We consider what happens when linear signal transduction cascades are connected in series and in parallel, including positive feedback and negative feedback. In general, the signaling is initiated by a signal or drug with time-domain signal $D(t)$ and results in signal $T(t)$ of activated targets, usually activated transcription factors in the nucleus. The signal transduction cascade has a transfer function $H(t)$, leading to a time-domain response $T(t) = \int_{-\infty}^{t} dt' H(t-t')D(t')$, Laplace-domain response $\tilde{T}(s) = \tilde{H}(s)\tilde{D}(s)$, gain $G = \lim_{s\to 0} H(s)$, and activation time $\tau = \lim_{s\to 0}(-d/ds)\ln\tilde{H}(s)$. We will assume that the signaling began in the distant past, dropping the lower limit $-\infty$ in the time integral and ignoring the boundary term.

1. Series circuits. Suppose that two signaling networks are combined in series through a signaling intermediate x:

$$x(t) = \int^{t} dt' H_1(t-t')D(t')$$

$$T(t) = \int^{t} dt' H_2(t-t')x(t).$$

   The individual transfer functions have gains G_1 and G_2 and activation times τ_1 and τ_2 that are all positive. Provide the overall transfer function $\tilde{H}(s)$, the overall gain, and the overall activation time in terms of the individual Laplace-space transfer functions, gains, and activation times.

2. Parallel circuits. Suppose the two networks are combined in parallel through signaling intermediates x_1 and x_2:

$$x_1(t) = \int^{t} dt' H_{1a}(t-t')D(t')$$

$$x_2(t) = \int^{t} dt' H_{2a}(t-t')D(t')$$

$$T(t) = \int^{t} dt' H_{1b}(t-t')x_1(t') + \int^{t} dt' H_{2b}(t-t')x_2(t').$$

   Provide the overall Laplace-space transfer function, the overall gain, and the overall activation time in terms of the properties of the four individual transfer functions.

3. Positive feedback. Suppose signaling intermediate x in a serial pathway is subject to positive auto-regulation:

$$x(t) = \int^{t} dt' H_1(t-t')D(t') + \int^{t} dt' H_x(t-t')x(t')$$

$$T(t) = \int^{t} dt' H_2(t-t')x(t).$$

   (a) Provide the overall Laplace-space transfer function, the overall gain, and the overall activation time in terms of the properties of the three individual transfer functions.

(b) In the weak feedback regime, the overall gain and activation time remain finite. In the strong feedback regime, the signaling intermediate x reaches saturation and a linear approximation is no longer appropriate. What condition determines whether the feedback is weak or strong, and non-physical result does the linear model predict for strong feedback?

4. Negative feedback. Suppose signaling intermediate x in a serial pathway is subject to negative feedback:

$$x(t) = \int^t dt' H_1(t-t')D(t') - \int^t dt' H_x(t-t')x(t')$$

$$T(t) = \int^t dt' H_2(t-t')x(t).$$

   (a) Provide the overall Laplace-space transfer function, the overall gain, and the overall activation time in terms of the properties of the three individual transfer functions.

   (b) Does negative feedback have strong and weak regimes?

5. Interpretation of feedback transfer functions.

   (a) Suppose that $y = 1/(1 \pm x)$. The Taylor series about $x = 0$ is $y = \sum_{n=0}^{\infty} c_n x^n$. Provide these Taylor series.

   (b) Use these Taylor series to interpret a feedback loop in terms of a feedback-free network with parallel connections.

6. Multiple feedback loops.

   (a) Suppose that two signaling components have independent feedback loops:

$$x(t) = \int^t dt' H_1(t-t')D(t') + \int^t dt' H_x(t-t')x(t')$$

$$y(t) = \int^t dt' H_2(t-t')x(t') + \int^t dt' H_y(t-t')y(t')$$

$$T(t) = \int^t dt' H_3(t-t')y(t).$$

   Provide the overall Laplace-domain transfer function, gain, activation time, and the condition that separates weak feedback from strong feedback.

   (b) Suppose that two signaling components have nested feedback loops:

$$x(t) = \int^t dt' H_1(t-t')D(t') + \int^t dt' H_a(t-t')y(t')$$

$$y(t) = \int^t dt' H_b(t-t')x(t') + \int^t dt' H_c(t-t')y(t')$$

$$T(t) = \int^{t} dt' H_2(t-t') x(t).$$

Provide the overall Laplace-domain transfer function, gain, activation time, and the condition that separates weak feedback from strong feedback.