Title

Immediate reuse of patch-clamp pipettes after ultrasonic cleaning

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# equal contribution

Abstract

The patch-clamp technique has revolutionized neurophysiology by allowing to study single neuronal excitability, synaptic connectivity, morphology, and the transcriptomic profile. However, the throughput in recordings is limited because of the manual replacement of patch-pipettes after each attempt often also being unsuccessful. This has been overcome by automated cleaning the tips in detergent solutions, allowing to reuse the pipette for further recordings. Here, we developed a novel method of automated cleaning by sonicating the tips within the bath solution wherein the cells are placed, reducing the risk of contaminating the bath solution or internal solution of the recording pipette by any detergent and avoiding the necessity of a separate chamber for cleaning. We showed that the patch-pipettes can be used consecutively at least 10 times and that the cleaning process does not negatively impact neither the brain slices

nor other patched neurons. This method, combined with automated patch-clamp, highly improves the throughput for single and especially multiple recordings.

Introduction

Patch-clamp recording is a widely-use and powerful technique to study single-cell electrophysiology, especially in neuroscience. It led to the characterization of several aspects of neuronal physiology, *in vitro* and *in vivo*, such as ion channel activity underlying action potential [1,2], intrinsic excitability [3,4], synaptic integration [5,6] plasticity [7] and network activity [8,9]. Patch-clamp also allows to record in the different compartments of neurons [10–12] including very distant dendritic branches or axonal sections. In the *whole-cell* configuration, it is possible to dialyze cells with fluorescent dyes for morphological reconstructions and to collect the cytoplasm to analyze single-cell transcriptomic profiles [13,14]. Altough the patch-clamp technique is well suited to characterize the heterogeneity of neurons in the brain, it is highly laborious and time-consuming with variable success rates resulting in a low-throughput. To overcome this issue, engineering advancements managed to automate patch-clamp *in vitro* and *in vivo* [15–17]. When automated, the software is able to track the patch pipette and individual neuron positions in order to approach and record them. While it reduces the human interaction for the recording, it is still required to manually change the patch pipette after each attempt.

To obtain a successful recording, one crucial parameter is to have a perfectly clean patch-clamp pipette tip filled with a filtered internal solution [18]. In this condition, there is a high chance to form a seal of high-resistance (≥ 1 GΩ, which is called “gigaseal”) with the membrane of the neuron. After each successful or failed attempt, the used pipette has to be manually changed. Therefore, the human factor can be a big issue

when it comes to multiple recordings because of vibrations from the exchange that can affect the seal of other pipettes attached to neurons in the context of neuronal network characterization. It can also be an issue for *in vivo* patch-clamp studies as the manual pipette replacement could disrupt the animal and the following recordings. To prevent that, an automated method for cleaning patch pipettes has recently been developed [19]. This approach consists of immersing the tip of used patch-clamp pipettes in a separate chamber containing the detergent Alconox while applying cycles of positive pressure and negative pressure to the pipette tip. Coupled with automated patch-clamp [20], the throughput of patch-clamp recording is significantly increased. However, the necessity of a separate bath cleaning chamber and the risk of contaminating the recording bath solution nor pipette internal solution with detergent reducing the usability of this cleaning method.

Since patch-clamp pipettes are made of borosilicate glass and can also be cleaned by sonification, we developed a novel cleaning system based on that method preventing the use of detergent. The sonification is performed by a piezo-element mounted on the recording headstage and connected to a pressure control system (Fig.1a). The ultrasonic cleaning is performed in artificial cerebrospinal fluid (aCSF) and allows to reuse the same patch-clamp pipette at least 10 times without affecting the recording quality. We also showed that the cleaning procedure within the same bath does not affect the stability of seal of other patch-clamp pipettes with their respective neurons. Therefore, ultrasonic cleaning is a powerful improvement offering significant advantages in particular for multiple simultaneous patch-clamp recordings.

Methods

Cleaning procedure

We used a now commercially-available pressure control system (LN-PCS, Luigs & Neumann, Germany) to deliver positive and negative pressure to the patch-pipette. The procedure is set and triggered with a SM10 Remote Control Touch (Luigs & Neumann, Germany). The cleaning protocol consisted of 6 steps alternating positive (+500 mBar for 3s) and negative pressures (-300 mBar for 3s) and the frequency of sonification is set at 40 kHz. Different pressures or frequencies can be used. A safe cleaning distance (> 5 mm from the brain slice) was defined before the beginning of the experiment to prevent any energy transfer from the pipette being cleaned to other pipettes attached to neurons in *whole-cell* configuration.

Electrophysiology

All procedures were performed with the approval of local authority (animal license 50154A4, LANUV). Adult C57BL/6 mice of both sex were anesthetized with isofluorane (AbbVie, UK) and decapitated. 300 µm-thick coronal slices were cut with a vibratome (Leica VT1200s) in an ice-cold modified cutting solution containing (in mM) : 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 Glucose, 6 $MgCl_2$, 1 $CaCl_2$, pH 7.4 (95% O_2 / 5% CO_2 and 310 mOsm/l). Slices were incubated for 30 min at 34°C in aCSF containing (in mM) : 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 Glucose, 1 $MgCl_2$, 2 $CaCl_2$, pH 7.4 (95% O_2 / 5% CO_2 and ~310 mOsm/l) and recovered at room temperature.

Patch-clamp recording were performed using a LNscope (Luigs & Neumann, Germany) equipped with a 40x water immersion objective (Zeiss, Germany), infrared-Dodt gradient contrast and a CMOS camera (Chameleion USB 3.0 monochrome Camera, Point Grey, Canada). Patch pipettes (5-8 MΩ) were pulled from borosilicate glass (GB150F-10, Scientific Products GmbH, Germany) with a horizontal puller (P-1000, Sutter Instruments, Novato, CA, USA). The internal solution contained (in mM):

100 K-gluconate, 20 KCl, 10 Hepes, 4 Mg-ATP, 0.3 Na-GTP, 10 Na_2-phosphocreatine, 0.3 % biocytine, pH 7.2 (~300 mosm/l). Data were acquired with an EPC-10 USB amplifier (Heka, Lambrecht, Germany) and Patchmaster Next software (Heka, Lambrecht, Germany). Data were digitized at 20 kHz and lowpass filtered at 10 kHz. Whole-cell patch-clamp recordings consisted of current steps from -100 pA to 300 pA in steps of 50 pA for 500 ms. Membrane resistance (R_m) was monitored before and after the cleaning procedure of non-recording pipettes. The resting membrane potential (RMP) was monitored during the cleaning process to evaluate any impact of the sonification on the stability of recording. The resistance of the pipette (R_{pip}), the resistance of gigaseal (R_{GS}) and the access resistance (R_a) were monitored in voltage-clamp mode to evaluate the efficiency of the cleaning procedure.

Data analysis

Data were analyzed with Matlab (version 2022a) and appropriate statistical analysis were performed using GraphPad Prism 8. Representations in Fig. 1B and Fig. 3A were created with BioRender.com.

Results

Cleaning efficiency

Before approaching the patch-clamp pipette to the brain slice, a “cleaning position” (> 5 mm from the slice) was defined to safely clean the pipette without affecting the sample. This is determined by the micromanipulator control panel. When “cleaning” is selected, the micromanipulator automatically goes to the saved XYZ coordinates (Fig. 1B). The piezo mounted on the headstage performs the sonification of the patch-clamp pipettes by making it oscillate at 40 kHz. To help removing the membrane residue

131 inside the pipette, the sonification is coupled with a cycle of high positive and negative
132 pressure similar to the protocol used for cleaning in detergent [19]. After the cleaning
133 procedure the patch-clamp pipette can be placed into a “parking position” which
134 correspond to a determined XYZ distance to its original place. This prevents any
135 damage of the slice by the automatic replacement of the pipette and to freely move to
136 find another neuron to record.
137 For each attempt, R_{pip} was monitored once a *whole-cell* recording attempt was made,
138 and compared before and after the ultrasonic cleaning (Fig. 2A) to assess the efficiency
139 of the procedure. As expected, R_{pip} was reduced after sonification (before: 11.08 ± 0.22
140 MΩ; after: 3.98 ± 0.05 MΩ, n = 55 attempts, $p < 0.0001$, Wilcoxon signed-rank test).
141 Over ten cleaning cycles, R_{pip} remained constant ($p = 0.0625$ for fresh vs tenth cycle,
142 n = 5, Wilcoxon signed-rank test; Fig. 2B). These data show that ultrasonic cleaning
143 coupled with alternating high and low pressure can successfully remove remaining
144 membrane residues and repeatedly over at least ten attempts.
145 Next, we evaluated the quality of recordings by monitoring R_{GS} and R_a. R_{GS} was
146 obtained before breaking in *whole-cell*, when the value was steady. We managed to
147 patch 55 neurons with 5 pipettes that were cleaned 10 times and we observed no effect
148 of ultrasonic cleaning on R_{GS} ($p = 0.89$, Friedman test; Fig. 2C) and R_a ($p = 0.6217$,
149 Friedman test; Fig. 2D). These results show that with the novel sonification method
150 patch-clamp pipettes can be reused at least 10 times for successful *whole-cell*
151 recordings.
152
153 *Effect of sonification on cell survival*
154 We next tested whether ultrasonic cleaning could affect neuronal survival within the
155 same chamber. In the context of simultaneous paired recordings, it is crucial that

cleaning one pipette tip does not affect other neurons maintained in *whole-cell* by the patch-clamp pipettes. As mentioned above, the pipette is put at a safe distance (>5 mm) from the sample to prevent any harm or energy transfer to the other pipettes. In this condition, RMP of neurons being recorded simultaneously remained stable during the cleaning process (before: -65.0 ± 1.8 mV; after: -65.1 ± 1.9 mV, n = 5, p = 0.5625, Wilcoxon signed-rank test; Fig. 3A, B) and R_m is not affected (before: 110.3 ± 9.3 MΩ; after: 109.7 ± 7.7 MΩ, n = 5, p > 0.99, Wilcoxon signed-rank test; Fig. 3A, C). In one example case, we successfully recorded the same neuron 3 times with the same patch-clamp pipette after being cleaned and filled it with biocytin for confirmation (Fig. 3D, E). Its intrinsic parameters remained stable for the all 3 attempts. And the post-hoc reconstructed morphology did not show any sign of neuronal damage (Fig. 3E). Altogether, these data show that ultrasonic patch-clamp pipette cleaning does not harm neurons situated within the same bath chamber.

Discussion

The patch-clamp technique is a very powerful method but it is laborious and the success rate is not high. In order to increase its throughput, automatization of the patch-clamp technique has recently been the focus of engineering. While there have been several successful developments to automatize the whole procedure [15,17,20], it is crucial to overcome the changing of patch-clamp pipette. Others have shown that it is possible to clean the tips using either bleach [21] or detergent combined with automated pressure control [19,22]. However, even if not detected, there might be some trace left of these chemicals inside the patch-clamp pipette that can be harmful for the cells. We developed an ultrasonic cleaning system coupled with automated

pressure control, which allows the tips to be cleaning in a physiological solution and is less likely to harm the cells.

Cleaning patch-clamp remains to this day one of, if not, the key element to make patch-clamp routine. In the context of drug discovery, automated patch-clamp experiments are a game changer to explore the pharmacology of ion channels. Although we did not investigate the effect of ultrasonic cleaning on ion channels, Kolb and colleagues showed that GABA receptors pharmacology is not affected with pipettes being cleaned in Alconox [19]. With these observations combined with ours, showing that ultrasonic cleaning does not affect neuronal excitability, it is highly possible that our method is not affecting ion channels pharmacology.

Regarding the longevity of pipettes, we did not investigate how many times pipettes can be used before replacing it with a fresh one. Kolb and colleagues showed that it is not possible to reuse the same pipette indefinitely [19]. We suspect that our approach will allow reusing the same pipettes for a limited time, as internal solution contains chemicals that degrade when not refrigerated (e.g. ATP, GTP).

By being able to clean the pipette tip in aCSF, our method offers the possibility to avoid using a second compartment. This is convenient, especially for *in vivo* patch-clamp, as it would allow clean the pipette above the cortex and reduce the time spent to change it after each attempt. Coupled with recently automated *in vivo* patch-clamp recording [15], it will increase the throughput of that method and make it more accessible.

In conclusion, we developed a detergent free cleaning method for patch-clamp pipettes based on sonification coupled with a pressure control system that can be implemented to automated patch-clamp systems.

Acknowledgments

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277 Figure legend

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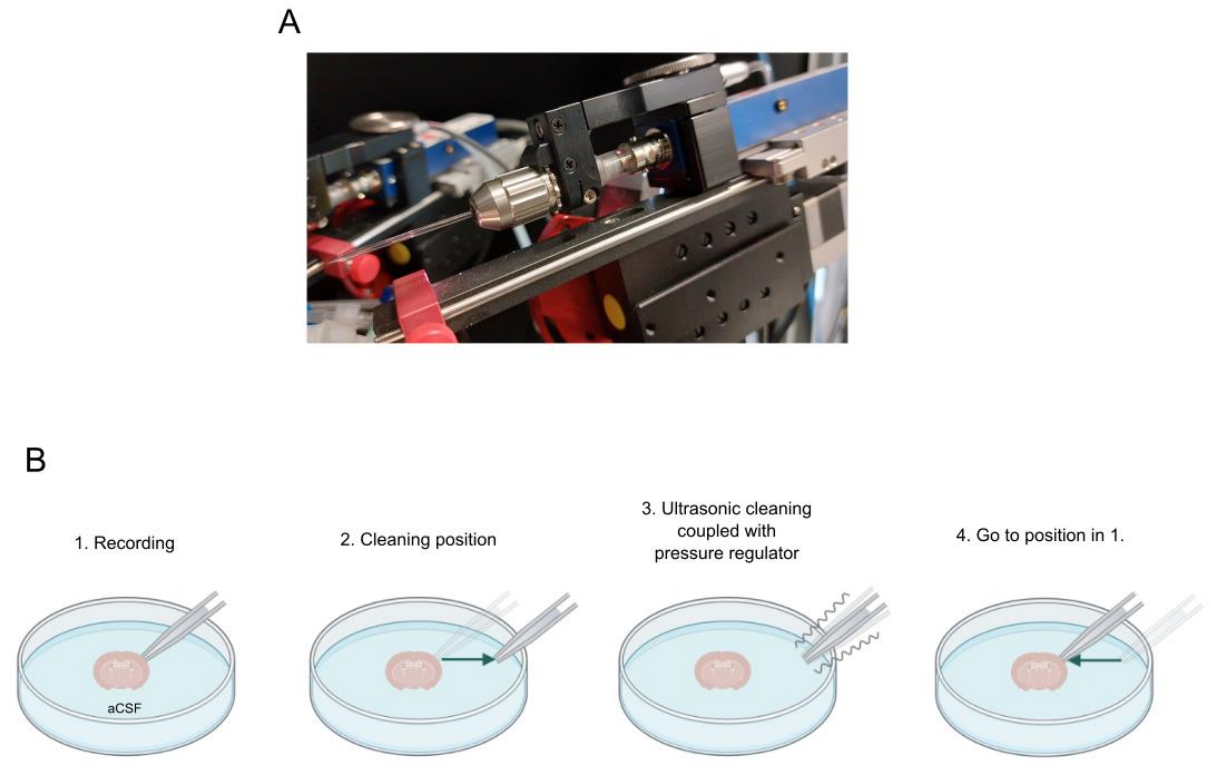

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280 **Figure 1: Ultrasonic cleaning is performed by a piezo coupled to a pressure**
281 **regulator. (A)** Picture of the headstage unit. This unit is connected to a pressure
282 regulator to allow the cleaning. **(B)** When the recording is finished (1), the cleaning
283 sequence can be launched. The manipulator put the tip to a pre-defined cleaning
284 position (2) where the sonification coupled with cycle of high positive and negative
285 pressure can occur (3). Once the procedure is finished, the manipulator put the tip back
286 to its initial parking position (4) before the cleaning was launched.

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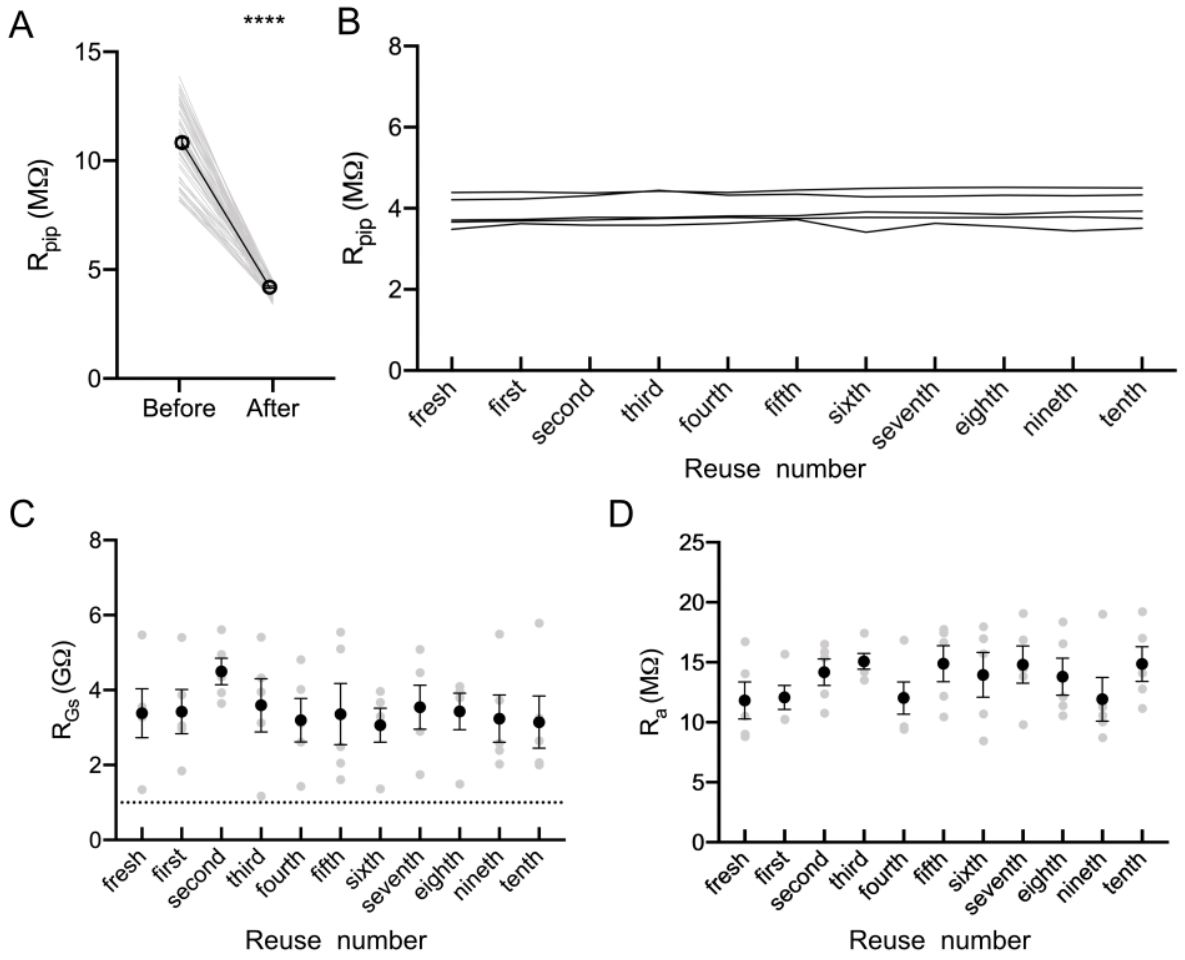

Figure 2: Ultrasonic cleaning allows reusing pipettes for multiple consecutive recordings. (A) and **(B)** indicate that ultrasonic cleaning can clean the tip as R_{pip} is recovered after each cycle. **(C)** shows that patch-clamp pipette can be successfully reused at least ten times and R_{Gs} is successfully reached. **(D)** show that R_a remains unaffected after each cleaning cycle, indicating that the tip is cleaned and the pipette can be reused for successful patch-clamp recordings.

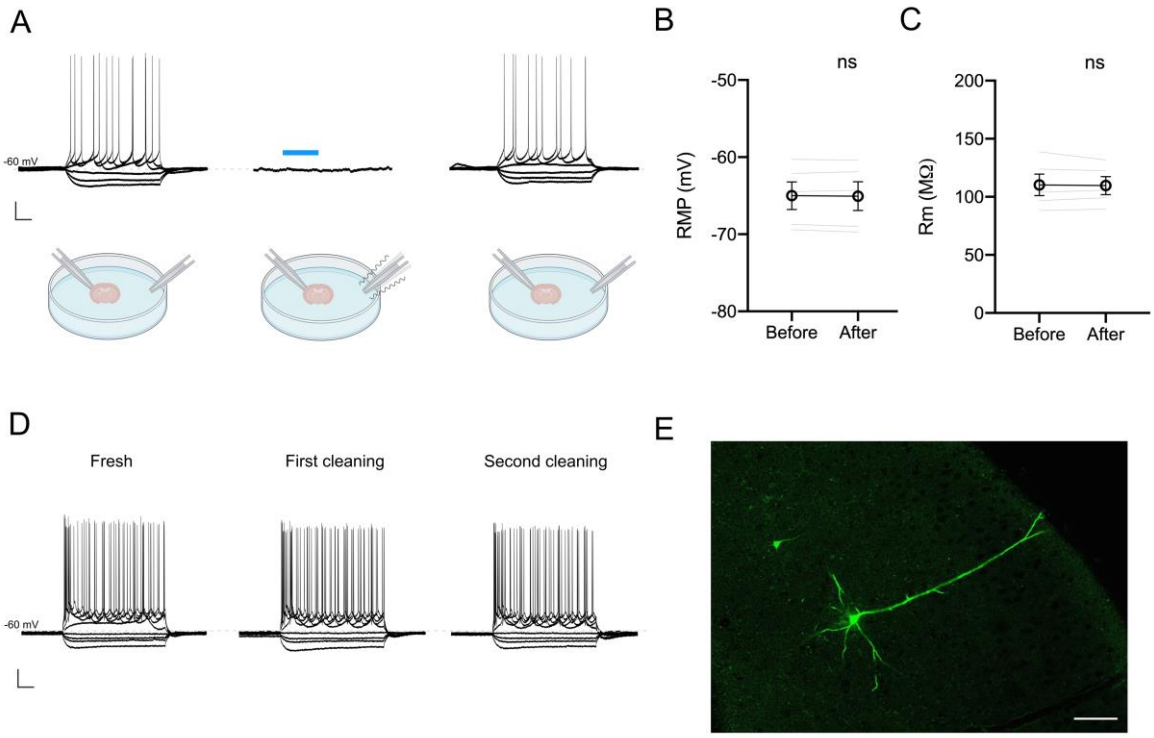

Figure 3: Ultrasonic cleaning is not harmful for neurons. (A) Example of a neuron being recorded before (left), during (middle) and after (right) ultrasonic cleaning of a patch-clamp pipette inside the same recording chamber. Scale bar is y = 20 mV (all panels) and x = 100 ms (left and right) and 20 s (middle). Blue bar corresponds to the moment when the non-recording tip is being cleaned. **(B)** and **(C)** show that the cleaning procedure does not interfere with simultaneous patch-clamp recordings as RMP and R_m of the recorded neurons remain unaffected. **(D)** Example a neuron being successfully recorded three consecutive times with the patch-clamp pipette after ultrasonic cleaning. Scale bar is y = 20 mV and x = 100 ms. **(E)** *Post-hoc* staining of neuron from **(D)**. Scale bar is x = 80 µm.