A MODEL FOR AXON GUIDANCE:
SENSING, TRANSDUCTION AND MOVEMENT

GIACOMO ALETTI, PAOLA CAUSIN, AND GIOVANNI NALDI

Abstract. Axon guidance by graded diffusible ligands plays a crucial role in the developing nervous system. In this paper, we extend the mathematical description of the growth cone transduction cascade of [2] by adding a model of the gradient sensing process related to the theory of [6]. The resulting model is composed by a series of subsystems characterized by suitable input/output relations. The study of the transmission of the noise-to-signal ratio allows to predict the variability of the gradient assay as a function of experimental parameters as the ligand concentration, both in the single and in the multiple ligand tests. For this latter condition, we address the biologically relevant case of silencing in commissural axons. We also consider a phenomenological model which reproduces the results of the experiments of [32]. This simple model allows to test hypotheses on receptor functions and regulation in time.

1. Introduction

In the developing nervous system, axons find the targets they will innervate navigating through the extracellular environment. Pathfinding crucially relies on chemical cues and, among the others, guidance by gradients of diffusible ligands plays a key role (see, e.g., [33, 27, 31]). Detection and transduction of navigational cues is mediated by the growth cone (GC), a highly dynamic structure located at the axon tip [15, 14]. The cascade that leads to motility decisions is initiated by binding of the ligand with receptors located on the GC surface and on filopodia, thin filaments that protrude out from the distal part of the GC.

the standard benchmark chemotaxis assay studies in vitro the response of GCs exposed to steady graded concentrations of a single attractive/repulsive ligand [36, 37, 26]. Axon turning angles are measured after a certain time interval from the onset of the gradient. Different mathematical and computational models have been developed to model this phenomena. In [12] and in the successive paper [35], the

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differential receptor binding across the GC diameter is connected to the likelihood of generating new filopodia. Filopodia production is enhanced/inhibited in the angular sector facing the attractant/repellent source. This effect represents a positive feedback mechanism. The new orientation of the GC is a combination of the previous orientation plus a function of the actual angle of maximum receptor binding. The two contributions are weighted the 97% and 3%, respectively, thus introducing an inertial (memory) effect. In [1], a step toward the introduction of intracellular mechanisms is carried out by relating the angular distribution of filopodia to the angular variation of ionic calcium diffused in the periphery of the GC. In [16, 17], guidance is driven by steady–state diffusible chemoattractants and chemorepellants, as well as by homophilic axon–to–axon attraction, for which a diffusive mechanism is supposed to exist as well. In [25], a system of ordinary differential equations describes the deterministic macroscopic motion of the GC and the dynamical evolution of its internal state, respectively.

In this article, we consider a model which extends the one proposed in [2]. The chemotactic GC system is described as a series of functional subsystems, ranging from gradient sensing to signal transduction, down to motion actuation. A characteristic time is singled out for each subsystem, representing the fact that independent concentration measures by receptors, internal reorganization preceding motion and discernible axon turning act on separated temporal scales, from the smaller to the larger one. The mathematical model describes input/output relations of signals of each functional subsystem, without reproducing intracellular chemical processes. The biological situation we address is the in vitro exposure to multiple diffusible cues, which interaction substantially modifies the GC response. This setting is representative of the case of commissural axons (see Fig. 1). In a first phase, these axons are attracted to the nervous system midline by a gradient of the protein netrin–1, but, after crossing the midline, receptors for the repellent Slit protein are upregulated and loss of response to netrin occurs, despite the fact that expression of the receptor for this latter ligand is maintained. This sequence of events leads axons to definitely depart from the midline to which they were attracted before (see [19, 7, 21, 22]). At our knowledge, guidance for commissural axons has been mathematically dealt with only in the paper [11], but a different aspect than the present work has been considered there. Namely, in [11] a theoretical model is proposed to explain sorting of commissural axons after crossing the midline due to the expression of different subfamilies of Slit receptors. Here, we focus rather on the
hypothesis of [32] that, in Drosophila but likely in vertebrates as well, the abrupt change of behavior of commisural axons is due to a gating effect of Slit receptors belonging to the Rondabout family [18] (Robo) on netrin receptors belonging to the Deleted in Colorectal Cancer family [20] (DCC). In [32], turning angles of axons after 1h of in vitro exposure to a gradient of netrin–1, Slit, or netrin–1 combined with Slit were measured. The results show that at Drosophila developmental stage 22, netrin–1 causes a net attraction of axons towards the source, while Slit as well as netrin–1 combined with Slit do not produce significant turnings. Moreover, at developmental stage 28, axons do not seem to be responsive any more to netrin–1, whilst they are strongly repelled by Slit and by netrin–1 combined with Slit. The conceptual idea to explain this behavior is that the silencing effect of Robo receptors on DCC receptors is partly or entirely responsible for the loss of responsiveness of commissural axons to netrin–1 (see, in particular, [32, Discussion]). This amounts to say that the different responsiveness is to be related to events occurring in the very early stages of the transduction chain.

As a matter of fact, mechanisms like the one illustrated above are still far to be completely unveiled. For example, how precisely ligand–receptor binding is converted into an intracellular signal is still a research issue. At present, receptor activation can be only monitored by observing biological responses, such as changes in neurite outgrowth [18]. This motivates the use of theoretical and computational models. In particular, the emerging area of the analysis of the cellular transduction system as a device that has to make decisions based on imperfect information about the environment can provide hints about the characteristics of the hidden processes of the transduction process. Imperfect information arises due to fluctuations in the field signalling molecules as well as throughout the entire GC intracellular network (for a discussion on this topic in eukaryotic cells or bacteria chemotaxis, see, e.g., [24, 28, 4, 3]). In [2], a study has been carried out on the propagation throughout the GC transduction cascade of the main statistical indexes relating signal and noise in guidance. Here, we extend this technique introducing a more detailed modeling of ligand–receptor binding and relating this process to the transduction mechanism. The study of the transmission of the noise-to-signal ratio allows, on the one hand, to predict the variability of the gradient assay as a function of experimental parameters as the ligand concentration, both in the single and in the multiple ligand tests. Experimental settings are indicated that produce most significant results (for example in the differentiation of responses). On the other hand,
Commissural axons are first attracted by netrin proteins secreted by the nervous midline cells. After crossing the midline, Slit receptors are upregulated and loss of response to netrin occurs, despite maintaining expression of DCC receptors. Axons are eventually repelled by the midline [19, 7, 21, 22].

This approach provides mathematical tools to address the issue of whether receptor silencing in commissural axons can explain loss of organized turning response.

In this work, we also consider a phenomenological model which reproduces the macroscopic outcome (turning angles) of the experiments of [32] on commissural axons. This simple model allows to test hypotheses on receptor functions and regulation in time.

The rest of the paper is organized as follows. In Sect. 2, we illustrate the model adopted for describing the axon chemotaxis, discussing the mathematical representation of the Sensing Device, Intracellular Transduction and Motor Actuator subsystems. In Sect. 3, we introduce the statistical indexes that will be used to characterize the system performance. In Sect. 4, we perform numerical simulations of the single and multiple ligand chemotactic assays, and we discuss the results. In Sect. 5, we present a simplified phenomenological model of axon response in presence of multiple cues that macroscopically reproduces the behavior of commissural axons.

## 2. Model of axon chemotaxis

The model of axon chemotaxis we consider, introduced in [2], provides a synthetic mathematical representation of the transduction cascade of the GC. Different subsystems are identified, which lead from sensing of ligand concentration gradients to motion (see Fig. 2). The model is especially tailored for studying 2D in vitro...
gradient assays, but it can be easily extended to deal with the 3D in vivo conditions. Measures of concentration differences in the environment are produced by the Sensing Device Subsystem (SDSys). The Intracellular Transduction Subsystem (ITSys) processes the input from the SDSys producing a signal which, through the Motor Actuator Subsystem (MASys), causes the deviation of the GC trajectory. The gradient sensing process takes place in a time of the order of tenths of seconds, signal transduction and internal reorganization in a time of the order of a few minutes, trajectory deviations in a time of the order of tenth of minutes.

2.1. Model of the gradient sensing function. Gradient sensing in axon guidance seems to fit a spatial mechanism (on this issue, see the discussion of [13]): GCs compare spatial differences in ligand concentration (e.g., front versus rear part) to determine the direction and the intensity of the gradient. The model we propose stems from the work of Berg and Purcell [6] on small sensing devices. Receptors, that play the role of sensing devices, are distributed all along the GC surface and the filopodia. Here we suppose that $N_1$ receptors are concentrated on the side of the GC facing the ligand source and $N_2$ receptors lie on the other side (see Fig. 3, left). If each receptor has a binding site capable of binding one molecule of ligand at a time, the expected time average occupation $\bar{p}$ of the receptor itself is linked to the local ligand concentration $c$ by

$$\bar{p} = \frac{c}{c + k_D},$$

where $k_D$ is the ligand dissociation constant. The history of the $i$-th site located on side $j = 1$ or $j = 2$ is described by a function $p_j^{(i)}(t)$ that assumes value 1 when the site is occupied and 0 when it is empty. The information about the surrounding concentration is then represented by the processes $p_j^{(i)}(t)$ recorded for a sampling
Figure 3. Left: a gradient of chemoattractant is established across the GC sides 1 and 2. The binding state (time average occupation) of the \( N_1 \) and \( N_2 \) receptors provides an estimate of the concentration difference. Right: ligands \( X \) and \( Y \) are present at the same time and they bind to their respective receptors (represented with a different symbol). Hierarchical interaction of receptors may take place and alter the GC turning response.

The difference in time occupancy \( \hat{\Delta p} = p_1 - p_2 \) provides an estimate of the difference of concentration across sides 1 and 2. In Sect. 2.1.1 and 2.1.2, we provide a model for the processes \( p_1^{(i)} \) in the case of single and multiple ligands, respectively.

2.1.1. The receptor binding process with one ligand. The binding of each receptor to a molecule of ligand is assumed as in [6] to be a continuous Markov chain on the state space \( S = \{ f, b \} \) (\( f \) = unbound, \( b \) = bound) with transition rate matrix

\[
Q = \begin{pmatrix}
-\frac{1}{\tau_f} & \frac{1}{\tau_f} \\
\frac{1}{\tau_b} & -\frac{1}{\tau_b}
\end{pmatrix},
\]

where \( \tau_b \) (resp. \( \tau_f \)) is the average time the receptor remains bound to (resp. unbound from) a molecule of ligand. The time \( \tau_b \) is estimated in [6, Eq.44] as

\[
\tau_b = (4Dsk_D)^{-1},
\]

\( D \) being the ligand diffusion constant and \( s \) the effective radius of the receptor. The state of a receptor is supposed to be statistically independent on the processes...
taking place at the other binding sites. A receptor switching from state \( f \) to \( b \) (resp. from state \( b \) to \( f \)) will remain in that state for a random time distributed as an exponential law of parameter \( \tau_b \) (resp. \( \tau_f \)), independent on its previous history. The characteristic times \( \tau_f \) and \( \tau_b \) are related to the average occupancy via the Ergodic Theorem \( \bar{P} = \tau_b / (\tau_b + \tau_f) \).

2.1.2. The receptor binding process with multiple ligands: silencing effect. When more ligands are present but non interacting, the binding processes are independent. Each process can be studied as in indicated in the previous section. We consider in the following the case of two ligands \( X \) and \( Y \). The transition matrix (3) of the joint process becomes

\[
Q_{XY} = \begin{pmatrix}
-(\frac{1}{\tau_f} + \frac{1}{\tau_f}) & \frac{1}{\tau_f} & \frac{1}{\tau_f} & 0 \\
\frac{1}{\tau_b} & -\left(\frac{1}{\tau_f} + \frac{1}{\tau_b}\right) & 0 & \frac{1}{\tau_f} \\
\frac{1}{\tau_b} & 0 & -\left(\frac{1}{\tau_f} + \frac{1}{\tau_b}\right) & \frac{1}{\tau_f} \\
0 & \frac{1}{\tau_b} & \frac{1}{\tau_b} & -\left(\frac{1}{\tau_f} + \frac{1}{\tau_b}\right) & \frac{1}{\tau_f}
\end{pmatrix},
\]

with state space \( S = \{f_X f_Y, b_X f_Y, f_X b_Y, b_X b_Y\} \). If one receptor of type \( Y \) in bound state can silence one receptor of type \( X \) in bound state, in the resulting process, the state \( b_X b_Y \) is then split into the two states \( b_X^b b_Y \) and \( b_X^b b_Y \), where the apex \( s \) (resp. \( u \)) stands for silenced (resp. unsilenced). The time that would have been spent in the state \( b_X b_Y \) in the non interacting case is now spent switching between the two states \( b_X^s b_Y \) and \( b_X^u b_Y \) with exponential laws of time parameter \( \tau_{on} \) and \( \tau_{off} \), respectively. The corresponding transition matrix on the state space \( S = \{f_X f_Y, b_X f_Y, f_X b_Y, b_X^s b_Y, b_X^u b_Y\} \) reads

\[
Q_{XY}^{\text{int}} = \begin{pmatrix}
-(\frac{1}{\tau_f} + \frac{1}{\tau_f}) & \frac{1}{\tau_f} & \frac{1}{\tau_f} & 0 \\
\frac{1}{\tau_b} & -\left(\frac{1}{\tau_f} + \frac{1}{\tau_b}\right) & 0 & \frac{1}{\tau_f} \\
\frac{1}{\tau_b} & 0 & -\left(\frac{1}{\tau_f} + \frac{1}{\tau_b}\right) & \frac{1}{\tau_f} \\
0 & \frac{1}{\tau_b} & \frac{1}{\tau_b} & -\left(\frac{1}{\tau_f} + \frac{1}{\tau_b}\right) & \frac{1}{\tau_f}
\end{pmatrix},
\]

2.2. Model of the Intracellular Transduction. Intracellular transduction is a highly complex network. Here, we do not consider single physical processes, but we directly model the input/output relation of the subsystem. A gradient of chemoattractant (resp. chemorepellent) concentration orients the GC motion toward (resp. away from) the direction of the concentration source. According to a mechanical description, we ascribe the trajectory deviation to an equivalent action vector \( \tilde{P} \). This latter quantity is continuously compared against a vector \( \tilde{P} \).
produced by the SDSys, directed along the maximum gradient and related to the difference in receptor occupancy \( \Delta p \) (this latter issue will be addressed more in detail later). The output of the ITSys results from the new information \( \tilde{P} \) and from a memory effect which damps the response

\[
\mathbf{P}_{t+\delta t} = (1 - \lambda)\mathbf{P}_t + \lambda(\tilde{\mathbf{P}} + \tilde{\eta})
\]

(7)

where \( \lambda \) is a weighting factor (memory effect, see also [35, Sect. Mathematical models]), \( \tilde{\mathbf{P}} = \mathbf{P} + \sigma_s \sqrt{\frac{2}{\lambda}} \mathbf{Z}_s, \tilde{\eta} = \sigma_\eta \sqrt{\frac{2}{\lambda}} \mathbf{Z}_\eta \), where \( \mathbf{P} = E(\tilde{\mathbf{P}}) \), \( \mathbf{Z}_s \) and \( \mathbf{Z}_\eta \) are two-dimensional standardized random vectors independent of \( \mathbf{P}_t \), \( \sigma_s \) and \( \sigma_\eta \) are volatility parameters and the factor \( \sqrt{\frac{2}{\lambda}} \) appears due to a normalization choice. The contribution \( \tilde{\eta} \) represents a noise term purely coming from the intracellular transduction mechanism and it is not dependent on the external concentration field. The time interval \( \delta t \) is chosen to be long enough so that the new contribution \( \tilde{\mathbf{P}} + \tilde{\eta} \) can be assumed statistically independent from \( \mathbf{P}_t \).

Let \( \lambda = \frac{\delta t}{\tau} \), \( \tau \) being a persistence time characteristic of internal signal transduction. Then, Eq. 7 can be reformulated as

\[
\delta \mathbf{P} = -\frac{\mathbf{P}_t - \tilde{\mathbf{P}}}{\tau} \delta t + \sigma \sqrt{\delta t} \sqrt{\frac{2}{\tau}} \mathbf{Z},
\]

where \( \sigma = \sqrt{\sigma_s^2 + \sigma_\eta^2} \) is the process volatility. The term \( \delta \mathbf{P} = \mathbf{P}_{t+\delta t} - \mathbf{P}_t \) represents an incremental “kick” on the trajectory (see also [8]). Since the characteristic time scale of ITSys is larger than \( \delta t \), we can adopt as a model of the input/output relation of this subsystem the following continuous generalized Ornstein–Uhlenbeck (OU) process, which is assumed to obey to Itô calculus (see, e.g., [5])

\[
d\mathbf{P}_t = -\frac{\mathbf{P}_t - \tilde{\mathbf{P}}}{\tau} dt + \sigma \sqrt{\frac{2}{\tau}} d\mathbf{W}_t,
\]

(9)

where \( \mathbf{W}_t \) denotes a two-dimensional Wiener process. When \( \tilde{\mathbf{P}} \) does not depend on time, \( \sigma \) is constant and the solution of (9) reads

\[
\mathbf{P}_t = \tilde{\mathbf{P}} + (\mathbf{P}_0 - \tilde{\mathbf{P}}) e^{-t/\tau} + \int_0^t \sigma \sqrt{\frac{2}{\tau}} e^{(s-t)/\tau} d\mathbf{W}_s.
\]

(10)

Relation (10) shows that the mean value of \( \mathbf{P}_t \) tends exponentially fast in time to \( \tilde{\mathbf{P}} \). Moreover, Itô’s lemma [5] implies that, at steady–state, \( \mathbf{P}_t \) is a bivariate Gaussian distribution subjected to an isotropic random perturbation of bounded variance \( \sigma^2 \).
2.3. Model of the Motor Actuation. We suppose that the action $P_t$ coming from Eq. (9) induces an acceleration only along the direction transversal to the trajectory (see also [25] for a similar hypothesis). The law of the GC motion can be written as:

find for $0 \leq t \leq T$ the GC position $x_g = x_g(t)$, such that

$$
\begin{align*}
\dot{x}_g &= v_g, \\
\dot{v}_g &= |P_t| \sin \beta e^\perp, \\
dP_t &= -\frac{P_t - \hat{P}}{\tau} dt + \sigma \sqrt{\frac{2}{\tau}} dW_t, \\
x_g(0) &= x^0_g, \\
v_g(0) &= v^0_g, \\
P_0 &= P^0,
\end{align*}
$$

(11)

where $v_g = v_g e_g$ is the axon velocity vector ($v_g = 20 – 30 \mu m/h, [34]$), $e^\perp$ is the unit vector perpendicular to $e_g$, $x^0_g$ and $v^0_g$ are the given initial position and direction of the axon GC, $\hat{P}$ is the given initial equivalent force, $m$ is the GC equivalent mass, $\alpha$ is the angle that $e_g$ forms with the horizontal direction and $\beta$ is the angle between $e_g$ and $P_t$ (see Fig. 4).

Notice that in system (11), the effect of external gradient only concerns the direction of the unit vector $e_g$, leaving the velocity modulus constant. Moreover, notice that when $\hat{P} = 0$, the trajectory will not be deterministically deviated. This reproduces the physical fact that, in absence of ligand gradients and at least on in vitro experiments, axons tend to follow noised trajectories with no significant bias from their initial growth direction [9].

Eq. (11)\textsubscript{3} models the ITSys input/output relation, while Eq. (11)\textsubscript{1,2} model the MASys input/output relation. Eqs. (11) constitute a stochastic differential system and can be numerically solved by using a stochastic Runge–Kutta integration scheme (see for example [23]). Parameters of the model and their meaning are given in Table 1.

3. Study of the Coefficients of Variation in the Transduction Chain

In this section, we introduce statistical indexes that allow to characterize the degree of organization of the signal throughout the steps of the chemotactic system. The macroscopically observable quantities (as the turning angles in the gradient assay) allow to compute the indexes pertaining to the output of the last part of the
Figure 4. Notation for the Motor Actuation subsystem.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p_j^{(i)}$</td>
<td>time occupancy process of $i$-th receptor on side $j$ of the GC</td>
</tr>
<tr>
<td>$\Delta p$</td>
<td>estimate of difference in receptor time occupancy across the GC</td>
</tr>
<tr>
<td>$D$</td>
<td>Diffusion constant</td>
</tr>
<tr>
<td>$s$</td>
<td>Effective receptor radius</td>
</tr>
<tr>
<td>$k_D$</td>
<td>Dissociation constant</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Volatility parameter</td>
</tr>
<tr>
<td>$\tau_b, \tau_f$</td>
<td>average time of receptor in bound or free state</td>
</tr>
<tr>
<td>$\hat{P}$</td>
<td>New contribution due to the external gradient</td>
</tr>
<tr>
<td>$P_t$</td>
<td>Actual value of the contribution due to the external gradient with memory effect</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Noise due to the intracellular transduction</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Memory effect parameter</td>
</tr>
<tr>
<td>$\delta t$</td>
<td>Characteristic time of gradient sensing</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Characteristic time of intracellular transduction</td>
</tr>
<tr>
<td>$v_G$</td>
<td>GC velocity vector ($v_g$ velocity modulus, $e_g$ velocity direction)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Angle between the GC direction and the x-axis</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Angle between the GC direction and $P_t$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Measure of turning angle in benchmark experiments</td>
</tr>
</tbody>
</table>

Table 1. Model parameters and their meaning. For parameter values used to derive numerical results, see text.
we use the coefficient of variation, defined as the ratio between the standard deviation and the expected value of a stochastic distribution, to assess the weight of the fluctuating over the deterministic part of a signal arising from a subsystem input/output relation.

Experimentally recorded distributions of turning angles $\gamma$ provide data to estimate the coefficient of variation

$$CV_\gamma = \frac{\text{std}(\gamma)}{E(\gamma)},$$

which represents an information about the degree of organization of the GC macroscopic behavior. When $CV_\gamma \gg 1$, noise prevails on the signal and the motion is just a random walk. When $CV_\gamma \ll 1$, noise plays a very minor role and the motion is a deterministic path. When $CV_\gamma$ approaches values of the order of the unity, which is generally the case in GC chemotactic assays, noise and signal have almost the same weight. Local fluctuations do exist, but trajectories show a significant and detectable bias.

We now proceed back in the chain, relating $CV_\gamma$ to the coefficient of variation of $P_t$, (which is an hidden process) as

$$CV_{P_t} \approx \sqrt{\frac{t}{\tau}}CV_\gamma,$$

$\tau$ being the time parameter of the ITSys (see [2] for a detailed derivation of Eq. (13)). Observing that

$$E(\tilde{P} + \tilde{\eta}) = E(\tilde{P}) = E(P_t), \quad \text{Var}(\tilde{P} + \tilde{\eta}) = \frac{2\tau}{t} \sigma^2 = \frac{2\tau}{t} \text{Var}(P_t),$$

we further have

$$CV_{\tilde{P} + \tilde{\eta}} = \frac{\text{std}(\tilde{P} + \tilde{\eta})}{|E(P)|} = \sqrt{\frac{2\tau}{\delta t}}CV_{P_t} \approx \sqrt{\frac{t}{\delta t}}CV_\gamma,$$

where the time scale factor $\sqrt{t/\delta t} = \sqrt{1/\lambda}$ keeps into account the memory effect which weights the signal $\tilde{P} + \tilde{\eta}$ in $P_t$ (see Eq. (7)). We can now investigate the properties of the sensing function. With this aim, we introduce the quantity

$$\ell^2 := \frac{\text{Var}\tilde{P} + \tilde{\eta}}{\text{Var}\tilde{P}} = 1 + \frac{\text{Var}\tilde{\eta}}{\text{Var}\tilde{P}},$$

where the second relation at the right hand stems from the statistical independence of $\tilde{P}$ and $\tilde{\eta}$. Observe that $\ell^2 - 1 = \frac{\text{Var}\tilde{\eta}}{\text{Var}\tilde{P}}$ represents the ratio between the variability of the intracellular transduction subsystem and the variability of the gradient sensing subsystem. Using relations (15), (16) and the properties (14), we get

$$CV_{\tilde{P}} = \frac{CV_{\tilde{P} + \tilde{\eta}}}{\ell} \approx \frac{1}{\ell} \sqrt{\frac{t}{\delta t}}CV_\gamma,$$
which connects the experimentally observable coefficient of variation \( CV_\gamma \) with the coefficient of variation of the sensing process \( CV_\tilde{P} \). A link can be further drawn between the statistical indexes of \( \tilde{P} \) and the processes of single receptors introduced in Sect.2.1. Let \( \sigma_1 \) and \( \sigma_2 \) be the variances of a typical \( \frac{1}{\delta t} \int p_1^{(i)} dt \) or \( \frac{1}{\delta t} \int p_2^{(i)} dt \) process on side 1 or 2 of the GC. We have that \( \text{Var}_\tilde{\Delta}P = \text{Var}_{p_1} + \text{Var}_{p_2} = \frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2} \), and hence

\[
CV_{\tilde{\Delta}P} = \frac{\sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}}{E(\Delta P)}.
\]

When \( N_1 = N_2 = N \), the above relation reads

\[
CV_{\tilde{\Delta}P} = \frac{1}{\sqrt{N}} \frac{\sqrt{\sigma_1^2 + \sigma_2^2}}{E(\Delta P)} \propto \frac{1}{\sqrt{N}}.
\]

We assume that \( \tilde{P} \propto \tilde{\Delta}P \). Then, by Eq. (17), we can further obtain

\[
\ell = \sqrt{\frac{N t \delta t}{\delta t}} \frac{E(\Delta P)}{\sqrt{\sigma_1^2 + \sigma_2^2}} CV_{\gamma}.
\]

Numerical simulations based on the gradient sensing model can be used to evaluate quantities referred to \( \tilde{\Delta}P \) (see Sect. 4).

3.1. Statistical indexes for different experimental settings. The quantity \( CV_\gamma \) is macroscopically observable, but, generally, data are reported in literature in correspondence of specific experimental conditions. At our knowledge, only in [29] a systematic study is carried out testing the axon response for different concentrations and gradient steepness.

A sole fixed ligand concentration close to \( k_D \) is instead used in [32] in the gradient assays. For example, with ligand netrin–1 at stage 22, the value \( CV_\gamma \approx 0.25 \) is obtained [32, Fig.2B]. By Eq. (19), we can compute

\[
\ell_{k_D} = \sqrt{\frac{N t}{\delta t}} \frac{CV_{\gamma_{k_D}}}{CV(\Delta p_{k_D})} \approx 5\sqrt{N} \frac{|E(\Delta p_{k_D})|}{\sqrt{\sigma_1^2_{k_D} + \sigma_2^2_{k_D}}}.
\]

As a matter of fact, using Eq. (15) and (20), the statistical indexes of different experimental settings can be predicted. For example, we can consider the case
where a concentration of ligand different from $k_D$ is considered,

$$\left( \frac{CV_{\gamma|x}}{CV_{\gamma|k_D}} \right)^2 = \frac{\text{Var}_{\tilde{\mathbf{P}}_{x|k_D}} + \mathbb{E}_{\tilde{\mathbf{P}}_{x|k_D}}(E(\tilde{\mathbf{P}}_{x|k_D}))^2}{\text{Var}_{\mathbf{P}_{x|k_D}} + (\mathbb{E}_{\mathbf{P}_{x|k_D}}(E(\mathbf{P}_{x|k_D})))^2}.$$

$$= \frac{\text{Var}_{\mathbf{P}_{x|k_D}} + (\ell^2_{k_D} - 1)}{\ell^2_{k_D}} \left( \frac{\mathbb{E}_{\tilde{\mathbf{P}}_{x|k_D}}(E(\tilde{\mathbf{P}}_{x|k_D}))}{\mathbb{E}_{\mathbf{P}_{x|k_D}}(E(\mathbf{P}_{x|k_D}))} \right)^2.$$

We can also consider the case where arbitrary concentrations $x$ and $y$ of ligand of type $X$ and $Y$ are present and compute the ratio $CV_{\gamma|x,y}/CV_{\gamma|x,0}$, which allows to quantify the effect of silencing with respect to the unsilenced case for arbitrary concentrations.

4. Numerical simulations

In this section, we carry out numerical simulations using the mathematical model introduced in the Sect 2. Then, we compute the statistical indexes of the input/output relations of the subsystem as discussed in Sect. 3.

4.1. The single ligand case. We study the properties of the sensing process $\tilde{\Delta}p$. We set in the model of Sect. 2.1.1 $\tau_b = 0.83s$, concentration at the center of the GC ranging from 0.1nM to 100nM and gradient steepness of 2% across the GC diameter. The value of $\tau_b$ is computed from Eq. (4) considering the netrin–1 parameters $D = 10^{-7}cm^2/s$ [10], $k_D = 10nM$ [20, 30] and $s = 10\AA$ [13].

In Fig. 5, left, we plot the coefficient of variation $CV_{\Delta p}$ as a function of the ligand concentration. The variance is alternatively computed from:

1. the status $(0,1)$ of $N_1 = N_2 = 2000$ receptors for a number of binding events $\delta t/(\tau_b + \tau_f)$ (red bars)
2. the approximation of Berg and Purcell [6, Eq.(50)] ($N_1 = N_2 = 2000$ receptors, each with variance proportional to $\mathbb{P}(1-P)^2$, orange bars)
3. a Monte Carlo simulation (500,000 simulations) of the model of Sect. 2.1.1 (yellow bars)

We superpose in the same graph the quantity $E(\Delta P) = E(p_1) - E(p_2)$, which is analytically computed from the non-negative eigenvector of the transition matrix of the process, related to the probability at steadiness of each receptor to be in a
4.2. The multiple ligand case. We simulate the processes $p^{(i)}, i = 1, \ldots, N_1 + N_2$ for the receptors for ligand $X$ under the silencing effect of receptors for ligand $Y$. We use the model in Sect. 2.1.2 with $\tau_b^X = \tau_b^Y = 0.83\text{s}$, concentration at the center of the GC ranging from 0.1nM to 100nM for both ligands and gradient steepness of 2% across the GC diameter. The value of $\tau_b^X$ and $\tau_b^Y$ are computed from Eq. (4) considering for both netrin and Slit $D = 10^{-7}\text{cm}^2/\text{s}$, $k_D = 10\text{nM}$ and $s = 10\text{Å}$.

In Fig. 6 (resp. Fig. 7), we plot the ratio $\text{CV}_{\gamma_{|x,y}} / \text{CV}_{\gamma_{|k,D}}$ (resp. $\text{CV}_{\gamma_{|c,o}} / \text{CV}_{\gamma_{|k,D,o}}$) obtained from Eq. (21). The top graduation of each panel represents Slit concentration, while the bottom graduation represents netrin–1 concentration. Dependence on the number of receptors $N_1^X = N_2^X = N_1^Y = N_2^Y$ is shown in each group of bars (same legend as in Fig. 5, right panel).

4.3. Discussion of the results. We have analyzed the performance of the gradient sensing subsystem by introducing a model based on the Berg and Purcell theory.
Following this model, the maximum expected value of the output signal is attained at a concentration value corresponding to the dissociation constant (see Eq. (1)). However, the noise-to-signal ratio represented by $\text{CV}_{\Delta \rho}$ is monotonically decreasing (yellow bars of Fig. 5, left panel). An explanation of this trend may be found in the fact that, while $\tau_b$ is a chemical property independent on the ligand concentration, $\tau_f$ is instead a function of the concentration. The time $\tau_b + \tau_f$ represents the average time between two consecutive binding events. The quantity $\delta t/(\tau_b + \tau_f)$ relates to the number of binding events during time $\delta t$. Then, if one records the binding status of each receptor $\delta t/(\tau_b + \tau_f)$ times independently or performs a time average of the binding processes, very different behaviors are obtained (red bars vs. yellow bars of Fig. 5, left panel). In fact, the variance of a single binding measure is proportional to $\overline{p}(1 - \overline{p})$ (Bernoulli random variable), while the variance of the
Figure 7. Ratio $\frac{\text{CV}_{\gamma|x,\alpha}}{\text{CV}_{\gamma|x,0}}$. The top graduation of each panel represents Slit concentration, while the bottom graduation represents netrin–1 concentration (values in nM, logarithmic scale). Each group of bars contains results for different number of receptors (the same convention as in Fig. 5, right panel, is used). Groups of bars that do not appear, as in the rightmost part of the last panel in the bottom row, indicate that every form of organized directionality is lost.

As for the outcome of the entire system, in vitro experiments suggest that the highest guidance is observed for a ligand concentration equal to $k_D$ (see, e.g., [29, Fig.3c]), for which the most organized motion (most efficient response) is displayed. The ratio $\frac{\text{CV}_{\gamma|x}}{\text{CV}_{\gamma|x,D}}$ obtained from MonteCarlo simulations reproduces this property (see Fig. 5, right). The lack of monotonicity of this curve is due to the effect of the intracellular noise $\tilde{\eta}$, that we have supposed to be independent on the concentration field, produced in the transduction step. In the neighborhood of the dissociation constant, the sensing process signal is high, as well its variability ($\text{CV}_{\Delta p} \approx 1$). For ligand concentrations greater than the dissociation constant, the strength of the signal decreases, but the coefficient of variation remains of the
same order. This means that the variability has decreased. The contribution of the constant factor $\tilde{\eta}$ impacts more significantly for $c > k_D$ than for $c \simeq k_D$, producing the uprise of $CV_\gamma$ in the tail. Moreover, the parametrization with respect to the number of receptors suggests that a lower number of receptors produces a more reliable mechanism, in the sense that the quality of the signal does not change too much for different concentrations (cf. [29, Fig.3c]). A lower bound on the number of receptors, $\simeq 200$ with the data [32, Fig.2B], is provided by the relation $\ell^2 > 1$, which amounts to say that the intracellular contribution is adding noise (and not filtering out the signal).

The results obtained in the case of interacting ligands predict the behavior of the coefficient of variation of the turning angle in different conditions. The mathematical model makes the assumption that the silencing process is entirely due to the gradient sensing phase. At the same time, the model suggests the most favorable experimental settings in order to validate this hypothesis. More precisely, too large coefficients of variation should be avoided, since they do not carry precise information about the origin of the noise. Figs. 6 and 7 allow to select a couple of netrin–1 and Slit concentrations, which lead to a coefficient of variation $\approx 1$. In such a condition the hypothesis underlying the mathematical model is verifiable by in vitro experiments.

5. Phenomenological study of axon response to multiple cues

In this section, we propose a simplified phenomenological model to reproduce axon response in presence of multiple cues. This model does not consider the detail of the gradient sensing process. We deal with the case where two cues $X$ and $Y$ are interacting. In order to model the resulting contribution $\tilde{P}$ in Eq. (7), we introduce the time dependent weights $w_X$ and $w_Y$, related to the activity of receptors binding to cue $X$ and $Y$, respectively, and we set

\begin{equation}
\tilde{P} = w_X \tilde{P}_X + w_Y \tilde{P}_Y,
\end{equation}

where $\tilde{P}_X, \tilde{P}_Y$ arise from the single $X$ and $Y$ cues.

The time functional dependence of the weights $w_X$ and $w_Y$ is supposed to be governed by the following differential system which represents the evolution at
different developmental stages of the receptor activation

\[
\begin{align*}
\theta_X \frac{\partial w_X}{\partial t} &= \begin{cases} 
0 & \text{for } 0 < t < t_{a,X}, \\
\text{sign}(t_{d,X} - t)w_X^\beta (1 - w_X^\beta)^\beta - aw_Y w_X^\beta, & \text{for } t_{a,X} \leq t,
\end{cases} \\
\theta_Y \frac{\partial w_Y}{\partial t} &= \begin{cases} 
0 & \text{for } 0 < t < t_{a,Y}, \\
\text{sign}(t_{d,Y} - t)w_Y^\beta (1 - w_Y^\beta)^\beta - bw_X w_Y^\beta, & \text{for } t_{a,Y} \leq t,
\end{cases}
\end{align*}
\]

\[w_X(0) = w_{X0},\]
\[w_Y(0) = w_{Y0},\]

where \(\theta_X, \theta_Y\) are characteristic times for receptors of ligand X and Y, \(t_{a,X}, t_{a,Y}\) and \(t_{d,X}, t_{d,Y}\), are receptor activation and de-activation times, respectively, and where \(a, b\) are influence coefficients that represent the competition effects (if \(a > 0\), signals from type–Y receptors down-regulate signals from type–X receptors, if \(a < 0\), signals from type–Y receptors up-regulate signals from type–X receptors, and the same for \(b\)). The parameter \(\beta\) is an exponent in the range \((0,1)\). Notice that in this range the solution of the above system is not unique, the sets \((t, 0)\) and \((t, 1)\) being made of branching points. In any point of lack of uniqueness of the solution, the branch of the bifurcation in the image range \((0,1)\) is chosen. The choice of \(\beta \in (0,1)\) allows for obtaining a finite growth time for any initial positive condition. Analogously, for \(t > t_d\), a finite decay time is observed if \(y(t_d) \leq 1\); a corner point appears when \(y(t_d) < 1\).

We use system (23) in the model (22) to study the behavior of commissural axons (substance X being netrin–1 and substance Y Slit). As illustrated in [32], in the case of sole netrin–1 gradient, the decay time must occur between stage 22 and stage 28; in the case of sole Slit gradient, full activation of the receptors occurs after stage 22. These conditions may be reproduced by choosing \(\beta = 0.5, \theta_X = 0.5, \theta_Y = 2, t_{a,X} = 4, t_{a,Y} = 20.5\) and \(t_{d,X} = 25, t_{d,Y} = 30\). The behavior of sole netrin–1 and Slit receptors is represented in Fig. 8, continuous lines. Both \(w_X\) and \(w_Y\) follow a power-law growth with saturation till the respective decay time. In case of interaction, Slit silences netrin–1 while no effect is exerted by netrin–1 on Slit. We choose here for the simulation \(a = 5, b = 0\). The silenced behavior of netrin–1 receptors is represented in Fig. 8, dashed line. In this case, the decay time of \(w_X\) occurs much before due to the silencing effect of Y receptors.
Figure 8. Time evolution of the weight factors $w_X$ and $w_Y$ obtained from system (23).

Figure 9. Simulated final axon turning angles (°) in response to netrin–1, Slit and combined netrin–1 and Slit at different development stages (cf. [32, Fig.2B,2E]).

The relation between an assigned concentration gradient and the vector $\hat{P}$ can be found by experimental results using the relation [2]

\[ R_{c,\text{min}} = \frac{v^2}{|\hat{P}|}, \]

where $R_{c,\text{min}}$ is the minimum radius of curvature of the trajectory (see also [25, Eq. (28)]).

The calibration of the model is obtained by solving problem (11) coupled with system (23) using the test setting of [32]. Final turning angles after 1h of exposure to the gradient, computed with parameters corresponding to stage 22 and stage 28, are reported in Fig. 9.
This simple phenomenological model indicates that at stage 22 the repellent effect of Slit is weak (corresponding to a low weighting factor), whilst its silencing effect is relevant. This condition suggests that Slit activates slowly, but significantly before stage 22, because it needs a suitable time to cause decrease of the netrin–1 weight by hierarchical interaction.

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