AMPLIFICATION AND TEMPORAL FILTERING DURING GRADIENT SENSING BY NERVE GROWTH CONES REVEALED WITH A SHEAR FREE MICROFLUIDIC DEVICES

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ABSTRACT

Nerve growth cones (GCs) are chemical sensors that convert graded extracellular cues into oriented axonal motion. Ensuring a sensitive and robust GC response to directional signals requires the ability to amplify and filter external gradients. However, our knowledge of how these signal processing tasks are performed remains sparse.

Here we present a shear-free gradient-generating microfluidic device with an architecture that greatly facilitates the interface of cultured neurons with microcircuits. With this device, we probe the information-processing capabilities of single GCs during GABA directional sensing, and relate these properties to the kinetics and the lateral dynamics of $GABA_A$ receptors.

KEYWORDS

Axonal guidance, gradient sensing, single receptor imaging, shear-free microfluidic.

INTRODUCTION

This paper reports chemotaxis measurements on neurons obtained using newly developed membrane-based microfluidic devices that allow the generation of spatially and temporally controlled gradients in a shear-free environment [1]. In these microcircuits, soluble chemical compounds can diffuse out of the channels through well-defined and spatially organized microfabricated porous openings (Fig. 1), thus allowing stable diffusible concentration gradients and complex dynamic chemical landscapes under shear free conditions. Moreover, its simple architecture greatly facilitates the interface with neurons cultured in microwells in normal conditions, removing the need for long term culture under perfusion and multi-step labeling in microchannels.

Using this device, we have probed the information-processing capabilities of single nerve growth cones (GCs) during GABA directional sensing. Indeed, GCs are chemical sensors that convert graded extracellular cues into oriented axonal motion. Ensuring a sensitive and robust response to directional signals requires the ability to amplify and filter external gradients. However, our knowledge of how these signal processing tasks are performed at the single cell level remains sparse [3]. This is largely due to the limitations of conventional guidance assays that have precluded systematic measurements of the GC output response to variable input gradients.

By coupling precise gradient generation and measurement at the single molecule level of the polarization of $GABA_A$ chemoreceptors at the GC membrane, we find that GCs act as: (i) signal amplifiers over a narrow range of concentrations, (ii) low-pass temporal filters with a cut-off frequency independent of stimuli conditions. Furthermore, thanks to quantitative computational modeling, we relate these systems-level properties to the saturable occupancy response and the lateral dynamics of $GABA_A$ receptors, and, thereby, provide an integrative view of individual GCs as sensing devices.

EXPERIMENT

To investigate the dynamics of GABAAR spatial organization under a controlled gradient of guidance cues, we designed shear-free microfluidic devices that overcame the limits of conventional micropipette or flow-based microfluidic assays. We have recently developed a simple fabrication process to integrate a track-etched porous membrane in a microcircuit made of UV polymerizable glue [2]. We designed a microsystem based on two separate parts (Fig. 1 A): i) a fluidic microcircuit integrating a membrane interface, ii) an open micro-well on a glass coverslip in which cells are plated. The two parts of the device are assembled just before the experiment. Neurons can thus be cultured and labeled using standard protocols.

Upon assembly, the porous membrane acts as a hydrodynamic barrier, separating the fluidic channels where solutes are circulating from the micro-well where the cells are growing [4], and the microfluidic circuit allows the generation of spatio-temporally controlled, yet purely diffusive, gradients in the micro-well (Fig. 1 B). Different design of the microcircuit allows the generation of complex gradient [1] or parallelization of experiments (Fig. 1 C). In the following, we use a simple co-flow design to generate the gradient in a single micro-well. Dissociated spinal cord neurons are cultured in the micro-well and then submitted to a diffusive gradient of GABA (Fig. 2 A). Before experiments, single $GABA_A$ chemoreceptors diffusing at the GC membrane are labeled using Quantum Dots (Fig. 2 A). As already observed in previous experiments with micropipettes [5], in the presence of the gradient we observe that $GABA_A$ receptors start polarizing towards the higher concentration of GABA (Fig. 2 B), thus amplifying the external signal.

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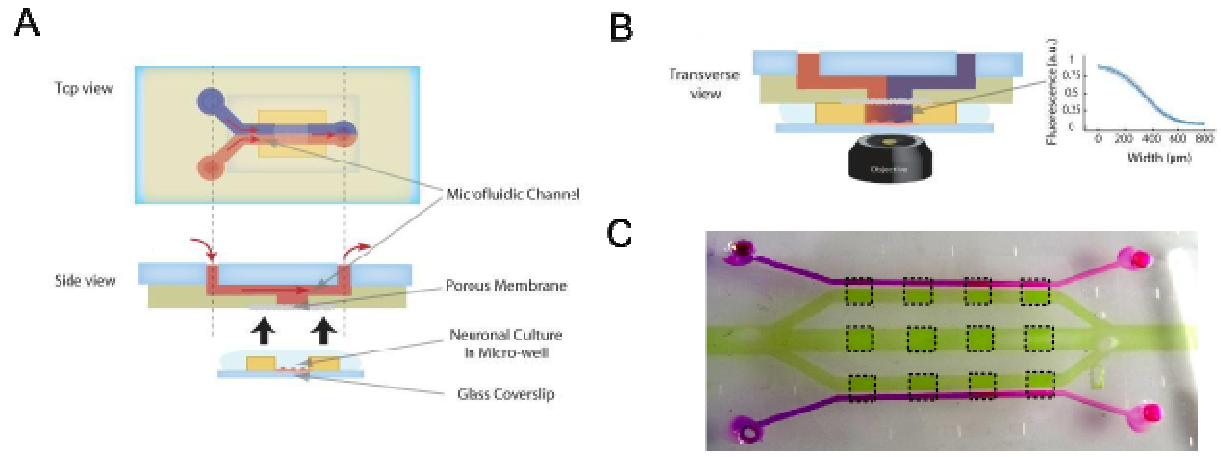

Figure 1: Membrane-based shear-free microfluidic device. A) Top and side schematic views of a simple microdevice allowing the generation of a gradient in a single micro-well. B) Transverse view of the device after gradient formation in the micro-well containing cultured neurons. The gradient profile has been obtained by confocal fluorescence microscopy of a FITC gradient. C) A more complex microcircuit to generate multiple gradients in independent wells (8 wells with possibly 2 different solutes, red and green) and a control condition (4 wells of medium, in green).

From all the analyzed GCs (94 out of 130 neurons, obtained from 6 independent primary cultures), we distinguished two populations, discriminated based on the area of the GC. In the first population, identified by their large footprints and considered as pausing GCs, labeled $GABA_A$Rs kept diffusing in the membrane throughout the experiment but no marked asymmetry in their distribution was observed (Fig. 2 B in blue) compared to control condition without GABA (Fig. 2 B in green). For thinner GCs, the mean position Y(t) reversibly shifted up gradient, indicating the formation of polarity at the cell membrane (Fig. 2 B in red). In the rest, we limit our analysis to the second population. From the polarization curve Y(t), we could extract two parameters with important functional relevance: (i) the polarization amplitude A at steady-state (expressed as a fraction of the GC lateral extension L), which is used as an estimator for amplification in gradient sensing; (ii) the polarization half-time T, which indicates the kinetics of the response.

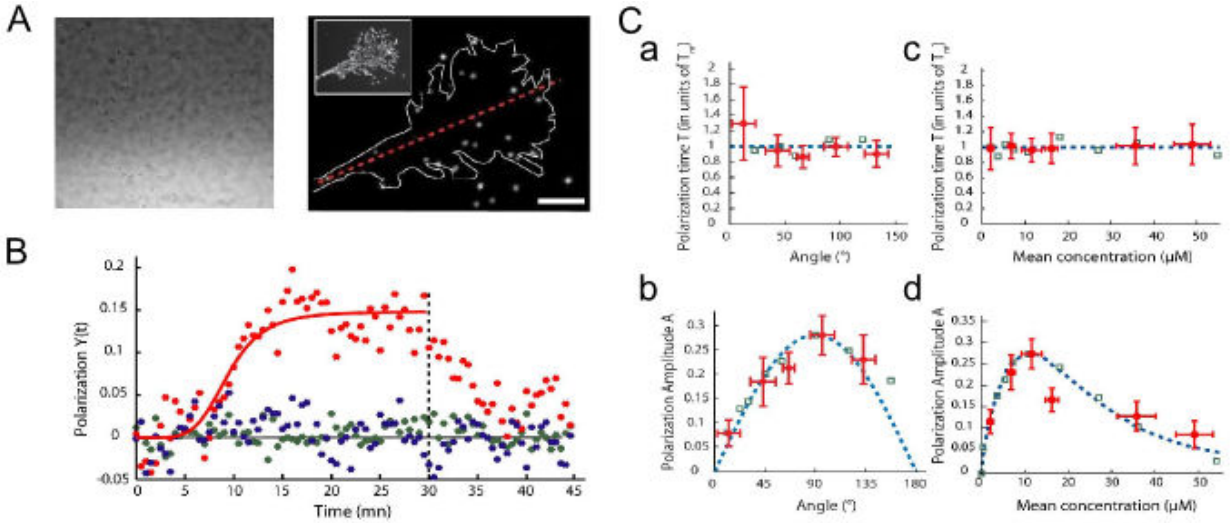

Figure 2: Single molecule experiments on dissociated neurons. A) Left: Spinal cord neurons submitted to a GABA gradient (visualized using FITC). Rigth: Single $GABA_A$ receptors in growth cones are labeled with quantum dots and their positions are tracked over time. Insert: maximum projection of fluorescence images. B) Mean position Y(t) of the labeled receptors perpendicular to the GC axis, for dynamic GCs (red, 54 cells), pausing GCs (blue, 40 cells) and control conditions without GABA (green, 28 cells). After 30 minutes (dotted line), the gradient was switched off. The red line is an adjustment of the polarization Y(t) with the phenomenological curve $At^n/(t^n+T^n)$ *(A = 0.15±0.02, T = 9.2±1.1 min, n taken equal to 5). D) Experimental results (red dots) and numerical simulations (green squares) for receptors polarization under different gradient conditions. a) Polarization time as a function of the angle* θ*. b) Amplitude as a function of the angle* θ*. The blue dotted line is proportional to sin(*θ*), i.e. to the projection of the gradient along the* Y_{GC} *axis. c) Polarization time T as a function of the mean concentration c at a relative steepness* $\delta = 7.5\ 10^{-3}\ \mu m^{-1}$*. d) Amplitude A for the same experimental conditions. The blue dotted line is proportional to f'(c) with* $f(c) = c^{h_m}/(c^{h_m}+K^{h_m})$.

For the different gradient conditions – orientation and mean concentration – we measured the polarization kinetics and found that the polarization time was constant, Tm ~ 9 min (Fig. 2 C, a and c). This points to the existence of a characteristic time scale over which the stimulation needs to be sustained in order to polarize the chemoreceptors. Since Tm can also be interpreted as a time over which GCs are able to filter out fluctuating signals, our results mean that GCs act as low-pass filters with a fixed cut-off frequency 1/ Tm ≈ 0.002 Hz.

Measuring the amplitude of the polarization for the same gradient conditions, we find i) an expected linear dependence with the effective gradient, defined as the projection of the gradient along the GC axis (Fig. 2 C, b), ii) a marked concentration dependence with a peak value around c ≈ 15 μM (Fig. 2 C, d), a concentration close to the GABA binding constant. This second observation suggests that GCs act as signal amplifiers over a narrow range of concentrations.

Furthermore, thanks to quantitative computational modeling, we relate these systems-level properties to the saturable occupancy response and the lateral dynamics of $GABA_A$ receptors (Fig. 2 C, green squares). Importantly, accounting for the non-linear concentration dependence in the chemoreceptor occupancy, imposed by the binding constant, is sufficient to capture the modulation of the integrative response of the cell.

CONCLUSION

Our measurements provide an integrative view of the GCs as chemical sensors and highlight how dynamic adaptation of the cellular organization is used for the processing of functional signals. They also illustrate how the workings of complex molecular and signaling circuits can be probed using controllable inputs made possible by microfluidic tools. Beyond the case of GABA guidance, shear-less microfluidic assays, which could be multiplexed to provide a simple and low-cost screening platform, will constitute an invaluable tool for deciphering, at a molecular or systems-level the rules by which nerve cells interpret chemical information and convert it into functional motility.

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