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OPTIMIZATION OF A MULTI-CHANNEL PUFFER SYSTEM FOR RAPID DELIVERY OF SOLUTIONS DURING PATCH-CLAMP EXPERIMENTS

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1. ABSTRACT

In biological experiments, especially in neuroscience research, it is important to manipulate the extracellular environment efficiently. We have developed a micro-puffing system for local drug delivery to single cells in electrophysiological experiments, and validated the kinetic properties of this instrument. Based on our results, the kinetics of the delivery of solutions and the territory controlled by this system are influenced by several factors: (1) inner diameter (I.D.) of the guide tubing; (2) I.D. of the puffing tip; (3) angle of the puffing tip; and (4) gravity or external pressure applied to the solution. The system can fully control a territory of 200 x 600 μm^2. The minimum delay in response to drug delivery is 10-20 ms. Switching between different solutions takes less than 100 ms. The minimum volume of solution required by the system is 0.2 ml. Taken together, our results provide useful data for designing and using an efficient drug/solution delivery system in electrophysiological experiments.

2. INTRODUCTION

In most electrophysiological experiments, manipulating the extracellular environment is crucial. In pharmacological experiments using patch-clamp recordings, it is common to monitor the response of a single cell in different external solutions. These experiments require a micro-puffing system that can deliver a given drug/solution to the cell during patch-clamp recording via electrical switching among different channels (1-5).

In the present work, we describe the RCP-II multi-channel micro-puffing system (Figure 1A,B). A glass pipette made in the lab is used for drug/solution delivery (puffing tip), and contains 7 polyethylene (PE) tubes (I.D. 0.28 mm, O.D. 0.61 mm) for 7-to-1 drug/solution delivery. The seven PE-tubes end at the terminal of the glass puffing tip (I.D. 0.1-0.4 mm). The estimated "dead-volume" is 1-2 µl, which arises from the ends of the 7 PE-tubes and their common outlet in the puffing tip. Each drug/solution channel is controlled by an electromagnetic valve with 25 µl dead volume. The small dead volume of the delivery tip permits very fast (less than 100 ms) switching between drug channels. The system permits the application of 7 different drugs/solutions, one after another, to a patch-clamped cell without interrupting the giga-seal. The TTL-based switch can be remotely controlled either by hand or by computer and provides very fast onset and offset of the selected drug/solution. The system does not introduce additional electrical noise into the patch-clamp recording.

During drug/solution delivery, the cell experiences a gradient from 0 to the final concentration of the source. To investigate the dynamics of drug/solution delivery, which determines the cell response, we measured key dynamic properties of the RCP-II puffing system.

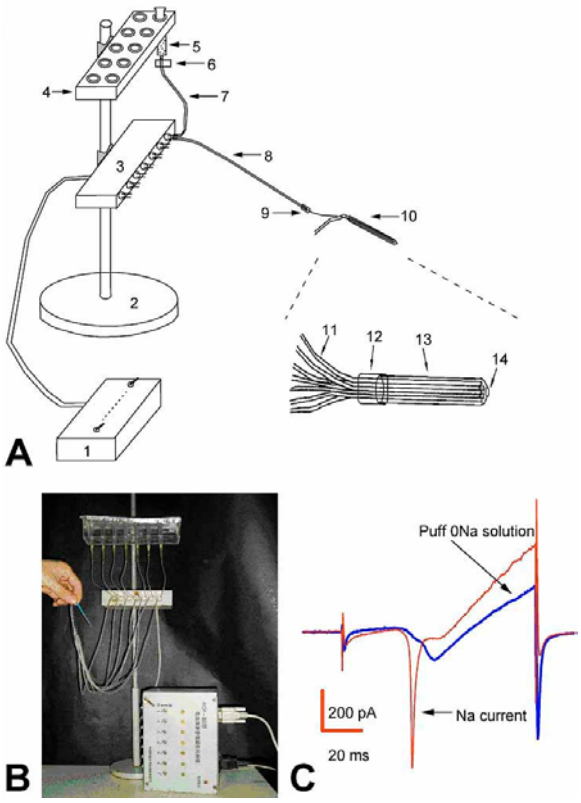

Figure 1. The RCP-II drug puffer system. A: Schematic diagram of the system. (1-controller, 2-base, 3-valves, 4-syringe holder, 5- syringes, 6-manual three-way stopcock, 7,8-emulsion tubes (I.D. >1 mm), 9-tubing adapter, 10,13-glass puffing pipette, 11-polyethylene tubes (I.D. = 0.28 μm), 12-adhesive, 14-pipette tip (I.D. = 100-200 μm). B: Photograph of the RCP-II system. C: Biological application: Blockade of sodium current during standard whole-cell voltage clamp recording from cultured chromaffin cells. In the presence of sodium-containing solution, a prominent sodium current was detected as the voltage ramp was applied (red trace). When sodium-free solution was applied through the puffer pipette of the RCP-II system, the same ramp failed to generate a current (blue trace).

3. METHODS

3.1. The drug/solution delivery system

The design of the micro-puffing system (RCP-II) is shown in Figure 1A,B. The puffing tip was made in the lab, and the micro-electromagnetic valves are commercially available (LFAA1209512H, Lee Company, Westbrook, Connecticut). The system is available from INBIO Inc. (Wuhan, Hubei 430074, China) or WPI Inc. (model MPS-2, WPI, Sarasota, FL 34240).

3.2. System maintenance

It is very important to wash the entire tubing system with distilled water after use. This is because solutions used in electrophysiological experiments contain salts, which crystallize out if allowed to dry. Small crystals in the fine PE tubes or valves alter the kinetics of delivery and can even block the flow completely. Typically, we wash the system 2-3 times using a total volume of 50 ml distilled water after use.

3.3. *In vitro* recording of drug/solution delivery

To test the response of the system, we included NaCl (150 mM) in one channel, and distilled water in another. The concentration of NaCl was sensed by a standard patch-clamp recording system using glass electrodes of 3-5 MΩ filled with 150 mM NaCl. The bath solution contained 150 mM NaCl. A voltage offset of 10 mV was applied between the patch electrode and the bath to induce a constant current, I(0), which was detected by the patch pipette (Figures 2-7). When distilled water (mimicking a drug or test solution) from the delivery system was puffed near the detecting electrode, the current decreased from I(0) to I(1) = 0 pA, because the distilled water isolated the sensing patch-electrode from the reference electrode. Thus, the current detected by the patch-clamp under voltage-clamp mode served as an indicator of the relative concentration of the drug (I = I(0), I(0)/2 and 0 pA representing 0, 50% and 100% of distilled water detected by the patch electrode). The territory under control was defined as the region where the steady-state concentration was ≥ 90% (C90, note that contour C50 > contour C90 > contour C100, because C90 include the territory of C100% plus the area where concentration is between 90% to 100% of the source) of that in the drug reservoir. Note that, because of differences in specific weights, our estimation of the kinetics may differ slightly from that with solutions containing drugs. The solutions were delivered from the puffer tip, which was in a fixed location, to the cell or sensor pipette, which could be moved up to 1200 μm away. The electronic switches were operated either by hand or by a software-controlled D/A interface.

To determination the time constant of puffing a drug, we used single exponential equation $C(\%)= 1- \exp(t_0-t)/\tau$ in the time range($t>t_0$) to fit the concentration curve. Where C(%) is the normalized drug concentration, t_0 is the time of half concentration, τ is the time constant of the concentration. This equation is used in Figs. 4-7. The time constant in Fig.3 is determined by equation $G(\%)=\exp(t_0-t)/\tau))$ ($t>t_0$), where G(%) is the normalized conductance of the puffing solution.

4. RESULTS

4.1. Application of the RCP-II system in biological experiments

The RCP-II system was initially devised to fulfill the requirement for fast drug-delivery in biological experiments in our lab(1, 6-11). Figure 1C illustrates a typical experiment using this system. Whole-cell recordings were made from single cultured chromaffin cells. A voltage ramp from –100 to +100 mV was applied for 100 ms in different extracellular environments. A large inward sodium current was recorded in sodium-containing solution. After applying sodium-free solution via the RCP-II system, the sodium current was completely blocked (Figure 1C) due to the absence of extracellular sodium ions

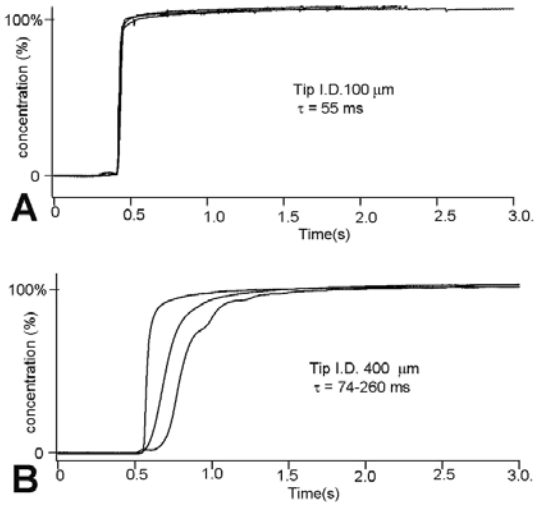

Figure 2. Consistency of drug-delivery kinetics among different tubes. Superimposed traces from a sensing electrode to puffs from each of the seven tubes in one puffing pipette. Note that the puffing tip with 100 μm tip diameter evoked fast and consistent responses (A), while in a 400 μm tip, different tubes evoked significantly different response kinetics (B).

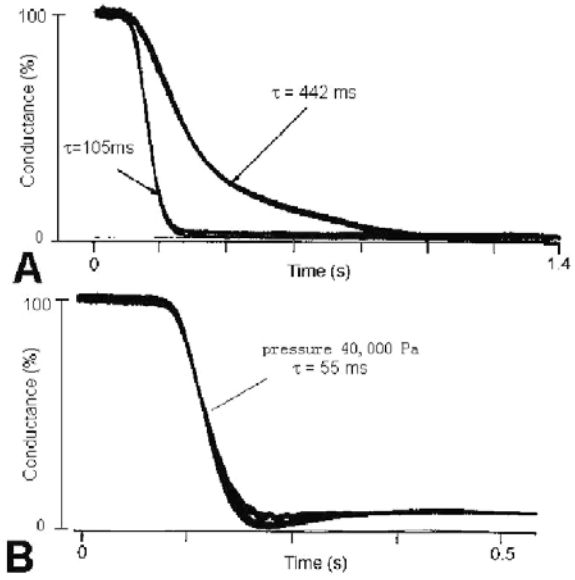

Figure 3. Effect of connecting tube diameter on puffing kinetics. (A) Puffing kinetics of two types of connection tubing (τ = 105 ms using 2 mm emulsion tubing, τ = 442 ms using 0.28 mm polyethylene tubing) at ambient atmospheric pressure. The "%-conductance" is the inverse value of "%-concentration" of the applied drug (distilled water in this case). (B) After applying an external pressure of $4*10^4$ Pa, the kinetics became the same for both types of tubing (τ = 55 ms).

around the cell. This result showed that the RCP-II system fully controlled the extracellular environment and allowed switching to different solutions during experiments(1, 2).

4.2. Consistency of drug-delivery kinetics among different tubes

Since seven polyethylene tubes (I.D. = 0.28 mm) occupy one glass delivery pipette, it is important that every tube delivers drugs/solutions with similar kinetics to avoid possible artifacts caused, for example, by differences in pressure. So we tested the delivery kinetics of each polyethylene tube in glass pipettes of various sizes. As shown in Figure 2A, the kinetics did not differ significantly among the tubes when using glass pipettes with tip diameters of 100 μm. But when a tip of 400 μm I.D. was used, the puffing kinetics displayed much more variance (Figure 2B), possibly because of greater differences in the relative positions of the polyethylene tubes within the larger tip. We further found that tip diameter of 200 μm gives similar result as 100 μm (i.e. no big kinetic difference among the 7 tubes, data not shown). Based on this result, we did not use glass pipettes with tip diameters larger than 200 μm.

4.3. Effect of connecting tube diameter on puffing kinetics

Most commercial drug-delivery systems use relatively thick emulsion tubing (I.D. > 1 mm) to connect the solution reservoirs (syringes in most systems) to the polyethylene tubing. But for expensive drugs, such as toxins, it is not cost-effective to use these systems(6-8). On the other hand, if a patch pipette or other substitute is used to apply the toxin, the quality of delivery is difficult to control and prone to artifacts. To optimize our system for small amounts of expensive drugs, we replaced all connections with thin polyethylene tubing of 0.28 mm I.D. We then tested whether this modification altered the kinetics. Our results showed that, when no pressure was applied to the syringes, the time constant of delay in delivery of solution was 105 ms for emulsion tubes 2 mm in diameter, and 442 ms for thin polyethylene tubes of 0.28 mm (Figure 3A). When a pressure of 4×10^4 Pa was applied to both syringes, the delay was reduced to 55 ms in both conditions (Figure 3B). Thus, a combination of thin polyethylene tubing and external pressure reduced the minimum volume of drug required to fill the delivery tube (0.2 – 1 ml).

4.4. Effect of solution height on flow

Since the driving force of the system is gravity, changing the relative heights of the solutions in the syringes can affect flow. We estimated the speed of flow as the time for one drop of solution to flow from the puffing pipette. We tested this relationship with 10, 2.5 and 1 ml syringes. As shown in Figure 4A, flow decreased with decreasing height. To ensure a similar flow, 1 ml syringes should be mounted 5 cm higher than 2.5 and 10 ml syringes.

4.5. Factors affecting the territory controlled by the delivery system

To use the puffing system in biological experiments, it is crucial to know exactly the territory throughout which the drug concentration can be clamped. Factors such as flow rate, the angle of the pipette, and its

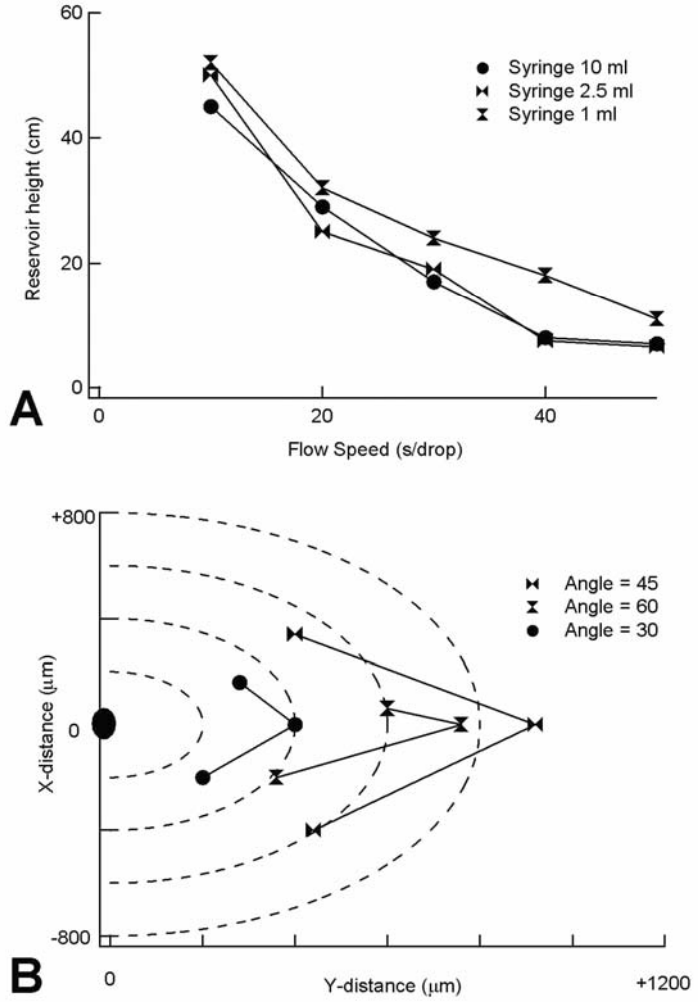

Figure 4. Effect of reservoir height and pipette angle. (A) Effect of reservoir height (cm) on flow rate (seconds per drop) for 10 ml, 2.5 ml and 1 ml syringes. (B) Effect of pipette angle on territory under control. Test conditions: reservoir height = 40 cm, flow rate = 18 s/drop, tip I.D. = 100 μm. The recording pipette was placed at various distances up to 1200 μm from the puffing tip, which was fixed at 0,0, indicated by the small grey circle on the x-axis.

diameter, can influence the territory over which conditions are under control.

Firstly, we tested the effect of pipette angle and found that the territory was largest at 45° and smallest at 30° (Figure 4B). Secondly, we tested the effect of flow rate and found no effect in the vertical axis over the range of 18 to 35 s/drop. However, in the horizontal axis, the controlled region was twice as large with the faster rate (18 s/drop; Figure 5). Thirdly, we tested the effect of the tip diameter of the glass pipette at the optimal flow rate (18 s/drop) and pipette angle (45°). We found that the tip of 100 μm controlled a larger region than those of 250 and 400 μm (Figure 6A), and the latter two did not differ significantly. To maintain the same flow, the pipette with the smaller diameter had to have a faster efflux, which resulted in a much larger region under control. To optimize the diameter of the glass pipette, we also measured the kinetics of 100 and 400 μm tips. Under the conditions of a 45° angle, and 20 s/drop, the 100 μm tip showed a much faster response, meaning a shorter delay of drug/solution delivery (Figure 6B). Taken together with the results outlined in 4.2, the tip diameter should be less than 200 μm.

4.6. Comparison of 100% and 50% control regions

In cell experiments, it is also important to know the concentration profile of an applied drug/solution. As shown in Figure 7, under the conditions of a 100 μm tip, 35 s/drop and a 45° angle, the territory of "100% concentration" (C100) was only slightly smaller than the "50% concentration" (C50) region under control. It is therefore important to place the delivery tip within 200 μm of the experimental target.

5. DISCUSSION

From the kinetics data, the RCP-II system can be optimized as follows:

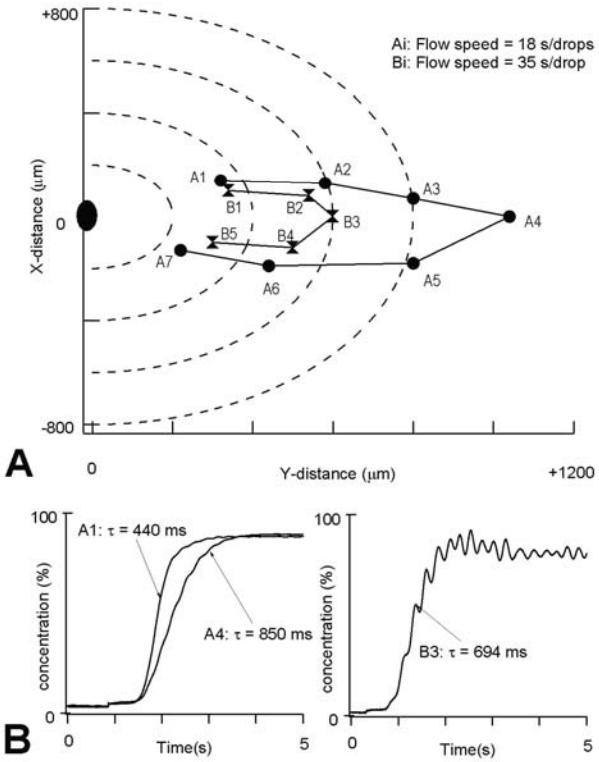

Figure 5. Effect of flow rate on C90. (A) The regions under control at different flow rates; arrangement as for Fig. 4B. Test conditions: reservoir height = 35 cm, Angle = 45°, pipette I.D. = 100 μm. Ai and Bi represent the positions at which C90 responses were measured. (B) Traces recording from locations A1, A4 and B3.

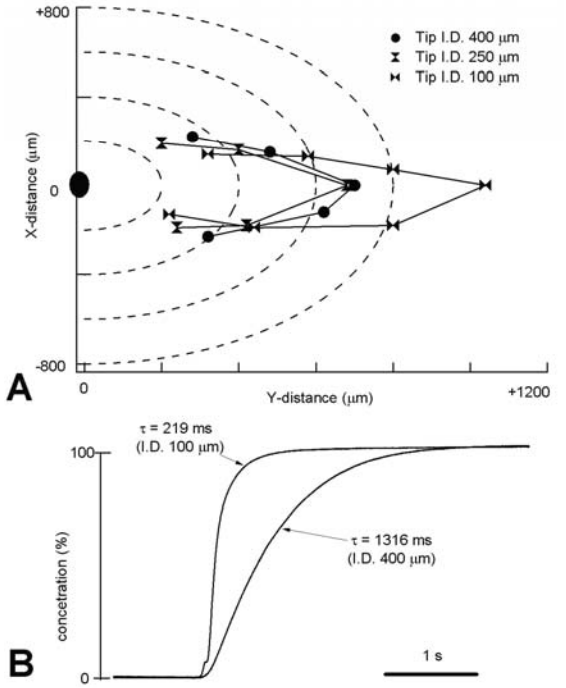

Figure 6. Effects of delivery tip I.D. (A) C90 with three pipettes of differing I.D. Test condition: reservoir height = 40 cm, Angle = 45°, Flow rate = 18 s/drops; arrangement as for Figure 4B. (B) Examples of delivery kinetics with 100 and 400 μm I.D. pipettes.

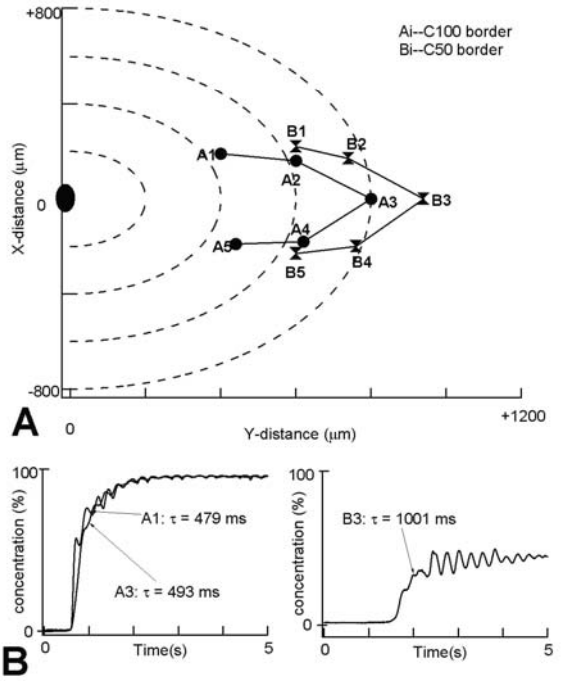

Figure 7. Comparison of C100 and C50 areas under control. (A) The 100% and >50% concentration areas under control; arrangement as for Figure 4B. Test conditions: reservoir height = 40 cm, angle = 45°, diameter of pipette = 100 μm, flow rate = 18 s/drops. (B) Traces of recordings from A1, A3 and B4 in panel A.

1. The delivery kinetics (Figure 2), the region under control and the kinetics of pipettes with different diameters (Figure 6) show that a tip diameter of 100 μm is best.

2. With a 100 μm tip, the territory under control increases with flow rate, which can be adjusted by the relative height of the solution. At a flow of 18 s/drop, the system has a controlled territory approximating that of the area (400*250 μm2) viewed under a microscope at 400× magnification. To obtain a standard flow of 10 s/drop, the drug or other solutions should be mounted 30 cm above the pipette when using 10 or 2.5 ml syringes, and 35 cm when using 1 ml syringe.

3. A pipette angle of 45° is optimal to maximize the territory under control.

4. To minimize the volume of solution wasted, especially when using expensive drugs, all tubing can be replaced with polyethylene tubes of 0.28 mm I.D., and additional external pressure should be applied to maintain the kinetics of drug/solution delivery.

6. ACKNOWLEDGMENTS

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