+1} = (1+\widetilde{E}_n)w_n^* + \frac{1}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}}\left(\overline{D}w_n^* + \overline{W}_n^* d_T\right) \\
&= \left(1+\widetilde{E}_n + \frac{\overline{D}}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}}\right)w_n^* + \frac{\overline{W}_n^*}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}}d_T
\end{aligned}$$

$$\begin{aligned}
&= \left(-\left(1+\widetilde{E}_n + \frac{\overline{D}}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}}\right)(P_1P_2P_3) + \frac{\overline{W}_n^*}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}}\right)d_T \\
&= \left(-\left(1+\widetilde{E}_n + \frac{\overline{D}}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}}\right)(P_1P_2P_3) + \frac{\overline{W}_n^*}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}}\right)d_T \\
&= P_3\left(-(1+\widetilde{E}_n + \frac{\overline{D}}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}})(P_1P_2) + 1\right)d_T.
\end{aligned}$$

Since we have already shown that $P_3 < 1$, in order to prove that $\frac{|z_n|}{|d_T|} < 1$, it suffices to show that

$$0 < (1+\widetilde{E}_n + \frac{\overline{D}}{\overline{W}_n^* + \frac{\overline{a}_{n+1}}{a_{n+1}}})(P_1P_2) < 1.$$

Since every term in this expression is positive, the left hand inequality follows. In order to show the right inequality, we expand P_1 and P_2 and simplify as follows:

$$
\begin{aligned}
& (1+\widetilde{E}_n+\frac{\overline{D}}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}})(P_1P_2) = \\
& (1+\widetilde{E}_n+\frac{\overline{D}}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}})\frac{T_n\overline{W}_{n-1}^*}{1+T_n\left(\overline{W}_n\Psi_{n-1}+\overline{W}_{n-1}^*\frac{1}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}}\overline{D}\right)} \\
= & \frac{1+\widetilde{E}_n+\frac{\overline{D}}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}}}{\frac{1}{T_n\overline{W}_{n-1}^*}+\frac{\overline{W}_n}{\overline{W}_{n-1}^*}\Psi_{n-1}+\frac{1}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}}\overline{D}} \\
= & \frac{1+\widetilde{E}_n+\frac{\overline{D}}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}}}{1+\widetilde{E}_n(1+\frac{\overline{k}_n}{k_n}(1+\frac{K_n}{\overline{W}_{n-1}^*}))+\frac{\overline{W}_n}{\overline{W}_{n-1}^*}\Psi_{n-1}+\frac{1}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}}\overline{D}} \\
= & \frac{(1+\widetilde{E}_n+\frac{\overline{D}}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}})}{(1+\widetilde{E}_n+\frac{\overline{D}}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}})+\widetilde{E}_n\frac{\overline{k}_n}{k_n}(1+\frac{K_n}{\overline{W}_{n-1}^*})+\frac{\overline{W}_n}{\overline{W}_{n-1}^*}\Psi_{n-1}},
\end{aligned}
$$

which shows that $(1+\widetilde{E}_n+\frac{\overline{D}}{\overline{W}_n^*+\frac{\overline{a}_{n+1}}{a_{n+1}}})(P_1P_2)<1$ as required.

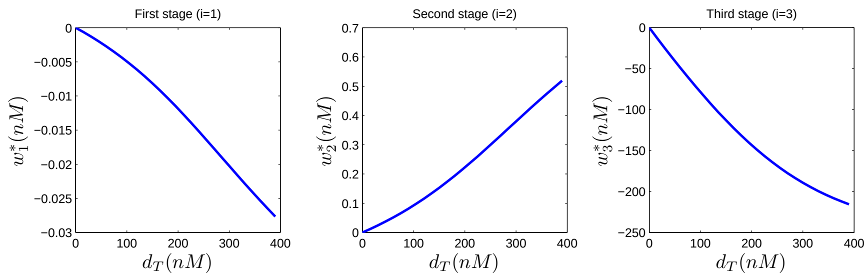
1.7 Derivation of the rate constants for a weakly activated pathway

The differential equation modeling cycle i is given by

$$
\begin{aligned}
\dot{X}_i &= a_iW_{i-1}^*(W_{iT}-W_i^*-X_i-Y_i-X_{i+1})-(\overline{a}_i+k_i)X_i \\
\dot{W}_i^* &= k_iX_i-b_iW_i^*(E_{iT}-Y_i)+\overline{b}_iY_i-\dot{X}_{i+1} \\
\dot{Y}_i &= b_iW_i^*(E_{iT}-Y_i)-(\overline{b}_i+\overline{k}_i)Y_i.
\end{aligned}
$$

The assumption of having a weakly activated pathway implies that $W_i^*, Y_i, X_{i+1} \ll W_{iT}$. Also, since $a_i, \overline{a}_i, b_i, \overline{b}_i \gg k_i, \overline{k}_i$, we can assume that the first and last equations are at the quasi steady state, that is, $\dot{X}_i \approx 0$ and $\dot{Y}_i \approx 0$. This leads to the reduced model for the cycle given by

$$\dot{W}_i^* = \frac{k_iW_{iT}W_{i-1}^*}{K_i+W_{i-1}^*}-\frac{\overline{k}_iE_{iT}W_i^*}{\overline{K}_i+W_i^*}.$$



SI Figure 1: **Attenuation and sign-reversal in a three-stage cascade: Example 1.** The x-axis shows the value of the perturbation d_T and the y-axis shows the steady state value of the resulting perturbations w_1^*, w_2^*, and w_3^*. Simulation is performed on the full nonlinear ODE model given by equation (2). The parameters of each stage i are taken from (30) and are given by $k_1 = 240(\text{min})^{-1}$, $k_2 = 6.3(\text{min})^{-1}$, $k_3 = 9(\text{min})^{-1}$, $\overline{k}_1 = 360(\text{min})^{-1}$, $\overline{k}_2 = 360(\text{min})^{-1}$, $\overline{k}_3 = 60(\text{min})^{-1}$, $a_1 = 18(\text{nM min})^{-1}$, $a_2 = 198.1(\text{nM min})^{-1}$, $a_3 = 978.3(\text{nM min})^{-1}$, $a_4 = 978.3(\text{nM min})^{-1}$, $\overline{a}_1 = 960(\text{min})^{-1}$, $\overline{a}_2 = 25.2(\text{min})^{-1}$, $\overline{a}_3 = 36(\text{min})^{-1}$, $\overline{a}_4 = 36(\text{min})^{-1}$, $b_1 = 115(\text{nM min})^{-1}$, $b_2 = 115(\text{nM min})^{-1}$, $b_3 = 4545.5(\text{nM min})^{-1}$, $\overline{b}_1 = 1440(\text{min})^{-1}$, $\overline{b}_2 = 1440(\text{min})^{-1}$, $\overline{b}_3 = 240(\text{min})^{-1}$, $E_{3T} = 3.2\text{nM}$, $E_{2T} = 224\text{nM}$, $E_{1T} = 224\text{nM}$, $W_{3T} = 360\text{nM}$, $W_{2T} = 180\text{nM}$, $W_{1T} = 200\text{nM}$, $\overline{W}_0^* = 100\text{nM}$, and $\overline{D}_T = 0\text{nM}$.

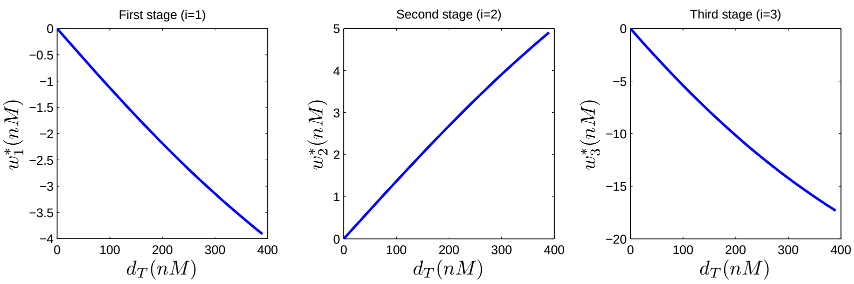
Hence, if $K_i \gg W_{i-1}^*$ and $\overline{K}_i \gg W_i^*$, we have that the phosphorylation rate constant is given approximately by $k_i W_{iT}/K_i$ while the dephosphorylation rate constant is given approximately by $\overline{k}_i E_{iT}/\overline{K}_i$.

1.8 Examples of attenuation in three-stage cascades

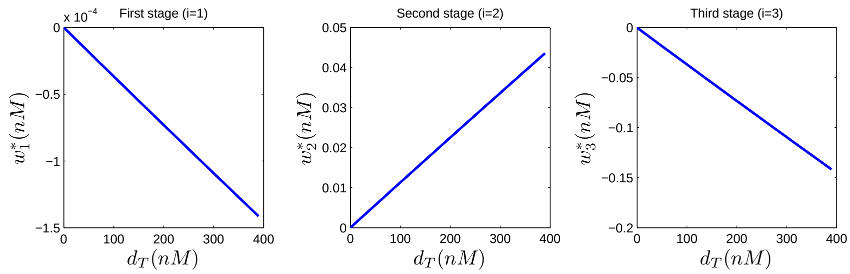
Here, we illustrate three more examples of three-stage cascades. As explained in the main text, the theoretical predictions hold for all parameter values if the perturbation d_T is sufficiently small. SI Figures 1, 2, and 3 provide three examples for nominal parameter values given in (30) and (31), and for extreme values in the interval constructed about the parameter set of (31), respectively. In all cases, surprisingly, the relationship between d_T and w_i^* is approximately linear even for large perturbations d_T.

1.9 Convergence of probability in the numerical simulations

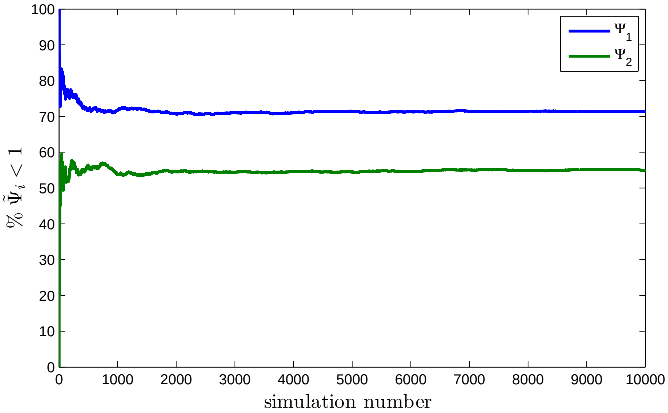
SI Figures 4, 5, 6, 7, and 8 show the convergence of the probabilities to their final values.



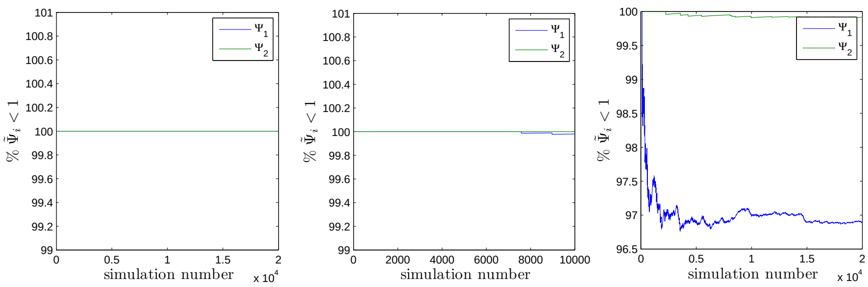
SI Figure 2: **Attenuation and sign-reversal in a three-stage cascade: Example 2.** The x-axis shows the value of the perturbation d_T and the y-axis shows the steady state value of the resulting perturbations w_1^*, w_2^*, and w_3^*. Simulation is performed on the full nonlinear ODE model given by equation (2). The parameters of each stage i are taken from (31) and are given by $k_i = 6(\text{min})^{-1}$, $\overline{k}_i = 6(\text{min})^{-1}$, $a_1 = 0.06(\text{nM min})^{-1}$, $a_2 = 0.198(\text{nM min})^{-1}$, $a_3 = 1.2(\text{nM min})^{-1}$, $a_4 = 1.2(\text{nM min})^{-1}$, $\overline{a}_1 = 24(\text{min})^{-1}$, $\overline{a}_2 = 25.2(\text{min})^{-1}$, $\overline{a}_3 = 36(\text{min})^{-1}$, $\overline{a}_4 = 36(\text{min})^{-1}$, $b_1 = 0.03(\text{nM min})^{-1}$, $b_2 = 0.6(\text{nM min})^{-1}$, $b_3 = 0.3(\text{nM min})^{-1}$, $\overline{b}_1 = 30(\text{min})^{-1}$, $\overline{b}_2 = 48(\text{min})^{-1}$, $\overline{b}_3 = 24(\text{min})^{-1}$, $E_{3T} = 300\text{nM}$, $E_{2T} = 200\text{nM}$, $E_{1T} = 300\text{nM}$, $W_{3T} = 400\text{nM}$, $W_{2T} = 200\text{nM}$, $W_{1T} = 300\text{nM}$, $\overline{W}_0^* = 200\text{nM}$, and $\overline{D}_T = 0\text{nM}$.



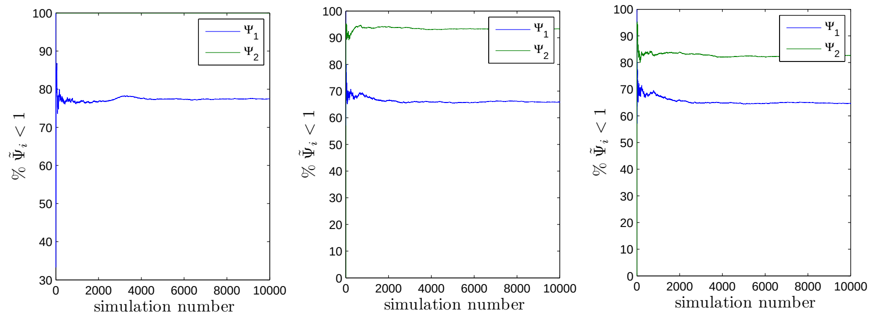
SI Figure 3: **Attenuation and sign-reversal in a three-stage cascade: Example 3.** The x-axis shows the value of the perturbation d_T and the y-axis shows the steady state value of the resulting perturbations w_1^*, w_2^*, and w_3^*. Simulation is performed on the full nonlinear ODE model given by equation (2). The parameters of each stage i are taken from the extreme values of the intervals constructed about the parameters in (30). These are given by $k_1 = 6.3(\text{min})^{-1}$, $k_2 = 6.3(\text{min})^{-1}$, $k_3 = 600(\text{min})^{-1}$, $\overline{k}_1 = 600(\text{min})^{-1}$, $\overline{k}_2 = 6.3(\text{min})^{-1}$, $\overline{k}_3 = 600(\text{min})^{-1}$, $a_1 = 4540(\text{nM min})^{-1}$, $a_2 = 18(\text{nM min})^{-1}$, $a_3 = 4540(\text{nM min})^{-1}$, $a_4 = 18(\text{nM min})^{-1}$, $\overline{a}_1 = 25.2(\text{min})^{-1}$, $\overline{a}_2 = 2400(\text{min})^{-1}$, $\overline{a}_3 = 2400(\text{min})^{-1}$, $\overline{a}_4 = 25.2(\text{min})^{-1}$, $b_i = 4540(\text{nM min})^{-1}$, $\overline{b}_1 = 25.2(\text{min})^{-1}$, $\overline{b}_2 = 2400(\text{min})^{-1}$, $\overline{b}_3 = 25.2(\text{min})^{-1}$, $E_{3T} = 224\text{nM}$, $E_{2T} = 3.2\text{nM}$, $E_{1T} = 3.2\text{nM}$, $W_{3T} = 180\text{nM}$, $W_{2T} = 360\text{nM}$, $W_{1T} = 360\text{nM}$, $\overline{W}_0^* = 100\text{nM}$, and $\overline{D}_T = 0\text{nM}$.



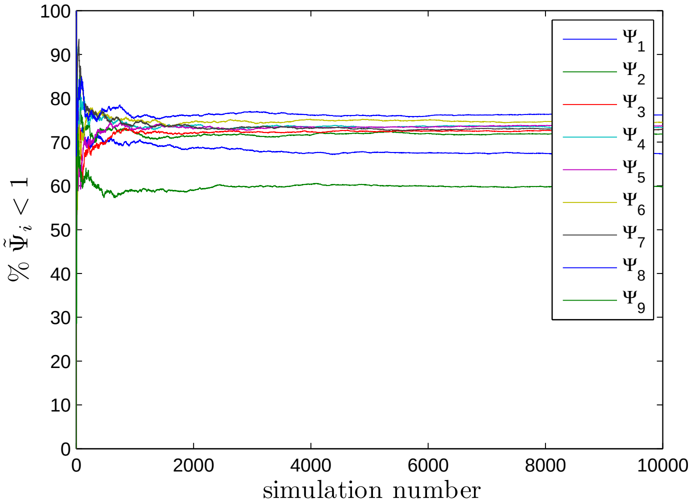
SI Figure 4: Three-stage cascade. Percentage of Ψ_i that are less than one calculated after every new simulation run. The plots converge to the values given in Table 2.



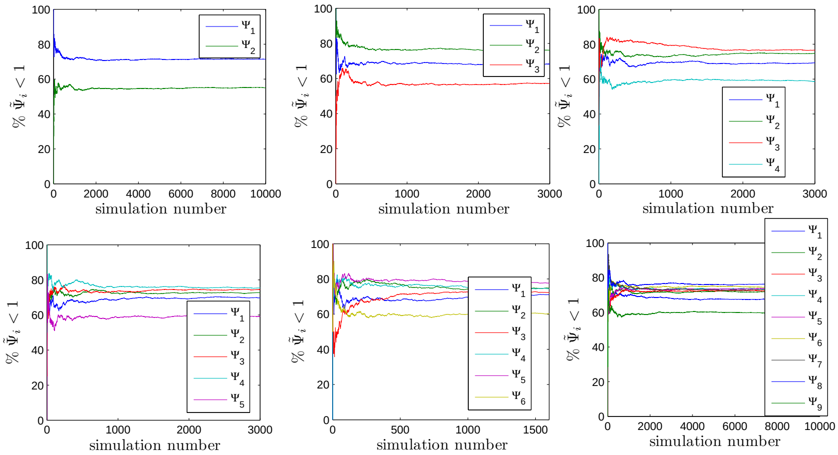
SI Figure 5: Three-stage cascade. Percentage of Ψ_i that are less than one calculated after every new simulation run for the three intervals about the nominal parameter values taken from (30) ($x = 2$ in the left plot, $x = 5$ in the center plot, and $x = 8$ in the right side plot). The plots converge to the values given in Table 3.



SI Figure 6: Three-stage cascade. Percentage of Ψ_i that are less than one calculated after every new simulation run for the three intervals about the parameter values taken from (31) ($x = 2$ in the left plot, $x = 5$ in the center plot, and $x = 8$ in the right side plot). The plots converge to the values given in Table 4.



SI Figure 7: Ten-stage cascade. Percentage of Ψ_i that are less than one calculated after every simulation. The plots converge to the values given in Table 5.



SI Figure 8: Cascade with increasing number of stages. Percentage of Ψ_i that are less than 1 calculated after every simulation run. All the plots converge. This simulation data was used to generate Figure 5.