

Figure 1: Parameters of a concentration gradient

formalism as described by Fick (1855). **B**) At the molecular level, diffusion of a guidance cue can be described by a random walk. Left panel presents a sample molecular trajectory, while a formalism of the mean particle displacement is presented to the right, where D is the diffusion constant (Einstein 1905). **C**) Simulated concentration gradient of guidance molecules. Each purple dot moves according to a random walk in order to model the process of molecular diffusion. **D**) Quantification of the simulated linear concentration gradient. The plot is made by calculating the number of molecules per rectangle as schematized by the blue box in C. The uneven nature of the curve reflects the inherent uncertainty resulting from the stochastic random walk underlying molecular diffusion. I define the steepness of the gradient as the fractional change in concentration, $\Delta C/C$. In a linear gradient (the case for all gradients considered in this paper), the change in concentration across a given distance (ΔC) is the same at any position, however the concentration increases as we move up the gradient. Therefore, the steepness of the gradients, $\Delta C/C$, is highest at the low end of the gradient, and vice versa.

A) Sample concentration gradient and its formalism as described by Fick (1855). **B)** At the molecular level, diffusion of a guidance cue can be described by a random walk. Left panel presents a sample molecular trajectory, while a formalism of the mean particle displacement is presented to the right, where D is the diffusion constant (Einstein 1905). **C)** Simulated concentration gradient of guidance molecules. Each purple dot moves according to a random walk in order to model the process of molecular diffusion. **D)** Quantification of the simulated linear concentration gradient. The plot is made by calculating the number of molecules per rectangle as schematized by the blue box in C. The uneven nature of the curve reflects the inherent uncertainty resulting from the stochastic random walk underlying molecular diffusion. I define the steepness of the gradient as the fractional change in concentration, $\Delta C/C$. In a linear gradient (the case for all gradients considered in this paper), the change in concentration across a given distance (ΔC) is the same at any position, however the concentration increases as we move up the gradient. Therefore, the steepness of the gradients, $\Delta C/C$, is highest at the low end of the gradient, and vice versa.

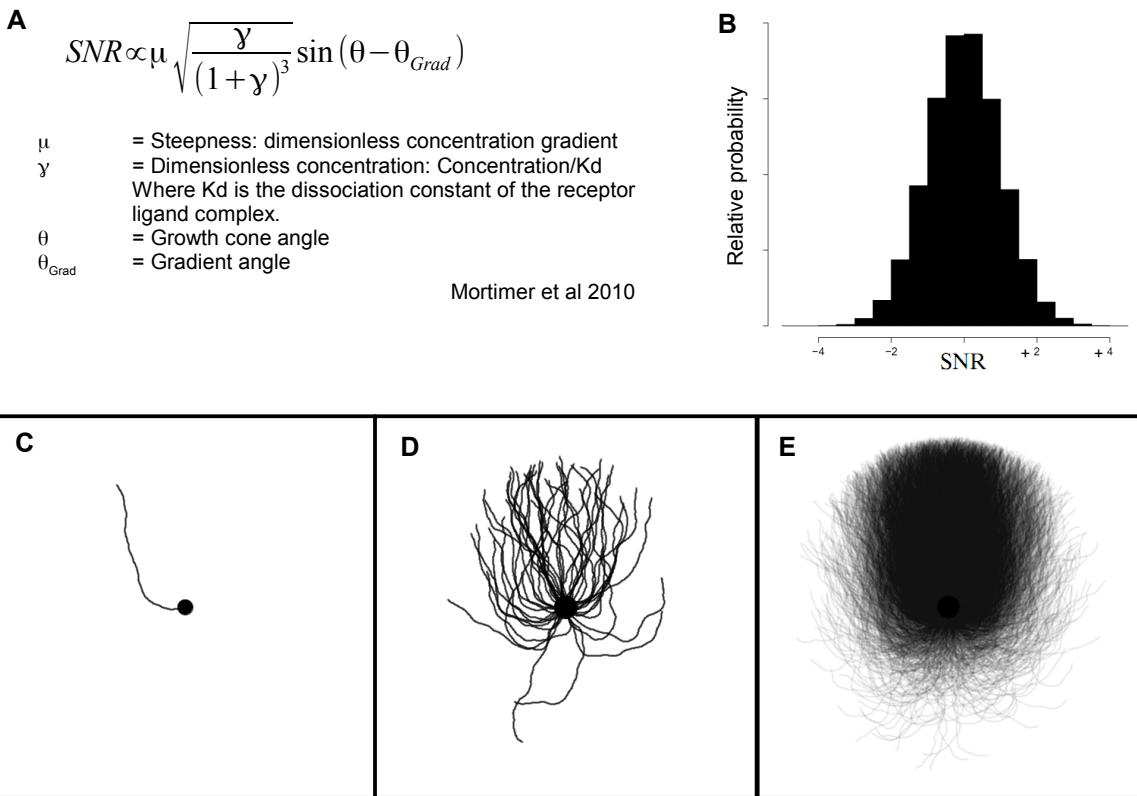


Figure 2: Axon turning model (Model 1)

A) Equation derived by Mortimer et al. (2010) that is used to describe the computation that a growth cone makes with respect to the direction of the concentration gradient, given the knowledge of the activation and location of bound receptors at its surface. In this model, the growth cone initially moves forward in a random direction θ with respect to the guidance cue gradient (θ_{Grad}). At each time-step, the growth cone computes a signal to noise ratio (SNR) as a function of the steepness of the gradient, the concentration of guidance cue, and the orientation of the growth cone with respect to the external concentration gradient. **B)** The stochastic component of the model involves a sample being drawn from a random normal distribution, with a mean of the calculated SNR and a standard deviation of 1. If the sample is a positive value, the growth cone will turn an angle of $\pi / 30$ to its right, and vice versa. Thus, the direction an axon will turn is stochastic, but biased by the magnitude of the SNR. **C)** A sample axon trajectory in a moderately steep gradient. The cell body is represented by the ellipse in the center of the panel. **D)** Sample simulation of 100 axons, showing that each simulation run is unique. **E)** A compilation of 5000 axons, representing the actual number of neurons that were simulated for each condition tested for this model.

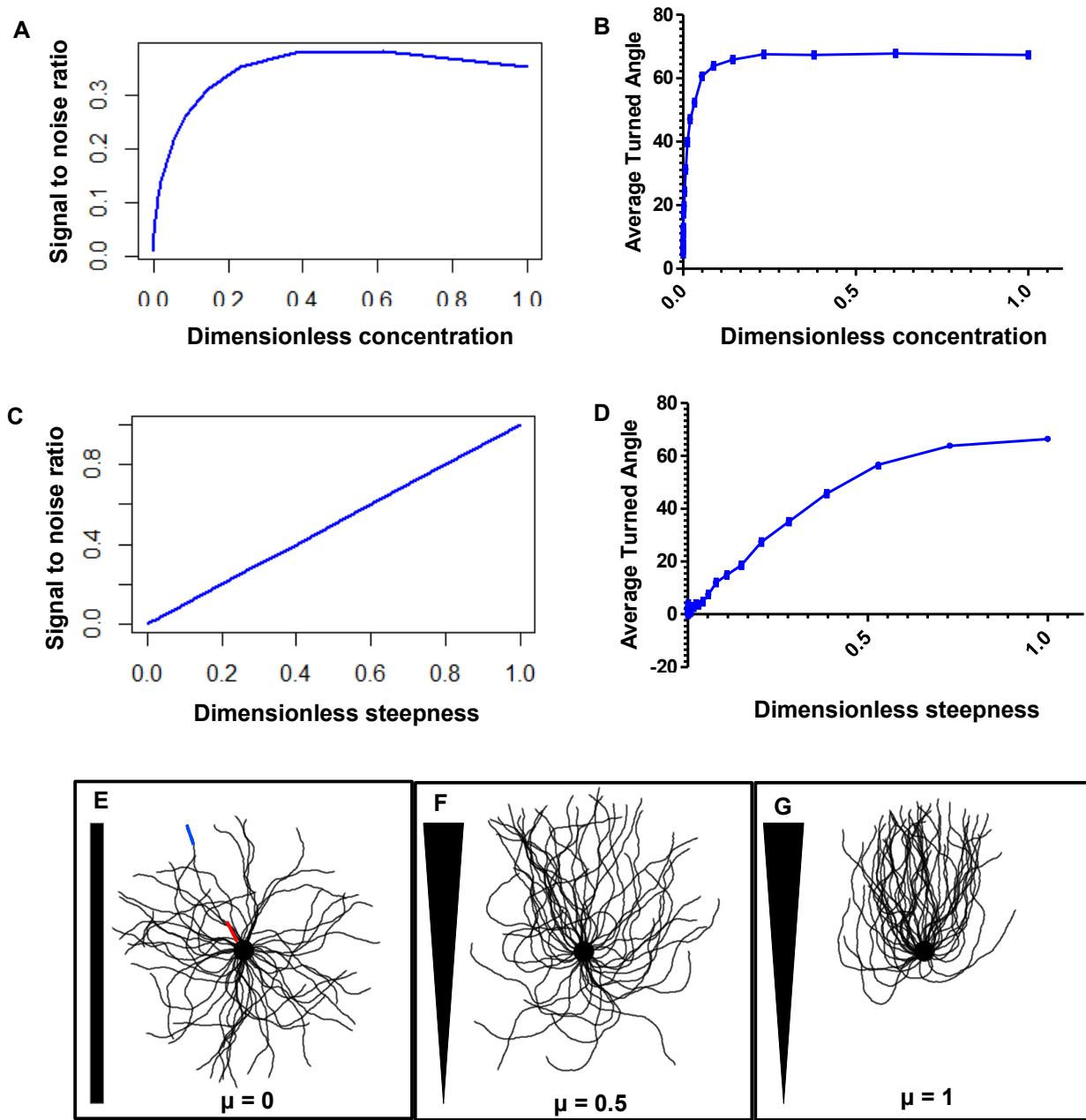
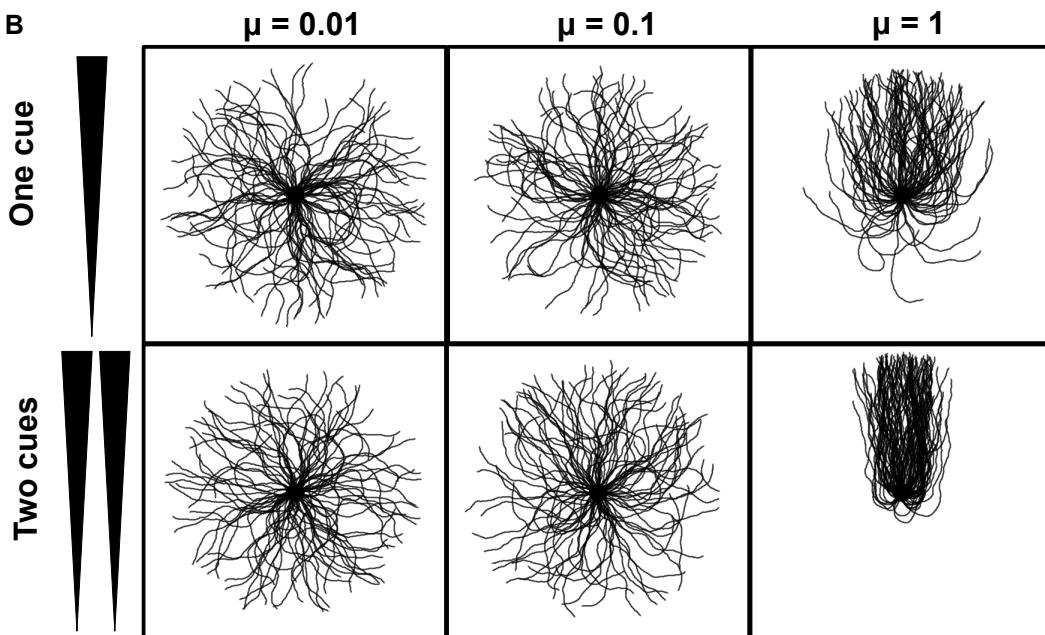
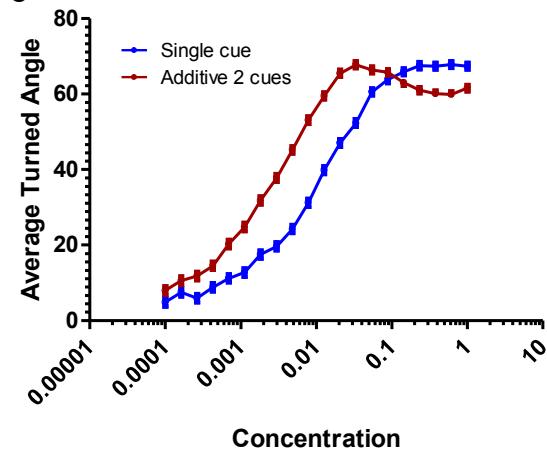
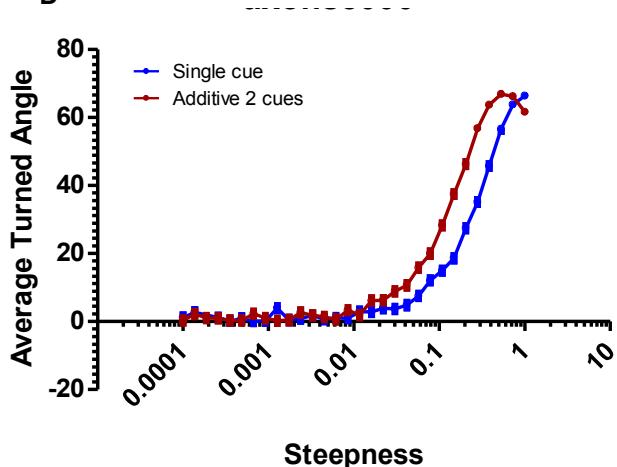


Figure 3: Exploration of the parameters of the axon turning model

Panel A shows the calculated value of the signal to noise ratio (SNR) as a function of the dimensionless concentration. The SNR increases rapidly from zero at low concentrations and plateaus around 0.35 at higher concentrations. Panel B shows the average turned angle for 5000 simulated axons as a function of dimensionless concentration. The angle increases rapidly and plateaus around 65 degrees. Panel C shows the calculated SNR for a range of increasing gradient steepness. The SNR increases linearly with steepness. Panel D shows the simulated axons follow a mostly linear trend in their average turned angle with increasing steepness, however unlike the calculated SNR, the turning response saturates, as axons which already turned to align with the gradient cannot improve any further. Panel E shows sample trajectories of 100 simulated axons (sharing the same origin) without a concentration gradient (steepness of 0). The axons turn entirely randomly, leading to disorganized trajectories. The tall black rectangle represents no gradient. Panel F shows sample trajectories of axons in a shallow gradient (steepness = 0.5), although stochastic, there seems to be a bias towards axons turning to align with the gradient. Panel G shows simulated axons in a steep gradient (steepness = 1), in this condition, the gradient is so steep that the growth cones can unambiguously detect the gradient direction, as only a few axons have not aligned properly. The black wedge represents the direction of the gradient, such that the wider edge represents the higher concentration. Error bars in B and D represent SEM of 5000 simulation runs.

A

$$SNR \propto \sum_{n=1}^N \mu_n \sqrt{\frac{\gamma_n}{(1+\gamma_n)^3}} \sin(\theta - \theta_{Grad})$$

Gradient steepness**B****C****D****Figure 4: Extending the axon turning model to multiple guidance cues****A)** I extended the equation in Figure 2A to be applicable to combining gradients, which is formalized here for an arbitrary number of guidance cues, N. The combination assumes that the signaling pathways each make their own contribution to the SNR additively. For the following models, I have simulated the results considering the case where $\gamma_1 = \gamma_2$ and $\mu_1 = \mu_2$.

B) Simulated axon trajectories for a logarithmically spaced range of steepness for one or two guidance cues. At a low steepness (0.01), axons in both conditions perform equally poorly, and there is no directed turning visible in the trajectories. When the steepness is higher (0.1), there begins to be a few more simulated axons which turn up the concentration gradient in the 2 cue condition, whereas with a single cue alone there is no such effect. At a steepness of 1, all axons in either condition are oriented in the correct direction, however there continues to be visual difference, where the 2 cue gradient attracts the axons more quickly, so the simulated trajectories are less spread out across space. **C)** Plot comparing the 1 and 2 cue models across a logarithmic range of concentration. The 2 cue gradient outperforms the single cue gradient until a sufficient concentration of ~0.1 is reached. **D)** Plot comparing the 1 and 2 cue models across a logarithmic range of gradient steepness. As seen in A, the two cue gradient attracts axons at a lower steepness than a single cue gradient, and plateaus around a steepness of 1. Error bars (C-D) represent the SEM of the 5000 axons per simulation condition.

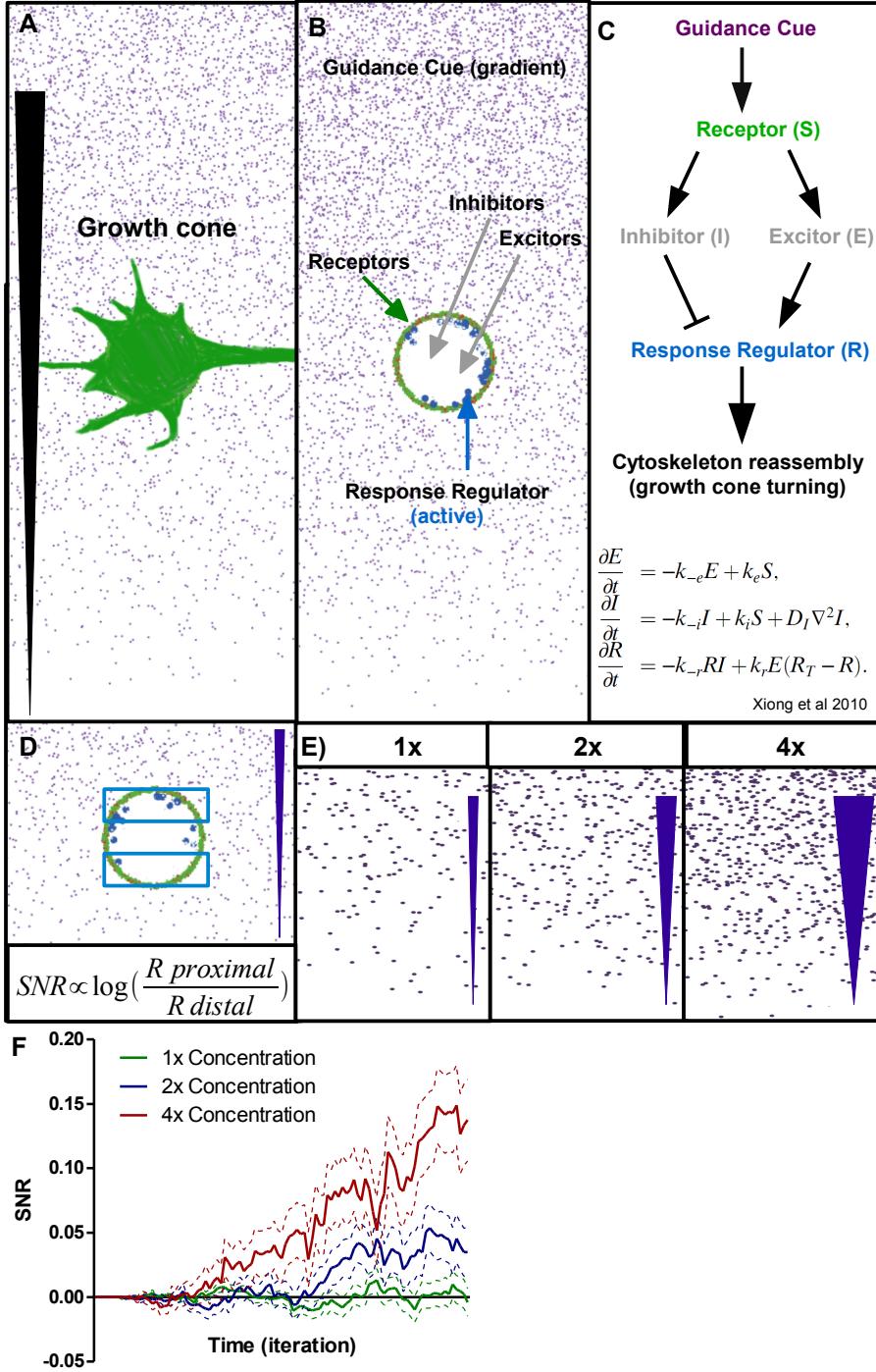


Figure 5: Gradient sensing model (Model 2) **A)** Schematic growth cone in a concentration gradient, the direction of which is represented by the black wedge. Each purple spot represents the position of 1 guidance molecule. **B)** Idealized circular growth cone which is used in this model. The receptors (green) form a ring that makes a boundary, and become red when active. The excitors and inhibitors are molecules which diffuse within the growth cone, colored white for clarity. These two species have opposing influence on the response regulators. Active response regulators (blue) are the output of the model, and represent the growth cones internal representation of the external gradient, which I consider to be analogous and proportional to the SNR calculated in model 1. **C)** A schematic of the logic of the molecular interactions that were programmed in a multi-agent model using NetLogo. A mathematical formalism (Xiong et al 2010) is presented at the bottom of the panel as a reference, where S, E, I, and R represent the level of active receptors, excitors, inhibitors and response regulators, respectively. For each molecular species, k is a rate constant for the (+) activation or (-) inhibition of the species indicated by the subscript. R_T represents the total number of response regulators. **D)** The output of the model, SNR, is calculated as the log ratio of the number of active response regulators on the proximal third / the distal third of the ideal growth cone. **E)** Visual representation of the simulated gradients with concentrations increasing by 2-fold. **F)** Influence of increasing the concentration on the SNR, proportional to the log ratio of the number of activated response regulators on the side proximal vs distal to the gradient. Dashed lines represent the SEM of 1000 simulation runs.

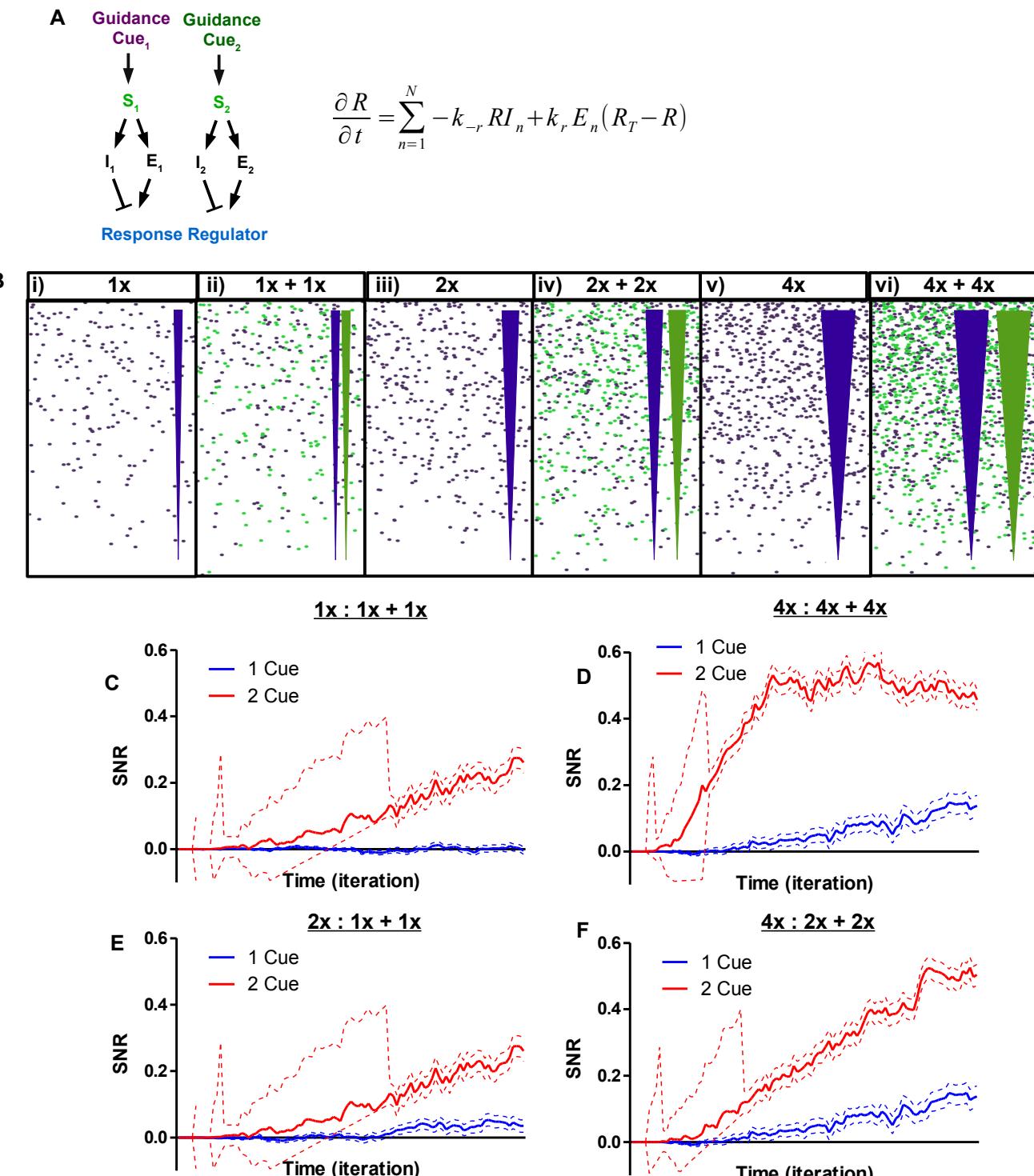


Figure 6: Exploration of the gradient sensing model and extension to multiple guidance cues **A)** The simplest model for extending the model to multiple cues. Two guidance cues signal through their specific receptors and intracellular signaling molecules, and converge on the same response regulators in an additive manner. The model is formalized to the right, with the same symbols as in Figure 5C. **B)** Panels i-vi visually represent the gradients used in the simulations, included as a reference for C-F. **C)** When the concentration is low, there is no asymmetric response for 1 cue, but moderate response with 2 cues. **D)** At a higher concentration (4x), there is a stronger response with 1cue than seen in D, however the combined gradient immediately outperforms the single gradient. **E)** When the simulation is controlled for the total number of guidance molecules, the 2 cue gradient still outperforms the single cue gradient. **F)** The same is true when the concentration is increased, the response of the combined gradient is a clear example of synergy. Dotted lines represent the SEM of 1000 simulations for each condition.