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Micro-agar salt bridge in patch-clamp electrode holder stabilizes electrode potentials

Xuesi M. Shao* and Jack L. Feldman

Department of Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095-1763, USA

Abstract

Maintaining a stable electrode potential is critical for patch-clamp measurements. The electrode potential of conventional patch electrode-holder assembly, where an Ag/AgCl wire is in direct contact with the patch pipette filling solution, is subject to drift if the pipette solution contains a low concentration of chloride ions (Cl^-). We developed an agar bridge of 3 M KCl filled in a polyimide microtubing which forms an electrical connection between an Ag/AgCl wire and the pipette solution. We examined the offset potentials of the micro-agar salt bridge electrode assembly in parallel with a conventional electrode assembly in generic recording conditions (the pipette solution contained 5 mM NaCl). The junction potential between the Ag/AgCl wire and the pipette filling solution in the conventional electrode contributed to most of the offset potential drift observed during the course of 30 min recordings. The drift was up to 27.3 mV after several changes of the glass pipette. In contrast, the micro-agar salt bridge stabilized the electrode potential within typically 2 mV without affecting the patch electrode resistance, capacitance or noise level. Numerical simulations showed that Cl^- diffusion from the agar bridge to the tip caused a negligible 0.4 μM Cl^- concentration change at the pipette tip within 30 min. This method is easy to implement and provides long-term recording stability. The micro-agar salt bridge can fit in most commercial patch electrode holders and can be conveniently maintained.

Keywords

Patch-clamp electrode; Electrode potential; Electrode holder; Salt bridge; Polyimide microtubing; Chloride; Patch pipette solution

1. Introduction

For accurate quantitative electrophysiological measurements, a stable electrode potential is essential, especially for patch-clamp studies of voltage-gated membrane channels. In conventional whole-cell patch-clamp experiments, the filling solution of the glass pipette exchanges with the cytoplasm (Marty and Neher, 1995). To emulate the intracellular environment, researchers use low concentrations of Cl^- in the patch pipette filling solution (normal mammalian neuron intracellular Cl^- concentration is in the range of 5–15 mM) in

many whole-cell patch-clamp experiments (Kay, 1992), as well as for outside-out patches. In addition, for some biophysical experiments examining the permeability of ionic channels, pipette solutions contain a variety of anions other than Cl^- (Bormann et al., 1987). In conventional patch electrode-holder assemblies, an Ag/AgCl wire provides the electrical connection between the headstage of a patch-clamp amplifier and the pipette solution. At the junction between an Ag/AgCl wire and the pipette solution, the reaction is $\text{Ag} + \text{Cl}^- \leftrightarrow \text{AgCl} + \text{e}^-$. When the pipette solution has a low Cl^- concentration, the interface becomes non-reversible (polarized), and the electrode potential is vulnerable to drift with time (Purves, 1981; Raynauld and Laviolette, 1987; Snyder et al., 1999). This electrode potential drift can significantly confound measurements of cell membrane potentials or currents and bias the command voltages during voltage-clamp experiments.

In order to overcome this problem, efforts have been made to build salt bridges that provide high concentrations of Cl^- interfacing the Ag/AgCl wire and are independent of the Cl^- concentrations in the pipette solution (Bormann et al., 1987; Snyder et al., 1999; Strong, 1984). In these designs, diffusion and bulk mixing between the salt bridge with a high concentration KCl (1–3 M) in fluid form and the pipette solution is a major concern. This diffusion induces junction potential drift and contaminates the pipette solution. Also the extra volume of the KCl solution increases the electrode capacitance and noise. In addition, these salt bridges are difficult to fabricate and are inconvenient to use. For example, the application of an intrapipette salt bridge designed by (Kleene, 1993) is limited by the requirement of addition of 0.07% agarose to the solution in the tip of every patch pipette which is inconvenient. Moreover, it has not been tested in whole-cell recording configuration.

Agar salt bridges are widely used with reference electrodes in contact with the bath solution to provide stable junction potentials when changing Cl^- concentrations in the bath is required during electrophysiological experiments. However, it is challenging to make a bridge with an immobilized high Cl^- salt solution sufficiently small that it can be easily inserted into patch pipettes without any adverse side effects such as introduction of bubbles, or increase in resistance, capacitance or noise level. Here we describe a patch-clamp electrode-holder assembly containing a micro-agar salt bridge of 3 M KCl in a polyimide microtubing that can be easily constructed. We tested the stability of the electrode potential of this agar salt bridge electrode in parallel with the conventional patch electrode in generic patch-clamp experimental conditions. We also examined the stability of conventional electrodes with Ag/AgCl wires coated with a few different methods. Using computational modeling techniques, we analyzed how diffusion of Cl^- from the micro-agar bridge affects the Cl^- concentration of the pipette solution at the pipette tip, where it could potentially alter the intracellular Cl^- concentration in whole-cell recording mode. We also examined and compared the resistance, capacitance and noise of these electrodes.

2. Materials and methods

2.1. Construction of the micro-agar salt bridge and electrode-holder assembly

New Ag wires (50 mm long and 250 μm in diameter), provided with a widely used patch electrode holder (HL-U, AXON instruments/Molecular Devices, CA, USA), were cleaned

with 100% alcohol and thoroughly rinsed with distilled water. They were then coated by soaking one end (~30 mm) in Clorox™ bleach (Ultra Clorox, Clorox Professional Product Company, CA, USA) for 20 min and then rinsed with distilled water.

A polyimide tubing (i.d. = 410 μm and o.d. = 520 μm , MicroLumen Inc. Tampa, FL, USA) was cut to 50 mm in length with a razor blade (Fig. 1). Polyimide tubing is an excellent choice for this application because: (i) even with a very thin wall it has adequate strength and rigidity, so with an o.d. sufficiently small to allow easy insertion into a patch pipette of 0.8–1.0 mm i.d. without generating bubbles, the inner diameter can be large enough to allow space for the agar filling that covers a chlorided 250 μm diameter Ag wire; (ii) agarose gel can readily fill the tubing by capillary action due to the adhesion between the molecules of polyimide and water that also tends not to generate bubbles when immersed into the patch pipette solution; (iii) polyimide tubing is transparent, allowing the agar level to be seen while filling; (iv) its low coefficient of friction and smooth inside and outside surfaces allow easy feeding of a wire inside and easy insertion into the patch pipette; (v) it is easy to cut to a desired length by a razor blade; (vi) it is chemically inert. We marked the tubing in the middle, and designated one end as “tip”. The chlorided Ag wire was inserted from the back of the tubing to a position close to but not beyond the mark. Agarose (3%; Gibco BRL, Life Technologies Inc. MD, USA) was dissolved in a 3 M KCl solution. Five to six milliliters of this solution in a 10-ml beaker (depth ~ 20 mm) was heated in a water bath at 75–80 °C. Then, the tip of the microtubing was immersed into the hot agarose gel, allowing the gel to fill into the tubing gradually by capillary action. When the gel got to the mark, we immediately inserted the Ag/AgCl wire into the gel in the tubing leaving 2–4 mm between the tip and the end of the wire. The Ag/AgCl wire was immersed for about 20–25 mm in the gel. Then the microtubing with the wire was taken out of the beaker and allowed to cool (Fig. 1A). The back of the tubing can be sealed with epoxy or cyanoacrylate. The salt bridge tubing with 2–4 mm of Ag wire extending out the back was installed in a commonly used patch-clamp electrode holder, e.g., HL-U (AXON instruments/Molecular Devices, CA, USA) or Q Series holders (Warner Instruments, CT, USA) as usual, and sealed with a rubber gasket (Pierced Seal, WS-3, Warner Instruments, CT, USA). This micro-agar salt bridge holder was used in the same way as a conventional holder with an Ag/AgCl wire (Fig. 1B).

2.2. Other methods for chloriding Ag wires

To examine if the electrode potential drifts are affected by the method of chloriding the Ag wires, we coated Ag wires electrolytically. Ag wires were cleaned with alcohol and rinsed with distilled water. They were chlorided by making them positive with respect to a 0.9% NaCl solution and passing a current at 1–2 mA/cm² for 25 min. The polarity was reversed once for 1–2 s during the 25-min coating period. For additional comparisons, we coated Ag wires by dipping them into molten AgCl (Sigma–Aldrich Co., MO, USA) (Thomas, 1978). We also purchased sintered Ag/AgCl electrodes (200 μm in diameter) from A-M Systems, Inc. (WA, USA).

2.3. Testing the micro-agar salt bridge electrode

Patch pipettes were pulled from thick wall borosilicate glass pipettes (8250, o.d. = 1.65 mm, i.d. = 1.0 mm, with filament. Garner Glass Company, CA, USA) with a horizontal puller

(Model P-97, Sutter Instruments); the back end of every pipette was fire polished before pulling. The tip size was 1–1.5 μm (resistance: 4–7 $\text{M}\Omega$). To simulate our routine experimental conditions, we used a pipette filling solution containing (in mM): 135 K-gluconate, 5.0 NaCl, 0.1 CaCl_2 , 1.1 EGTA, 10 HEPES, 2.0 ATP (Mg^{2+} salt) and 0.3 GTP (Na salt), pH adjusted to 7.25 with KOH. The bath solution contained 142 NaCl, 3 KCl, 1.5 CaCl_2 , 1.0 MgSO_4 , 10 HEPES and 10 glucose; pH adjusted to 7.4 with NaOH.

To be consistent, we filled the pipettes to a level that when inserted into the holder 5 ± 1 mm of the Ag/AgCl wire or the micro-salt bridge was immersed in the filling solution, unless otherwise stated. When we filled the patch pipettes, we were careful to assure there was no excessive solution on the inner wall of the pipette that might contact the part of the Ag wire without coating, which might result in an unstable junction potential. We were also careful not to scratch the Ag/AgCl wire while changing pipettes. We looked at the Ag/AgCl wires with a dissection microscope after several changes of pipettes to check if there were scratches.

A petri dish was filled with the bath solution. An Ag/AgCl reference electrode cell (3 mm diameter Ag/AgCl pellet, A-M Systems, Inc. WA, USA) made contact with the bath and was connected to the headstage grounding plug. In order to test two electrodes at a time under the same conditions, we used a two-channel patch-clamp amplifier (MultiClamp 700B, Axon Instruments/Molecular Devices, CA, USA). Two holders were mounted on the two headstages of this amplifier and the two pipette tips were immersed into the same bath at room temperature (24 ± 1 $^{\circ}\text{C}$). Electrode potential was measured in current-clamp mode with the current set at zero. The offset potential was also set at zero. Two channels of signals were amplified and low-pass filtered at 40 Hz, digitized at 200 Hz sampling frequency with DIGIDATA 1440A and software CLAMPEX 10 (AXON Instruments/Molecular Devices, CA, USA) and stored on a Pentium-based computer.

2.4. Measurements of resistance, capacitance and noise

Resistance was measured with the built-in feature of Multi-Clamp 700B Commander with voltage-clamp mode setting voltage at zero. MultiClamp Commander generates square pulses and the resistance of the electrode is calculated from Ohm's law. Open circuit electrode capacitance was measured with the Cp fast Auto capacitance compensation feature in MultiClamp 700B Commander with voltage-clamp mode while setting the Cp slow to zero pF. The value of capacitance was displayed as Cp fast in pF and in μs in the V-Clamp panel of the Commander. The open circuit RMS noise was determined by checking the Irms checkbox in MultiClamp Commander with voltage-clamp mode and voltage was set to zero. The measurement (in pA) was in the bandwidth of 30 Hz to 5 kHz (four-pole Butterworth filter).

2.5. Numerical simulations

Time-dependent diffusion processes of ions are governed by Fick's second law. For three-dimensional diffusion, Fick's second law is expressed as

$$\frac{\partial C}{\partial t} = D \left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} + \frac{\partial^2 C}{\partial z^2} \right) \quad (1)$$

where C is the concentration of diffusing substance; t the time; D the diffusion coefficient; x , y and z are the space coordinates (Crank, 1975). A finite element modeling program FlexPDE 5 (PDE solutions Inc. Antioch, CA, USA) was used to solve the partial differential Eq. (1) and to simulate the diffusion process between the 3 M KCl micro-agar bridge and the pipette solution.

2.6. Maintenance

The micro-agar salt bridge should be kept immersed in 3 M KCl solution when not in use. We sealed the tip of a glass pipette (same size as the patch pipette) with heat, filled it with a 3 M KCl solution from the back so that the solution covered the tip of the salt bridge when this pipette was inserted into the holder. Then, we tightened the cap nut of the holder. The side port for applying suction in the electrode holder should be sealed by a rubber cap if the electrode is not going to be used for more than a day to prevent the solution in the pipette from evaporating or, if the side port is connected to a suction tubing with a three-way valve as common practice, this valve should be turned to the suction tubing off position.

2.7. Data analysis

The electrode potential drift data shown in Fig. 2B were analyzed with a data analysis software SAS (V 9.1, SAS Institute Inc., Cary, NC, USA). The statistical significance between the drifts of micro-agar salt bridge electrode versus that of conventional electrode was tested with a two-way repeated measures ANOVA model using the procedure “MIXED”. The criterion for statistical significance was set to $p = 0.05$.

3. Results

3.1. Comparisons of micro-agar salt bridge electrode with conventional electrode

We used pairs of patch pipettes, each pair pulled from the same piece of glass so that they would have the same tip size, to test and compare the stability of potentials of the two electrode-holder assemblies: a conventional holder with an Ag/AgCl wire (chlorided by soaking it into Clorox™ as described in Section 2) and the other with the micro-agar salt bridge. We recorded the potential in current-clamp mode (current was set to zero) for a period of 30 min. Then we exchanged both pipettes for a new pair as we did during our day-to-day patch-clamp experiments and repeated the 30-min recording. As shown in the upper panel of Fig. 2A, the potential was quite stable in the first test period with a newly coated Ag/AgCl wire in the conventional holder. Potential drift started from the second test and it drifted up about 10 mV. Larger drifts were observed in the third to sixth tests, and were in the range of 20–25 mV. In the seventh test, 10 mm of the Ag/AgCl wire was immersed in the filling solution in the pipette. The drift was up to 28 mV. We looked at the Ag/AgCl wires with a dissection microscope after several changes of pipettes and we saw no apparent scratching on the AgCl coating. As can be seen in Fig. 2A, most of the drift was within the first 12–15 min of the test; the potential stabilized after 15 min.

Using a micro-agar salt bridge electrode (Fig. 2A lower panel), the drift was greatly reduced in the first 30-min test of the newly made salt bridge. The electrode potential remained extremely stable (drift within 3 mV) starting from the second test, even after repeated changes of the pipettes (tests 1–7 in parallel with those tests of conventional electrode using paired pipettes; Fig. 2A upper panel). These data suggest that the junction potential of the Ag/AgCl wire interfacing with the pipette filling solution of low Cl^- concentration is the major source of the potential drift.

Over a period of 3 months, we tested a micro-agar salt bridge electrode for patch-clamp recordings from brainstem slices. The salt bridge was maintained in a 3 M KCl solution when it was not in use. After 3 months, excellent stability was still maintained. A series of tests showed a very similar profile as traces 1–7 in the lower panel of Fig. 2A. The eighth trace was the second test recording after storage in 3 M KCl. These results suggest that the micro-agar salt bridge electrode provides long-term stability for electrophysiological experiments.

To illustrate how the magnitude of potential drift varied with the number of times we switched pipettes, drifts during 30-min tests versus sequential numbers of tests with different pipettes are shown in Fig. 2B. The data were averaged maximum drift during the course of 30-min in three series of experiments using the protocol described in panel A. Each series started with a pair of new Ag wires coated simultaneously by soaking them in Clorox™. One of the pair was used in the conventional electrode and the other was used in a newly made micro-agar bridge. In the first test, the potential drift was 7.8 ± 4.41 mV (mean $\pm$ S.D.) with the conventional electrode and 4.9 ± 1.42 mV with the micro-agar bridge electrode. In subsequent tests, the conventional electrodes showed consistent drifts of 20 mV or greater whereas drift with the micro-agar bridge electrode was consistently less than 2 mV ($p < 0.05$).

3.2. Comparisons between electrodes with the Ag wires coated three different ways

To examine if the potential drift depends on how the Ag wires are coated with chloride, we tested an electrolytically coated Ag/AgCl wire in the electrode holder in a series of seven tests (30 min each). We changed patch pipettes in every test as in typical patch-clamp experiments. In another series of seven tests with paired glass pipettes with the dual channel patch-clamp amplifier, we tested one electrode with a dipped-in AgCl wire and the other with a sintered Ag/AgCl wire. As shown in Fig. 3, in all cases, the magnitude of drift was smallest in the first test and increased in subsequent tests up to 10 mV with electrolytically coated Ag/AgCl wire (sixth and seventh tests in upper panel of Fig. 3), up to 15 mV with the sintered Ag/AgCl wire (seventh test, middle panel of Fig. 3), and as big as 20.6 mV with the dipped-in Ag/AgCl wire (second test, lower panel of Fig. 3).

3.3. Electrode resistance, capacitance and noise

We also examined the resistance, capacitance and noise level for the micro-agar salt bridge electrode-holder assembly versus a conventional electrode-holder assembly with the Ag/AgCl wire coated using Clorox (Table 1). There was no significant difference in electrode resistance between the micro-agar salt bridge electrode and the conventional one ($p > 0.05$,

paired *t*-test). The capacitance and noise of the micro-agar salt bridge electrode holder appeared to be larger than those of the conventional holder. However, when filled pipettes were inserted into the two holders, the capacitance and noise of each of the two electrode-holder assemblies were not significantly different.

3.4. Numerical simulations

To examine quantitatively how 3 M KCl in the micro-agar bridge diffuses to the pipette solution and how the concentration of Cl^- at the pipette tip changes with time during patch-clamp experiments, numerical simulations based on Fick's second law were performed. The geometry of the diffusion system including the micro-agar salt bridge and the pipette solution is shown in Fig. 4A. The diffusion coefficient for Cl^- is $2.032 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ in water (Lide, 2006) and is $1.3 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ in 3% agar (Djelveh et al., 1989) at 25 °C; these numbers were incorporated into the model. In whole-cell recording configuration, since the cell volume is tiny compared with the pipette solution, we defined flux = 0 at the tip, i.e., plugged tip, in the model. The initial value of Cl^- was 3 M in the micro-agar bridge and was 0 M in the pipette solution. The diffusion process started at $t = 0$. The modeling showed that, at $t = 30 \text{ min}$ (the duration of many patch-clamp experiments), the concentration of Cl^- at the pipette tip was 406 nM. This concentration increased further with time. At $t = 60 \text{ min}$, the Cl^- concentration was 230 μM (Fig. 4B and C). In commonly used experimental conditions (such as our pipette solution that contained 5 mM NaCl, the concentration changes at the tip would be much smaller as the driving force for diffusion is smaller compared with initial value of 0 Cl^- in the model), these concentration changes are negligible.

The changes of Cl^- concentration at the pipette tip were greatly affected by the distance from the agar surface in contact with the pipette solution to the tip. Increasing this distance by 10 mm (Fig. 4A and E, the distance from the agar surface to the tip was increased from 15 to 25 mm), diffusion caused minuscule changes in Cl^- concentration (at the levels of 10^{-10} M) at the tip within 1 h and only 8.6 μM change within 2 h (Fig. 4E).

As shown in Fig. 4D, the model predicted that the Cl^- concentration in the micro-agar bridge at the interface with the pipette solution falls rapidly in the first few minutes as the Cl^- diffuses into the pipette solution. However, in the agar bridge a few mm away from the interface the Cl^- concentration is maintained near 3 M (panels B and E). Thus the calculations indicate that the micro-agar bridge in this geometry can effectively immobilize Cl^- ions, maintaining a high and stable concentration of Cl^- contacting the Ag/AgCl wire.

Since K^+ has a slightly smaller diffusion coefficient than Cl^- and the pipette solution contains a high concentration of K^+ (130–140 mM) in typical whole-cell patch-clamp experiments, based on our simulation results for Cl^- , the effects of K^+ diffusion into the pipette solution on the cell would not be a concern.

4. Discussion

In this study, our data show that electrode potential drift during the course of patch-clamp experiments can be a serious concern (up to 27.3 mV during a 30-min recording in

commonly used conditions). Although some methods to coat an Ag wire, such as electroplating or sintering AgCl, reduced potential drift, the drift was still substantial and not optimal for patch-clamp experiments. We developed a practical method for constructing a micro-agar salt bridge of 3 M KCl in the patch electrode holder that practically eliminates substantial drift of electrode potential during the course of experiments. This salt bridge does not affect electrode resistance, capacitance and noise compared with a conventional electrode-holder assembly. Numerical simulations for the Cl^- diffusion process in the micro-agar bridge and the pipette solution indicate an insignificant effect on intracellular Cl^- level in generic experimental conditions. This micro-salt bridge electrode is suitable for most applications with a variety of patch pipette filling solutions containing from zero to high concentrations of Cl^- . Experiments examining the selectivity of Cl^- channels in particular can benefit from this technique. The use of an agar-filled microtubing also prevented the Ag/AgCl wire from scraping while changing pipettes. This method provides stable electrode potential during real-life patch-clamp experiments and provides long-term stability with minimal maintenance. The micro-salt bridge is easy to make and can be used in most commercial patch electrode holders.

We observed a total junction potential in the range of -75 to -90 mV with the micro-salt bridge electrode which is the sum of the junction potentials between the Ag/AgCl wire and the 3 M KCl salt bridge, between the salt bridge and the patch pipette solution, between the pipette solution and the bath solution at the pipette tip, as well as the junction potential between the bath solution and AgCl reference electrode. This value is consistent with previous calculations and measurements (Raynauld and Laviolette, 1987). The potential can be offset by the patch-clamp amplifier in electrophysiological experiments and is not a concern as long as it is stable during the experiments. This potential can be reduced to close to zero by placing an agar salt bridge of 3 M KCl at the reference electrode.

The electrode potential of a conventional electrode-holder assembly drifted substantially during the course of experiments. Although the junction potentials between the Ag/AgCl wire and the patch pipette solution, between the pipette solution and the bath solution as well as between the bath solution and the AgCl reference electrode all contribute to the total junction potential, our data showed that when the Ag/AgCl wire made connection with the pipette solution via the micro-salt bridge instead of making direct contact, the potential drift under the same conditions became negligible. These results suggest that the junction potential of the Ag/AgCl wire in contact with the pipette solution is the primary source of offset potential drift during experiments.

Our tests with a conventional Ag/AgCl wire in the electrode-holder assembly showed that the potential drifted considerably within the first 12–15 min, then gradually stabilized. This waiting period for stabilization can be very time consuming if one has to wait 15 min every time after changing a pipette between patching on cells. Therefore, the micro-agar bridge in the electrode-holder assembly described in this study is recommended.

The electrode potential of the conventional patch-clamp electrode drifted substantially, starting from the second or third test. Since electrode potential drift can be minimized by the 3 M KCl salt bridge, the primary cause of the drift is the low concentration of Cl^- in the

patch pipette filling solution (5 mM). These results are consistent with the idea that the interface of Ag/AgCl wire with low Cl^- solutions becomes non-reversible (Purves, 1981; Raynauld and Laviolette, 1987; Snyder et al., 1999). Some solid AgCl on the silver wire dissolves and the Cl^- concentrations in the vicinity of the wire increase causing the 12–15 min initial rise of the electrode potential. In addition, fluid currents change the local concentrations of Cl^- ; therefore, the junction potentials are unstable (Raynauld and Laviolette, 1987). These problems have been resolved with the micro-agar salt bridge method in this study.

We noticed that with the micro-agar salt bridge electrode there was a monotonic drift of electrode potentials of a few millivolts in consecutive 30-min tests and a moderate drift in the first test (Fig. 2A lower panel and panel B). The offset potential returned to ~ -90 mV after the salt bridge was maintained in 3 M KCl solution overnight (Fig. 2A trace 8 in the lower panel). This was due to the diffusion between the immobilized 3 M KCl and the pipette solution. The ionic composition at the tip of the agar salt bridge tends to approach that of the pipette filling solution (Fig. 4B, D and E) (Barry and Diamond, 1970). The drift is slow and small compared with that of a conventional electrode-holder assembly. An electrode potential drift of 1–3 mV within an experiment is usually acceptable (see below). Additional drift between experiments is not a concern since it can be offset by the patch-clamp amplifier. For the concern regarding the drift in the first experimental use of a newly made micro-agar salt bridge or the first use of a micro-agar bridge maintained in 3 M KCl, an easy solution would be to place the tip of the micro-agar bridge in contact with the patch pipette solution for 20–30 min before starting patch-clamp experiments.

When measuring anion permeability using 0 Cl^- in the pipette solution over 30 min, there may be concerns about the micromolar changes in Cl^- concentration at the pipette tip. We suggest two protocol modifications. First, one can immerse the micro-agar bridge tip into the pipette solution for 10–20 min before starting patch-clamp experiments. As shown in Fig. 4D, the concentration of Cl^- at the surface of the micro-agar bridge in contact with the pipette solution rapidly decreased within first few minutes and then stabilized at 0.2–0.3 M. After 10–20 min in contact with the pipette solution, the starting KCl concentration at the agar surface for a new pipette would be 0.2–0.3 M instead of 3 M. Therefore, the changes of Cl^- at the pipette tip would be much smaller. Secondly, one can shorten the microtubing and the Ag/AgCl wire, increasing the distance from the micro-agar bridge surface to the tip. Simulation results in Fig. 4E showed, when this distance was increased by 10 mm, there was almost no change in Cl^- concentration at the tip within 1 h and only 8.6 μM change within 2 h.

In addition to conventional whole-cell and outside-out patch recordings, this micro-agar salt bridge method is also applicable and may greatly improve perforated patch recordings (Horn and Marty, 1988) when the Cl^- concentration in the patch pipette solution is low. This argument also holds for patch pipette internal perfusion techniques (Cull-Candy et al., 1981; Velumian et al., 1993), if the pipette filling solution level or the Cl^- concentration is going to change during internal perfusion.

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Fig. 1.

(A) Schematic of the micro-agar salt bridge. Three percent agarose in 3 M KCl solution was heated in a water bath at 75–80 °C. Then a polyimide tubing (i.d. 410 μm, o.d. 520 μm) was filled 20–25 mm long with the hot agarose gel by capillary action. A chlorided Ag wire ($d = 250 \mu\text{m}$) was inserted into the hot agarose gel in the tubing. The back of the tubing was sealed with epoxy or cyanoacrylate. The salt bridge can be installed in commonly used commercial holders with the rubber gasket provided. (B) Photograph of the micro-agar salt bridge in position in an AXON HL-U holder. When the pin cap is tightened, the 90° bent Ag wire on the rubber gasket makes contact with the 1 mm pin that fits in the headstage of the patch-clamp amplifier. When the filled patch pipette is inserted into the electrode holder, the pipette filling solution makes contact with the agar salt bridge at the tip of the polyimide tubing instead of making direct contact with the Ag/AgCl wire. For clarity, the threaded collar of HL-U holder for securing the holder on the headstage is omitted in the photograph.

Fig. 2.

(A) Electrode potentials during the course of 30-min tests recorded using a conventional electrode-holder assembly with the Ag/AgCl wire (chlorided by soaking it in CloroxTM) in direct contact with the pipette filling solution (upper panel) or the micro-agar salt bridge electrode-holder assembly (lower panel). Numbered traces (1–7) indicate sequential trials with new pairs of pipettes. Trace 8 is a record obtained with the micro-agar bridge electrode after it had been used in patch-clamp experiments for 3 months (stored in 3 M KCl when not in use. Second test after storage in 3 M KCl). (B) Summary of data (mean $\pm$ S.E.) obtained in three series of experiments using the protocol illustrated in Panel A excluding trace 8. Drift was measured as the difference between the maximum and minimum potential of each trace. Asterisks indicate significant differences ($p < 0.05$) between conventional electrode and the micro-agar salt bridge electrode determined with a repeated measures ANOVA model.

Fig. 3.

Electrode potentials during the course of 30-min tests with conventional electrode-holder assembly with: electrolytically coated (electroplated) Ag/AgCl wire; sintered Ag/AgCl wire, or; dip-in Ag/AgCl wire in direct contact with the pipette solution. Refer to Fig. 2 legend for the testing procedure.

Fig. 4.

(A) Geometry for the finite element simulation of the diffusion of Cl^- in the micro-agar bridge electrode. The x and y dimensions have been drawn to different scales for clarity. The agar gel covered 20 mm of the Ag/AgCl wire. The interface between the agar surface and the pipette solution was at 23 mm. The distance from this interface to the pipette tip was 15 mm. The agar bridge microtubing was immersed 5 mm into the pipette solution. The initial Cl^- concentration in the agar was set to 3 M while the Cl^- concentration in the pipette filling solution was set to 0 M. See text for details of the calculations. (B) Calculated Cl^- concentration as a function of distance after 0.5 and 1 h of diffusion. Each curve consists of

two segments: one from 0 (back end of the micro-agar bridge) to 20 mm in agar at the midway between the surface of the Ag/AgCl wire and the inner surface of the polyimide tubing; the other from 20 mm at the center of Ag/AgCl wire to the pipette tip. The dashed line marks the position of the interface of the micro-agar bridge and the pipette filling solution. To the left of this line the graph indicates the concentration in the agar whereas to the right of this line the graph indicates the concentration in the pipette solution. The abscissas of (A) and (B) are equivalent. (C) Calculated Cl^- concentration at the pipette tip as a function of time. Note the 30 min delay before there is a detectable increase in Cl^- . (D) Calculated Cl^- concentration at the center of the interface between the micro-agar bridge and the pipette solution as a function of time. (E) Calculated Cl^- concentration as a function of distance after 1 and 2 h of diffusion in a model modified by increasing the distance between the agar/pipette solution interface and the pipette tip to 25 mm. The dashed line marks the position of the interface between the agar and the pipette filling solution. The small jitters in the 1 h trace at the tip are due to small instabilities in the numerical simulations at very low Cl^- concentrations when they are expressed in log scale. Panels (B) and (E) are log-scaled.

Table 1

Electrical properties of conventional and micro-agar salt bridge electrode-holder assemblies

	RMS noise (pA)	C_{fast} (pF)	τ_{fast} (μs)	Resistance (MΩ)
Headstage	0.56			
Conv holder	0.59	2.44	0.82	
Conv holder + filled pipette	0.62	2.9	0.83	5.77 $\pm$ 0.75
Agar bridge holder	0.62	2.57	0.83	
Agar bridge holder + filled pipette	0.63	2.88	0.84	5.82 $\pm$ 0.71

Conventional (conv) holder with Ag/AgCl wire coated using CloroxTM was immersed in the patch pipette solution 5 mm deep. Open circuit noise and capacitance of the two holders were measured with the same headstage and the same channel of the patch-clamp amplifier. RMS noise was determined by the built-in Irms feature in a bandwidth of 30 Hz–5 KHz in MultiClamp 700B in voltage-clamp mode. Open circuit capacitance was measured with the Cp fast Auto capacitance compensation feature in MultiClamp 700B in voltage-clamp mode where the capacitance is displayed in pF and in μ s (time constant τ_{fast}). Resistance values are means $\pm$ S.D. of six pairs of pipettes. Every pair was pulled from the same piece of glass pipette and was tested one with the conventional holder, and the other with the micro-agar salt bridge holder using the dual channel patch-clamp amplifier at the same conditions. Two pipette tips were immersed into the same dish of bath solution grounded with the same Ag/AgCl reference electrode.