Amplification and temporal filtering during gradient sensing by nerve growth cones probed with a microfluidic assay

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SUPPLEMENTARY MATERIALS

1. Effect of the shear stress on the morphology of a neuronal growth cone

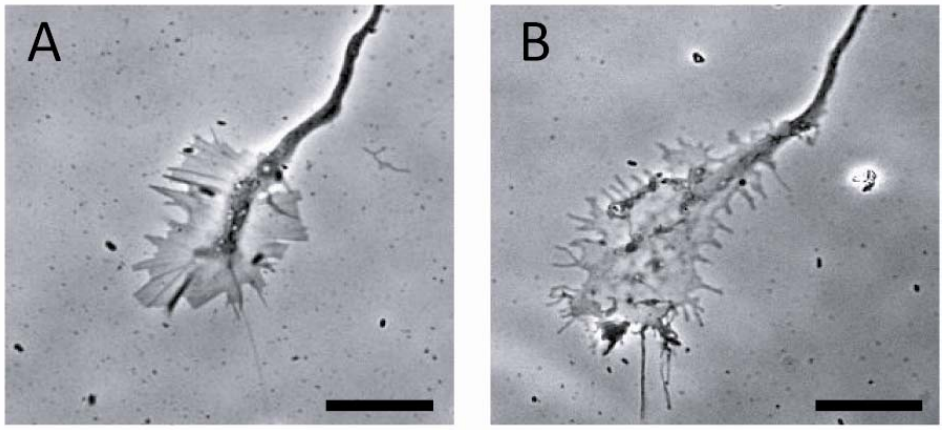

Fig. S1: *DRG growth cone in a flowing microchannel (vertical, up-down flow). A) At a mean flow speed of 5 µm.s^{-1} (approx. shear stress of 5.10^{-4} N.m^{-2}). B) Less than 2 minutes after an increase of the flow speed to 500 µm.s^{-1} (approx. shear stress of 5.10^{-2} N.m^{-2}). Scale bars 20 µm.*

2. Modeling of the diffusive properties in the microdevice

To test the performance of our devices, we have implemented a simple two-dimensional description of the diffusion in the microchamber (**Fig. S2 A**). We considered the diffusion equation in the *y, z* coordinates – the x axis is the flow direction in the microchannel – for a solute of diffusion coefficient *D* and concentration *c(y,z,t)* .

$$\frac{\partial c}{\partial t} = D \cdot \left(\frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2} \right)$$

We modeled the membrane as a semi-absorbent boundary with permeability κ and the walls and the glass coverslip as reflective boundaries:

$$D \cdot \left. \frac{\partial c}{\partial z} \right|_{z=0^+} = \kappa \cdot (c(y, z=0^-, t) - c(y, z=0^+, t))$$

$$\left. \frac{\partial c}{\partial y} \right|_{\pm w/2} = 0 \quad \text{and} \quad \left. \frac{\partial c}{\partial z} \right|_{L} = 0$$

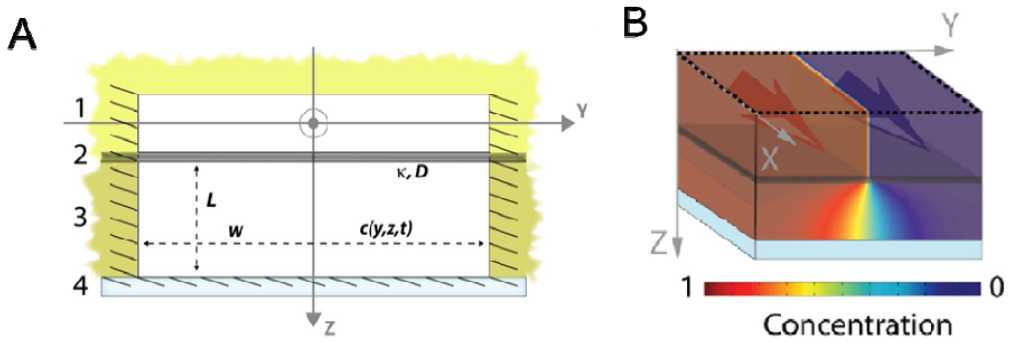

Fig. S2: *A) Scheme used for modeling diffusive processes in the microdevice. The microfluidic channel (1) imposes the concentration condition c(y,z=0,t) at the membrane. The membrane (2) transfers the concentration to the micro-well (3) assuming a semi-permeable boundary with permeability coefficient κ. The solute diffuses freely in the micro-chamber from the membrane (2) to the coverslip (4). B) The gradient generated by diffusion in the micro-well can be simulated using finite-elements modeling.*

In our experiments, the membrane was thin compared to the chamber height (ratio~10) and had pores with characteristic size 400 nm, much larger than the size of GABA ligands (MW 103, D_{GABA} = 700 $\mu m^2/s$) or fluorescein markers (MW 389, $D_{Fluorescein}$ = 450 $\mu m^2/s$). Therefore, we assumed the limit of infinite permeability κ, where the concentration profile in the microchannel $c(y,z=0^-,t)$ was completely transferred to the other side of the membrane within the microchamber.

3. <u>Generation of an exponential concentration profile.</u>

For our gradient sensing assays, we used a Y-shaped geometry with a co-flow in the microchannel (**Fig. S2 B**). One inlet was filled with a solution of GABA (C_0 = 200 µM) and fluorescein (0.5 µM) in air-buffered medium, and the other with medium only. In order to obtain a gradient with fixed relative steepness $\delta = \nabla c/c$ over a large region, we carefully adjusted the relative pressure between the two streams with pressure regulators (OEM-EP 0-2 psi, Parker) and placed the interface at 1/5 of the channel width (y_0 = 200 µm). For the ligand, this corresponds to a profile $c(y,z=0^+) = C_0(1-H(y-y_0))$ where $H(y)$ is the Heaviside function (equal to 0 for $y < 0$ and 1 for $y > 1$) and y_0 is the position of the interface between the two streams. The diffusion equation was numerically solved using a partial differential equation solver (Matlab, MathWorks), for an initially empty chamber $c(y,0<z<L,t=0)=0$,

and with boundary condition $c(y,z=0^{+})=C_0(1-H(y-y_0))$. After diffusion of the ligand through the chamber height (L = 200 µm), the computation of the profile at the coverslip surface $c(y,L,t)$ resulted into an exponential profile (with constant relative steepness δ) in the central part of the coverslip (250 < y < 800 µm) (**Fig. S3 A**). For each measured GC, we determined the absolute concentration and steepness (**Fig. S3 B**).

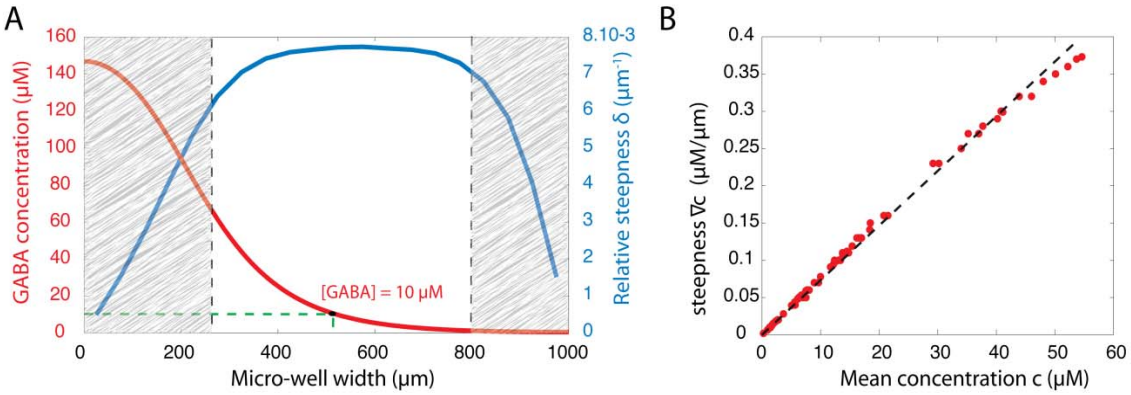

Fig. S3*: Generation of an exponential gradient in the micro-well. A) Simulation of the concentration profile at the bottom of a 200 µm high micro-well. A 200 µm wide coflow of 200 µM GABA is generated in the microchannel. B) Values of the gradient parameters (mean concentration and steepness) for the measured growth cone (red dots). The slope corresponds to δ = 7.5±0.4 10-3 µm-1.*

4. Determination of the parameters h and K

In our experimental conditions, the gradient was sufficiently shallow and the amplitude *A(c)* could be approximated by a function proportional to *f'(c)* (Fig. 6 D). With a least square optimization procedure, we found that the best fit was obtained for h_m = 2.1 and K_m = 18 µM.

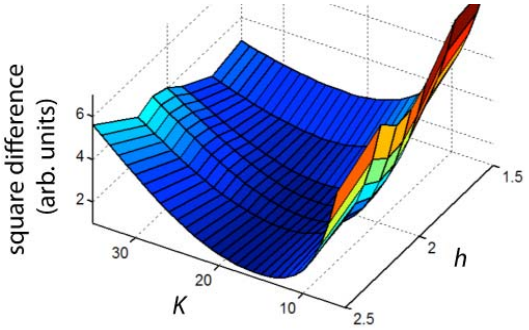

Fig. S4: *Square difference between the data A(c) in the experiments and a curve proportional to f'(c). The minimum value is for* h_m = 2.1 and K_m = *18 µM*