

Robotic Outside-out Patch Clamp Measurement Method with Gigaseal Preservation via Equilibrated Stretching of Aspirated Cell Membrane

Yuzhu Liu, Jinyu Qiu, Ruimin Li, Shaojie Fu, Pengfan Zhang, Biting Ma, Mengya Liu, Xin Zhao, and Qili Zhao*

Abstract—The outside-out patch clamp measurement method has great potential application values in ion channel function research due to its ability to record the picoampere-level current flowing through a single ion channel. To achieve this, a gigaohm-scale seal (gigaseal) between the recording micropipette and a square micron-sized aspirated cell membrane needs to be preserved in order to suppress the environmental noise while the latter is stripped off from the cell by the former. To preserve seal integrity during aspirated membrane stripping, this paper proposes a robotic outside-out patch clamp measurement method that maintains a gigaseal through equilibrated stretching of aspirated cell membrane. First, an electrical stripping model is developed to detect the stripping state of the aspirated cell membrane online. Then, a force analysis is performed to determine the appropriate retraction direction of the micropipette for providing an equilibrated stretching force. Furthermore, theoretical and simulated mechanical models are established to optimize the contact position on the cell surface and the inclination angle of the micropipette. Based on these analyses, a robotic outside-out patch clamp measurement process is constructed to ensure stable and reliable electrophysiological recordings. Experimental results on pyramidal neurons in mouse brain slices demonstrate that the proposed method achieves a 100% detection accuracy in stripping state identification. With favorable stretching parameters, the outside-out patch clamp success rate based on the whole-cell mode was improved by up to 80%, and the mean outside-out seal resistance reached 3.04 G Ω . Moreover, the increased seal resistance effectively reduces leakage conductance by 68% and suppresses baseline current fluctuations by 49%, resulting in lower measurement noise and improved recording fidelity. With the above advantages, our work lays a solid foundation for single ion channel function analysis in future.

Index Terms—Outside-out patch clamp measurement, robotic patch clamp, robotic subcellular manipulation.

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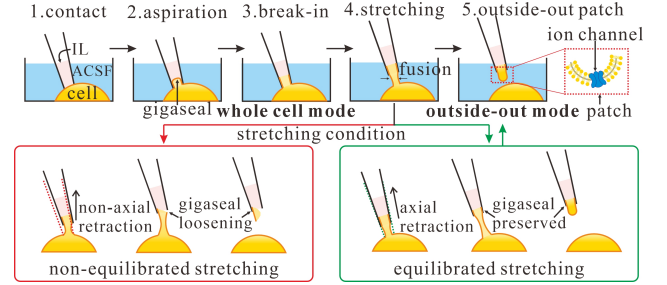


Fig. 1. Schematic illustration of the outside-out patch clamp procedure.

I. INTRODUCTION

THE patch clamp technique, invented by Neher and Sakmann in 1976, has become the “gold standard” in ion channel function studies of brain and neuroscience research [1]–[3], due to its ability to detect picoampere-level (10^{-12} A) ion channel signals. The reliability of such measurements fundamentally relies on the gigaohm-scale seal (gigaseal) between the recording micropipette and the cell membrane, which effectively suppresses environmental electrical noise and thereby enables precise detection of extremely weak ion channel currents. Measuring the electrical response of a single ion channel under stimulation is the key technique for studying ion channel functions in brain and neuron science. To achieve single-channel-level resolution, a square micron-sized cell membrane patch, which usually contains fewer than ten ion channels [4], needs to be stripped off from the whole-cell membrane using micropipette while preserving gigaseal between micropipette and stripped aspirated cell membrane. Depending on which side of the stripped aspirated cell membrane is exposed at the micropipette opening, single-channel patch clamp recordings are classified into two modes: the inside-out mode [5] and the outside-out mode [6]. Our previous work focused on the robotic inside-out patch clamp method [7]. This paper mainly focuses on the robotic outside-out patch clamp method.

Fig. 1 schematically illustrates the outside-out patch clamp procedure and the mechanical meaning of equilibrated stretching during membrane stripping. First, a micron-sized micropipette filled with intracellular liquid (IL) and plugged with an electrode wire (termed micropipette electrode) contacts the cell membrane. Then, an aspiration pressure is exerted inside the micropipette to aspirate a square micron-sized cell membrane into the micropipette opening, forming a gigaseal between the aspirated membrane and the micropipette inner

wall for shielding environmental electrical noise. A brief suction pulse or electrical stimulus is then applied to rupture the aspirated membrane inside the micropipette (break-in), connecting the whole intracellular environment and the electrode wire to form the whole-cell mode. The micropipette is then moved away from the cell membrane along a defined retraction path to strip the aspirated membrane off from the cell. During this process, equilibrated stretching is required to maintain the gigaseal while allowing the neck part of the aspirated membrane to gradually narrow, fuse, and detach from the whole-cell membrane. In contrast, non-equilibrated stretching may loosen the gigaseal or rupture the membrane, eventually leading to failure of outside-out patch formation. Therefore, equilibrated stretching that preserves the gigaseal during membrane stripping is a key requirement for stripping a stable outside-out patch. After successful stripping, a square micron-sized membrane patch remains at the micropipette opening with its outside surface exposed to the extracellular liquid, thereby forming the outside-out mode. In this state, the outside surface of the membrane faces the extracellular liquid, enabling the study of ligand-gated and voltage-gated ion channels under controlled extracellular stimulation.

In the outside-out patch clamp mode, the extremely weak picoampere-level signals of single ion channels are highly susceptible to environmental electrical noise and leakage currents. To enable reliable single-channel measurements, the gigaseal resistance between the aspirated membrane and the inner wall of the micropipette must be maintained throughout the membrane stripping process. To preserve gigaseal integrity, the membrane should be stretched in an equilibrated manner (see Fig. 1). However, two major challenges hinder the realization of equilibrated stretching during outside-out patch formation. First, due to the extremely small size of the aspirated membrane and the inclined mounting angle of the micropipette, a clear contour of the aspirated cell membrane is nearly impossible to be observed under the microscope during the stripping process. Therefore, the operator usually cannot monitor the stripping state through microscopic visual feedback during the stripping process of aspirated cell membrane. Second, the stripping process is influenced by multiple stretching operation factors including the retraction direction of micropipette, inclination angle and contact position on cell surface. Due to the absence of stretching model and the aforementioned visual feedback of stretching process, the selection of the above operational parameters heavily depends on the operator's experience, which easily generates a non-equilibrated stretching force during the stripping process of aspirated cell membrane. The above non-equilibrated stretching force may cause the loosening of the gigaseal and fusion failures of stretched membrane below micropipette opening in stripping process, finally leading to the failure of outside-out mode formation and signal recordings. These issues significantly degrade the stability and reliability of outside-out patch clamp measurement method, thereby limiting its application in ion channel function research. A new outside-out patch clamp method that provides an equilibrated stretching force during membrane stripping is therefore required to improve measurement reliability and recording quality.

In recent years, robotic patch clamp techniques, especially the robotic whole-cell mode patch clamp research, have made substantial progress [8]–[11]. For example, the planar patch clamp systems are the first type of commercialized automated patch clamp systems. These systems are usually equipped with a microchip with an array of micro-holes connecting to a pump to aspirate and measure multiple cells at one time [12]. Owing to their high throughput and standardized measurement format, planar patch clamp systems have been widely used in drug screening and ion channel pharmacology [13]. However, the hole-in-plane structure makes these systems only applicable for suspended cells and unsuitable for membrane stretching to form an outside-out patch clamp mode. Blind patch clamp systems target neurons *in vivo* or in brain slices, achieving gigaseal formation and break-in according to micropipette impedance measurements without visual feedback [14]. The above micropipette impedance may serve as an indicator to guide the stripping process for maintaining a sufficiently high seal resistance, without microscopic view. However, due to the lack of microscopic visual feedback, the cell searching success rate and whole-cell patch clamp success rate are significantly lower than the traditional method with microscopic visual feedback, significantly limiting the overall efficiency of outside-out patch clamp operation. As an improvement, differential interference contrast (DIC) imaging [15] and two-photon microscopy imaging [16] depicting label-free neurons in brain slices and the fluorescent dye-labeled neurons *in vivo*, respectively, have been utilized to guide robotic patch clamp on these cell types. However, because of the lack of microscopic visual feedback on the aspirated membrane and the stretching model to optimize the stretching operation parameters, no robotic outside-out patch clamp operation has been realized based on the above systems according to references.

In our previous research, we developed a robotic inside-out patch clamp method based on vesicle rupture control, where the key membrane events were vesicle formation after gigaseal formation without break-in, followed by vesicle rupture control. Although that work provides a conceptual basis for monitoring membrane state changes through electrical signals, outside-out patch clamp involves a different membrane evolution. In outside-out mode, membrane stripping is performed after break-in, and the key step is to stretch and fuse the aspirated membrane while maintaining the gigaseal, thereby stripping a stable outside-out patch from the whole-cell membrane. This difference in membrane structure makes the previous vesicle state model unsuitable for outside-out stripping-state detection. Moreover, because break-in may make the gigaseal more vulnerable to loosening or detachment, the simple stretching parameters used in the previous inside-out method cannot be directly applied. Therefore, a new electrical stripping model and a new mechanical stretching model are required in this study.

For the first time, a robotic outside-out patch clamp measurement method is proposed to improve electrophysiological recording reliability through preserving gigaseal by equilibrated stretching of the aspirated cell membrane. First, an electrical stripping model is developed to detect the stripping state

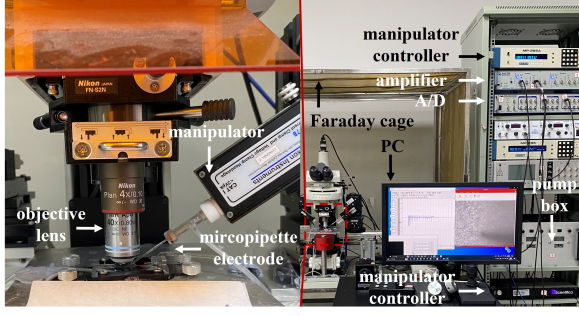


Fig. 2. Robotic outside-out patch clamp measurement system.

of the aspirated cell membrane online. Then, a force analysis is performed to determine the appropriate retraction direction of the micropipette for providing an equilibrated stretching force. Furthermore, theoretical and simulated mechanical models are established to optimize the contact position on the cell surface and the inclination angle of the micropipette. Based on these analyses, a robotic outside-out patch clamp measurement process is constructed to ensure stable and reliable electrophysiological recordings. Experimental results on pyramidal neurons in mouse brain slices demonstrate that the proposed method achieves a 100% detection accuracy in stripping state identification. With favorable stretching parameters, the outside-out patch clamp success rate based on the whole-cell mode was improved by up to 80%, and the mean outside-out seal resistance reached 3.04 G Ω . Moreover, the increased outside-out patch seal resistance effectively reduces leakage conductance by 68% and suppresses baseline current fluctuations by 49%, resulting in lower measurement noise and improved recording fidelity. In addition, a performance comparison with representative manual and robotic patch-clamp approaches is provided, further clarifying the methodological scope and incremental contribution of the proposed method. With these advantages, the proposed method provides a robust measurement foundation for high-fidelity single-channel electrophysiological analysis in the future.

II. SYSTEM SETUP

The robotic outside-out patch clamp measurement system used in this study was independently developed in our laboratory [17], as shown in Fig. 2. The system integrates an upright microscope, motorized objective positioning stages, a three-axis micromanipulator, a patch-clamp amplifier, a pneumatic pressure controller, a CCD camera, and a custom-developed Qt-based software platform. These subsystems provide visual feedback, micropipette positioning, pressure regulation, and electrophysiological signal acquisition for robotic outside-out patch clamp operation. The detailed hardware configuration, pressure-control specifications, and preparation procedures for brain slices and micropipettes are provided in Supplementary Section S1.

III. METHODOLOGIES AND KEY TECHNOLOGIES

A. Stripping State Detection of Aspirated Membrane Based on an Electrical Stripping Model

As mentioned above, the stripping state of the aspirated membrane is difficult to observe microscopically because of

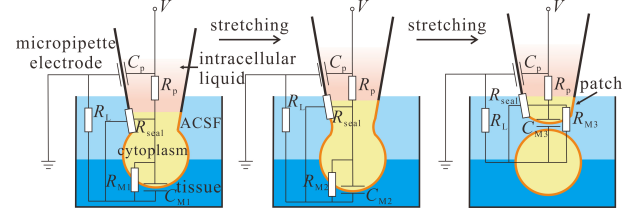


Fig. 3. Schematic of the electrical stripping model of aspirated cell membrane in outside-out patch clamp operation.

its small size and the inclination angle of the micropipette. To address this issue, we developed an electrical stripping model to provide feedback on the stretching state of the aspirated membrane during the patch stripping process [18]. Although this model follows the same electrical modeling principle as our previous robotic patch-clamp studies [7], it is adapted to the specific membrane configuration and stripping process in outside-out patch formation, as detailed in Supplementary Section S2.

Fig. 3 schematically illustrates the equivalent circuit of the measured impedance of the micropipette Z_M during the stripping process of aspirated cell membrane. After break-in, a whole-cell state is formed as shown in left image of Fig. 3. The value of Z_M consists of the following parts as

$$Z_M = \left(R_p + (R_{\text{seal}} \parallel R_M \parallel \frac{1}{j\omega C_M}) + R_L \right) \parallel C_p \quad (1)$$

where the symbols $+$ and $\parallel$ denote series and parallel connections, respectively. R_p denotes the micropipette resistance, including the silver wire resistance and the intracellular liquid resistance; R_{seal} denotes the resistance resulting from the gigaseal; R_M and C_M denote the membrane resistance and capacitance; R_L denotes the resistance of the ACSF; C_p denotes the capacitance of the micropipette wall. The cytoplasmic resistance is compensated by the amplifier, so its influence on the electrical stripping model is minimized [19].

After the micropipette retracts to stretch the cell membrane, the thickness of the cell membrane decreases due to the slight increase of the cell membrane surface area during stretching process. According to the parallel-plate capacitor equation $C = \epsilon A/d$, the value of C_M slightly increases after the stretching starts. Since the states of most ion channels remain unchanged, the resistance R_M approximately keeps constant. As aspirated membrane stretching proceeds, a neck shape generates between the aspirated cell membrane and other part of the cell membrane. As further stretching goes, the neck part becomes narrower and finally causes the aspirated cell membrane to be stripped from the other part of cell membrane to form a patch. During the stripping process, the neck part of aspirated cell membrane fuses together due to membrane fluidity [20], finally forming an outside-out patch adhering to micropipette opening. After patch stripping, only a small isolated patch remains at the micropipette opening. The membrane area decreases significantly, and the number of ion channels is greatly reduced, resulting in a rapid increase in R_M and a rapid decrease in C_M . As the inner environment of the micropipette and the extracellular environment remain constant during the whole stripping process, the other circuit

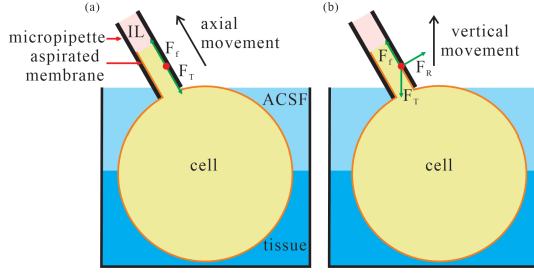


Fig. 4. Schematic of the force analysis of aspirated cell membrane and micropipette with different retraction directions during aspirated cell membrane stretching process. (a) Axial retraction along the micropipette axis. (b) Vertical upward retraction.

parameters, including R_P , C_P , and R_L , remain approximately unchanged. In summary, the variation trends of R_M and C_M are utilized to detect the stripping state of the aspirated cell membrane during outside-out patch clamp operation.

Specifically, a sharp decrease in C_M together with a large increase in R_M indicates the transition from the stretching state to the final outside-out patch state; therefore, the system stops micropipette retraction once this electrical change is detected.

B. Micropipette Retraction Direction Determination Based on Force Analysis

Maintaining the seal between the aspirated membrane and the inner wall of the micropipette is a prerequisite for the formation of the outside-out mode. Only with this stable seal can the aspirated membrane undergo equilibrated stretching during micropipette retraction. To determine an appropriate micropipette retraction direction during aspirated membrane stretching for better maintenance of the seal, we first conducted an analysis of the force interactions at the aspirated membrane–micropipette interface. As illustrated in Fig. 4, two retraction directions commonly used in cell manipulation were compared: axial retraction along the micropipette axis, which is usually utilized in cell poking retraction [21], and vertical upward retraction, which is usually applied in the retraction of cell pressing operation [22].

In the axial retraction case, the aspirated membrane is subjected to two main forces: an upward friction force F_f from the micropipette wall and a downward traction force F_T from the cell body, which arises from the resistance force of cytoskeletal structures and the mechanical coupling between the cell and the surrounding tissue. As shown in Fig. 4(a), since these two forces are collinear, no radial force is introduced, thereby preserving the seal between the membrane and the micropipette.

In contrast, when the micropipette is retracted vertically upward, the friction force F_f still follows the micropipette axis, while the cell-generated traction force F_T acts vertically downward. To maintain force equilibrium under these forces, a radial force F_R is generated perpendicular to the inner wall of the micropipette at the seal interface. This radial force tends to separate the aspirated membrane from the micropipette inner wall and thus break the seal. This analysis indicates that axial retraction of the micropipette facilitates the formation of an equilibrated stretching force and helps maintain the seal during membrane stretching. Thus, the axial direction is set

as the retraction direction of the micropipette during aspirated membrane stretching.

C. Contact Position and Inclination Angle Optimization Based on Mechanical Stretching Model

After the axial retraction direction was determined for seal preservation, the remaining problem was to determine how the micropipette should contact the cell surface so that axial retraction can generate equilibrated stretching of the aspirated membrane. Although axial retraction reduces the radial force at the seal interface, the resulting strain distribution is still affected by the contact position on the cell surface and the inclination angle of the micropipette. The qualitative cantilever-beam analysis and the preliminary spherical-model screening, provided in Supplementary Sections S3.1, S3.2, indicate that these two parameters jointly influence the balance between local strain concentration and strain uniformity. Therefore, based on the axial retraction strategy and the mechanically favorable contact region identified in the supplementary analysis, a more detailed finite element model was further developed to optimize the contact offset and micropipette inclination angle.

To further evaluate how contact parameters—including the contact position and the micropipette inclination angle—affect the equilibrium state of membrane stretching within the previously determined contact region, we developed a finite element (FE) model to simulate cell membrane deformation during aspirated membrane stretching. Different from the previous spherical model used for contact-region screening, this model incorporated the aspirated cylindrical region and applied axial displacement to better represent the robotic retraction process. The detailed geometric construction, boundary conditions, displacement setting, and angle-selection considerations are provided in Supplementary Section S3.3. Based on the FE model described in Supplementary Section S3.3, the strain distribution near the micropipette opening was extracted to quantitatively evaluate the equilibrium state of membrane stretching under different contact settings.

We first calculated the strain variance of the effective element ($\varepsilon \geq 1\%$) for each finite element simulation, where a smaller variance corresponds to a more uniform strain distribution within the evaluated region, as shown in Fig. 5(a). For example, a contact position of 0.5 offset exhibits distinct differences in variance under inclination angles of 30° , 45° , and 60° . In general, for small offsets (0 – 0.5), the strain variance remains relatively low under 30° and 45° conditions, while the 60° condition exhibits moderately higher values. When the offset exceeds 0.5—especially at 60° —the variance increases substantially, reflecting strongly non-uniform deformation in the local region. For very large offsets (> 0.8), the results become increasingly influenced by the proximity to the fixed boundary, leading to potential distortions in the simulation output.

The effective-element ratio, defined as the proportion of effective elements ($\varepsilon \geq 1\%$) to the total number of elements, for each finite element simulation is shown in Fig. 5(b). A higher ratio indicates that a larger portion of the effective deformation is localized near the contact region, reflecting

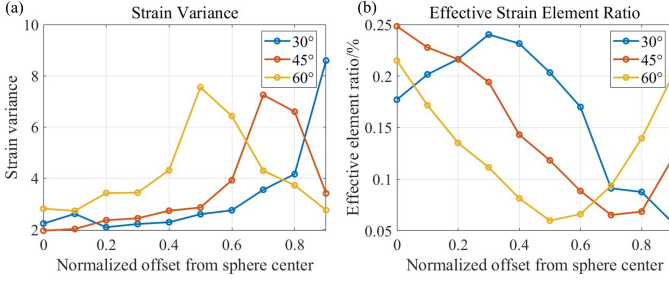


Fig. 5. Quantitative evaluation of membrane strain uniformity under varying contact parameters. (a) Distribution of strain variance with contact position and inclination angle. (b) Distribution of effective element ratio with contact position and inclination angle.

stronger spatial concentration of strain. It can be found that the ratio varies considerably with offset across different angle conditions. Under the 30° condition, the effective-element ratio remains relatively high overall and peaks at small offsets (0.2–0.6), indicating stronger spatial concentration near the contact region. In contrast, under the 60° condition, the ratio is substantially lower, indicating a misplaced strain distribution: regions that should bear the stretching remain largely unstretched, while irrelevant areas experience excessive strain, which may cause cell detachment.

These findings indicate that a combination of smaller inclination angles and small contact offsets facilitates more equilibrated strain distribution, making it a preferred strategy for achieving stable outside-out patch formation.

D. Robotic Outside-out Patch Clamp Procedure with Equilibrated Stretching of the Aspirated Cell Membrane

Based on the above work, a robotic outside-out patch clamp procedure was developed to enhance operation efficiency through equilibrated stretching of aspirated membrane. A constant low positive pressure of +0.5 psi was maintained inside the micropipette to generate a small outflow at the opening, thereby reducing clogging when the micropipette was immersed in the ACSF. The procedure begins with adjusting the micromanipulator so that the micropipette is mounted at a predetermined inclination angle. A healthy target neuron is then selected under microscopic observation, characterized by a smooth contour and a tear-drop morphology. Through a custom-developed software platform, the operator manually selects six points along the cell boundary, and a circle-fitting algorithm computes the geometric center and radius. A user-defined offset coefficient is entered, and the corresponding contact position along the radial direction is determined. After the focus is adjusted back to the micropipette plane, the visual servoing system automatically guides the micropipette tip to the computed location.

After entering the ACSF, the micropipette opening is maintained under the low positive pressure until it gently approaches the cell surface, which can be confirmed by a 4 MΩ increase of the micropipette resistance. Once contact is detected, the pressure is switched to a small negative value of approximately −0.5 psi to promote gigaseal formation. The negative pressure is gradually increased until the seal resistance reaches the gigaseal range. Subsequently, a brief

suction pulse of −2 to −3 psi with a duration of 0.5–1 s is applied to rupture the aspirated membrane, thereby achieving the whole-cell mode. Finally, the micropipette is retracted axially at a constant velocity of 300 μm/s, optimized from extensive experiments, to strip the aspirated membrane and form the outside-out patch.

The detailed robotic operation flow and control architecture are provided in Supplementary Section S4, together with Supplementary Video S1 showing the complete robotic outside-out patch clamp process.

IV. EXPERIMENTAL RESULTS

In this section, we present a series of experiments on pyramidal neurons in acute mouse brain slices to evaluate the proposed robotic outside-out patch clamp measurement method and the key methodologies described in Section III. First, stripping state detection experiments were performed to validate the electrical stripping model and its ability to track transitions of the aspirated membrane during stretching. Next, stretching operation parameter tests were carried out to examine how different inclination angles and contact offsets influence the success rates of outside-out patch formation. Finally, patch quality was assessed by comparing membrane resistance and capacitance before and after stretching under various operation parameter settings, with a focus on their implications for electrical measurement stability.

A. Stripping State Detection Model Experiments

In these experiments, we used the Clampex software for electrical signal acquisition. The software could not produce reliable impedance values during the rapid changes of the stretching phase. The instability of capacitance fluctuations during the transition is the primary factor that challenge the responsiveness and accuracy of the fitting algorithm. We analyzed the raw current traces recorded during the patch clamp experiments. In the “Membrane Test” mode, each square-wave voltage evokes a capacitive current response of the form:

$$I(t) = \frac{V}{R_M} e^{-t/\tau}, \quad \tau = R_M C_M \quad (2)$$

where V is the applied voltage, R_M is the membrane resistance, C_M is the membrane capacitance, and τ is the time constant.

When the membrane surface area slightly increases during the stretching state, C_M increases while R_M remains approximately constant. As a result, the peak amplitude of the current response progressively increases. In contrast, during the subsequent phase of outside-out patch formation, R_M increases and C_M decreases, leading to a rapid reduction in the peak amplitude.

As shown in Fig. 6, representative current responses were selected from three distinct membrane states: whole-cell mode, membrane stretching, and the final outside-out patch state. These traces show the characteristic evolution of the transient current response during membrane stripping. During stretching, the current response changes with the elongation and necking of the aspirated membrane. After outside-out

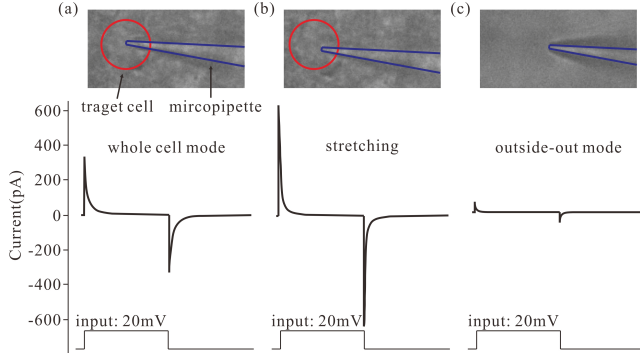


Fig. 6. Current responses at different membrane states: (a) whole-cell mode; (b) stretching state; (c) outside-out mode.

patch formation, the transient current peak decreases rapidly, corresponding to the rapid decrease in membrane capacitance and increase in membrane resistance predicted by the electrical stripping model. Therefore, the measured current trace provides direct real-time feedback for identifying the rapid transition from stretching to outside-out patch formation. A complete dynamic sequence, including the measured current response and the estimated C_M and R_M , is provided in the supplementary file “Supplementary Video S2”.

Based on a data set of 60 paired experiments, we applied the dual criterion of a relative resistance increase ($\Delta R \geq 1.1$) and a capacitance decrease ($\Delta C \geq 0.30$), where $\Delta C = (C_{\text{before}} - C_{\text{after}})/C_{\text{before}}$ and $\Delta R = R_{\text{after}}/R_{\text{before}}$. Using this criterion, outside-out patch formation was identified with an accuracy of 100%. Details are provided in Supplementary Section S5.1.

B. Operation Parameter Testing Experiments

1) *Outside-out Patch Clamp Results with Axial and Vertical Retraction:* To investigate the effect of the micropipette retraction direction on patch formation, we selected a 30°-mounted micropipette contacting the cell apex (0 offset) and compared two retraction modes: axial retraction along the micropipette axis and vertical retraction perpendicular to the cell surface. In each case, ten trials were conducted at a retraction speed of 300 $\mu\text{m/s}$. A clear difference in outcome was observed: axial retraction yielded 9 successful outside-out patches out of 10 trials, whereas vertical retraction succeeded in only 2 out of 10. These results indicate that axial retraction minimizes the radial force acting on the seal interface, thereby helping maintain a high-resistance seal and increasing the success rate of outside-out patch formation.

2) *Outside-out Patch Clamp Success Rate under Different Inclination Angles and Contact Positions:* To further evaluate how contact parameters influence the success rate of outside-out patch formation, we conducted experiments under three inclination angles of the micropipette (30°, 45°, and 60°) and two offset levels (0 and 0.5). For each condition, experiments were repeated until 10 whole-cell modes were successfully obtained, and the subsequent success rate of outside-out patch formation was recorded. The results are summarized in Table I, which lists the step-wise success rates of outside-out patch clamp under different inclination angles and offsets. Values in parentheses indicate the number of successful trials over the

TABLE I
STEP-WISE SUCCESS RATES OF OUTSIDE-OUT PATCH CLAMP

Angle	Offset	Whole-cell	Outside-out (overall)	Outside-out (conditional)
30°	0	37.0% (10/27)	33.3% (9/27)	90.0% (9/10)
30°	0.5	62.5% (10/16)	56.3% (9/16)	90.0% (9/10)
45°	0	41.7% (10/24)	33.3% (8/24)	80.0% (8/10)
45°	0.5	71.4% (10/14)	64.3% (9/14)	90.0% (9/10)
60°	0	55.6% (10/18)	38.9% (7/18)	70.0% (7/10)
60°	0.5	71.4% (10/14)	35.7% (5/14)	50.0% (5/10)

total attempts. A complete robotic outside-out patch clamp process is provided in the supplementary file “Supplementary Video S1.” On average, the entire robotic outside-out patch clamp operation takes approximately 60 seconds, with the membrane stripping phase lasting about 15 seconds.

The results show that both 30° and 45° achieved consistently high conditional success rates (around 90%) at both offsets based on whole-cell mode. This represents an improvement of up to 80% compared with the lowest conditional success rate in Table I. For each success rate reported in Table I, 95% confidence intervals (CI) were computed using the Wilson method for binomial proportions. The conditional success rates for 30° and 45° (9/10 and 8–9/10 across offsets) correspond to 95% CIs approximately in the range of [55%, 99%], whereas the lowest rate (5/10 at 60°, offset 0.5) corresponds to a CI of approximately [23%, 77%]. These intervals indicate that although the success rates vary across parameter combinations, smaller inclination angles and smaller offsets consistently exhibit higher success probabilities. These findings are consistent with our previous analysis, confirming that smaller angles and smaller offsets favor more reliable outside-out patch formation. However, from the experimental perspective, the overall success rate of outside-out patch clamp is strongly influenced by the yield of the whole-cell mode. Therefore, to achieve a high overall success rate for the entire outside-out process, both the efficiency of whole-cell operation and the equilibrated stretching of the membrane in outside-out formation need to be ensured.

3) *Patch Quality Evaluation under Varying Contact Parameters:* To further assess the impact of contact parameters on patch quality and operation repeatability, the electrical C_M and R_M values were compared before and after stretching under six experimental conditions. Paired t -tests showed that, in all tested configurations, the mean capacitance decreased and the mean resistance increased during the transition ($p < 0.05$). A Kruskal–Wallis test further revealed significant differences among groups for both resistance and capacitance after stretching ($p < 0.01$). Details are provided in Supplementary Section S5.2.

The statistical distribution of outside-out seal resistance after membrane stripping is further shown in Fig. 7 using box plots with overlaid individual data points. The robotic groups were arranged according to the inclination angle and contact offset. The manual group was included as a reference for the seal resistance after successful outside-out patch formation.

As shown in Fig. 7, different robotic operation parameters led to distinct distributions of outside-out seal resistance. The groups with smaller inclination angles and smaller offsets, including the 30°/0, 30°/0.5, and 45°/0 groups, exhibited overall higher resistance distributions than the 60° groups,

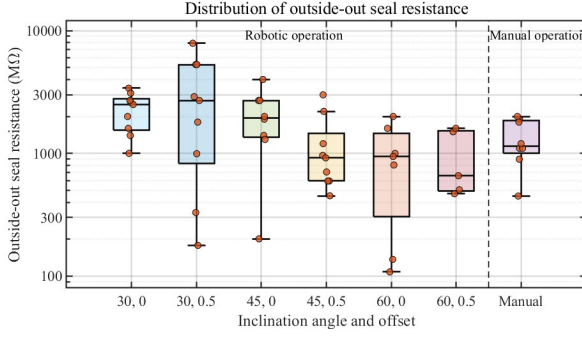


Fig. 7. Statistical distribution of outside-out seal resistance under different robotic operation parameters and manual operation. Box plots with overlaid individual data points show the median, interquartile range, and dispersion of the seal resistance after successful outside-out patch formation.

indicating that small-angle and small-offset conditions are more favorable for maintaining gigaseal during membrane stripping. Among these groups, the $30^\circ/0.5$ group achieved the highest mean seal resistance of $3.04 \text{ G}\Omega$, whereas the lowest mean resistance among the robotic groups was obtained under the $60^\circ/0$ condition. Compared with this lowest robotic parameter group, the mean seal resistance of the $30^\circ/0.5$ group was increased by 223.2%. Compared with the manual reference group, the mean seal resistance was increased by 133.2%.

Beyond improving the formation success of outside-out patches, optimized stretching parameters also enhanced the quality of electrical measurements by increasing seal resistance, reducing leakage conductance, and lowering baseline noise. These effects improve the reliability and fidelity of picoampere-level single-channel electrophysiological measurements. Details are provided in Supplementary Section S5.3.

V. DISCUSSION

A. Performance Comparison and Methodological Scope

Existing patch-clamp methods differ in experimental preparation, recording mode, and technical objective. Therefore, success rates and electrical metrics should be interpreted as representative performance ranges under different task conditions, rather than as a strict ranking based on a single evaluation standard. A detailed comparison with representative manual and robotic patch-clamp approaches is provided in Supplementary Section S6.

For blind *in vivo* whole-cell patch clamp, the main difficulty lies in searching for cells without visual feedback while maintaining stable recordings despite disturbances from respiration, heartbeat, and brain motion. In contrast, the main challenge of the robotic outside-out patch clamp mode in this work is to preserve the gigaseal after whole-cell break-in and further form a stable outside-out membrane patch through controlled membrane stripping.

Under the optimized inclination angle and contact position, the whole-cell success rate in this work reached 71.4%, which is comparable to the 70.0% whole-cell success rate achieved for pyramidal neurons in mouse brain slices in our previous morphology-guided robotic whole-cell patch clamp study. With optimized operation parameters, the conditional

success rate of outside-out patch formation based on successful whole-cell mode reached 90.0%, and the overall outside-out success rate reached 64.3%. The equivalent outside-out patch resistance after stripping was approximately $3.04 \text{ G}\Omega$, indicating that a $\text{G}\Omega$ -level resistance could still be maintained after membrane stripping.

B. Model Applicability and Mechanical Limitations

Another limitation of the present mechanical model is that the neuron was simplified as a spherical body with its lower hemisphere fixed by the surrounding tissue. This assumption is reasonable for screening contact parameters around the soma of pyramidal neurons in acute brain slices, where the micropipette mainly interacts with the exposed somatic surface. However, the model does not fully represent complex neuronal morphologies, such as branched dendrites, axons, or irregular somatic shapes. Therefore, the optimized inclination angle and contact offset obtained in this study should be interpreted as parameter ranges for soma-targeted outside-out patch formation in brain slices, rather than universal parameters for all cell types and tissue environments.

For cells with different geometries or boundary conditions, the same modeling framework can be adapted by redefining the cell geometry, material parameters, and mechanical constraints. For example, cultured adherent cells may be better represented by semi-ellipsoidal or spindle-shaped geometries due to basal substrate adhesion, rather than by a spherical model with the lower hemisphere constrained by surrounding tissue. In contrast, *in vivo* applications would require additional consideration of tissue deformation and physiological motion caused by respiration, heartbeat, and brain pulsation. In these cases, the finite element model should be re-parameterized using cell-type-specific mechanical properties and combined with real-time feedback, such as force sensing, impedance monitoring, and motion compensation, to update the stretching trajectory during operation. Future work will therefore focus on extending the model to non-spherical cell geometries, integrating real-time force or force-estimation feedback, and adapting the robotic outside-out patch clamp strategy to mechanically dynamic *in vivo* environments.

C. Measurement Quality and Future Work

The present work did not yet directly evaluate functional single-channel recordings from the formed outside-out patches. Future studies will incorporate single-channel current measurements to further verify the functional quality of the robotic outside-out patches. Membrane capacitance after stripping may reflect the approximate size of the formed membrane patch, but it cannot be directly equated with the number of functional ion channels because channel density may be spatially heterogeneous. In addition, the retraction speed was fixed at $300 \text{ }\mu\text{m/s}$ in this study. Since retraction speed determines the strain rate applied to the aspirated membrane, its effect on patch success rate, seal resistance, capacitance, and patch longevity should be systematically investigated in future work. Detailed discussions of capacitance interpretation and retraction-speed optimization are provided in Supplementary Sections S7.

VI. CONCLUSION

In this study, we presented a robotic outside-out patch clamp measurement system that enhances electrophysiological recording reliability by ensuring equilibrated stretching of the aspirated membrane. An electrical stripping model was developed to provide real-time detection of the stripping state, and a force analysis was performed to determine the appropriate retraction direction for maintaining a high-resistance seal during membrane retraction. Furthermore, theoretical and finite element mechanical models were established to optimize the contact position and micropipette inclination angle to achieve equilibrated stretching of the aspirated cell membrane.

Building upon these components, a robotic outside-out patch clamp measurement process was implemented and validated on pyramidal neurons in the visual cortex of mouse brain slices. The results demonstrated that the proposed electrical model achieved 100% accuracy in stripping state detection, while optimized operation parameters improved the outside-out patch clamp success rate based on the whole-cell mode by up to 80% and increased the mean patch seal resistance to 3.04 G Ω . Moreover, the increased seal resistance effectively reduced leakage conductance by 68% and suppressed baseline current fluctuations by 49%, resulting in lower measurement noise and improved recording fidelity.

It should be noted that the present validation was performed on pyramidal neurons in mouse brain slices, and the current mechanical model simplifies the target cell as a spherical body with fixed lower boundary constraints. Therefore, the optimized parameters should be interpreted as effective parameter ranges for soma-targeted outside-out patch formation under the tested brain-slice conditions, rather than universal settings for all cell types and tissue environments. Future work will extend the model to cells with more complex geometries and different boundary conditions, integrate real-time force or force-estimation feedback, and adapt the robotic outside-out patch clamp strategy to mechanically dynamic environments such as *in vivo* preparations.

Overall, the proposed robotic outside-out patch clamp measurement method improves the reliability of outside-out patch formation and reduces leakage- and noise-related measurement interference, providing a robust and automated measurement foundation for future single-channel electrophysiological recordings and ion channel function studies.

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