Topic Introduction

Single-Channel Recording of Ligand-Gated Ion Channels

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Single-channel recordings reveal the microscopic properties of individual ligand-gated ion channels. Such recordings contain much more information than measurements of ensemble behavior and can yield structural and functional information about the receptors that participate in fast synaptic transmission in the brain. With a little care, a standard patch-clamp electrophysiology setup can be adapted for single-channel recording in a matter of hours. Thenceforth, it is a realistic aim to record single-molecule activity with microsecond resolution from arbitrary cell types, including cell lines and neurons.

INTRODUCTION

Single-channel recordings are a label-free method to obtain microscopic kinetic information from the ion channels that participate in synaptic transmission in neurons (Hamill et al. 1981). The time course of synaptic currents depends on the kinetics of ligand-gated channels (Wyllie et al. 1998), and single-channel conductance determines charge transfer during a synaptic potential. In the age of super-resolution imaging, we can track individual ion channels as they diffuse in and out of synapses (Opazo et al. 2010). So, in general, microscopic properties of ion channels are highly relevant for understanding basal signaling in neurons and plastic changes in synaptic strength both short and long in duration. Furthermore, because they can yield rich kinetic information, revealing events from microseconds to seconds, single-channel patch-clamp recordings can be used to assess conformational changes (Burzomato et al. 2004) or the number of subunits (Rosenmund et al. 1998) in an ion channel complex.

DESIGN OF SINGLE-CHANNEL RECORDING EXPERIMENTS

First, the optimal recording configuration must be chosen. The three main options are cell-attached (with ligand inside the pipette), inside-out, and outside-out (Fig. 1). Intracellular ligand-gated channels (e.g., some transient receptor potential [TRP] channels) can be patched in analogous ways, with organelles obtained by fractionation (Kirichok et al. 2004) or by slicing cells open with a patch pipette (Dong et al. 2008). Further, biochemically purified channels can be reconstituted into membranes for bilayer recordings to verify their activity (Baranovic et al. 2013; Dellisanti et al. 2013). Some ligand-gated channels (e.g., glycine receptors [Fucile et al. 2000]) undergo substantial changes in activity following patch excision. Such receptors are best studied in the cell-attached configuration, allowing

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FIGURE 1. Recording configurations in mammalian cells. (A) Excising an inside-out patch from the cell-attached configuration often results in a bleb, from which no recording can be made. The outer surface can be removed by brief exposure of the patch to air. Outside-out patches form within the pipette from the resealing of the patch membrane after a smooth and assertive withdrawal of the patch pipette from the whole-cell configuration. For an organellar patch, the plasma membrane is sliced and the organelle of interest (preferably labeled; here shown as a black oval) is pushed out through the incision. Agonist molecules (black) are shown in the pipette or bath solution, as appropriate. The outer leaflet of the plasma membrane is shown in black to aid orientation. (B) A typical recording (30 sec) is shown for the rat glycine $\alpha 1$ receptor in cell-attached mode. Chloride-conducting openings are upward at negative membrane potential (pipette potential was approximately $+70$ mV in this recording). The bar under the trace indicates the magnified section (shown at *bottom*) illustrating a cluster with high open probability.

records to be collected for tens of minutes (Burzomato et al. 2004; Plested et al. 2007; Kussius and Popescu 2009; Purohit and Auerbach 2009). The ligand is included in the pipette and cannot be exchanged.

The noise in excised patches tends to be higher, but patch excision brings the opportunity to do both equilibrium and nonequilibrium experiments (Rosenmund et al. 1998), which can provide specific information about channel kinetics. It is also essential with a channel of unknown properties to start in a configuration where the patch can be perfused, to establish the dependence of activity on relevant agonists or drug compounds. For most receptors, this process means recording in outside-out configuration. The same logic applies to channels with intracellular ligand-binding sites (such as cyclic nucleotide-gated [CNG] channels), for which excision into the inside-out configuration is required. A precisely timed perfusion system is helpful for isolating specific activity induced by, say, an agonist from random noise, other channels, and breakdown. For glutamate receptors, we use a fast perfusion system with resolution of better than a millisecond (Carbone and Plested 2012), even though we generally apply glutamate for seconds at a time. Recording episodically with timed perfusion allows episodes that show spurious activity outside of the agonist pulse to be discarded.

For nonequilibrium experiments, it is generally necessary to obtain patches with one channel (Rosenmund et al. 1998), which is achieved only with very low expression. Channel activity may also run down during the recording, yielding a section with only one channel. To obtain useful equilibrium recordings, it is essential that the channel density in the membrane be neither too high nor too low. The aim is to isolate stretches of recording where we are certain only one channel is active. In this context, the ideal level of expression depends on the activity of the particular channel. If the channels have substantial open probability, it is likely that only patches with a few channels will be useful, otherwise activity will be overlapping. Normally, the random nature of expression will lead to some

patches being “blanks”—that is, no activity at all is observed. If the channel in question desensitizes substantially, it is not a problem to have multiple channels in the patch, because there is a good chance that for some of the record, only one of the channels will be active, and the rest will be desensitized.

A further critical consideration is what voltage to record at. We want the highest potential at which patches can survive and give a decent conductance. Most ligand-gated channels, including the nicotinic receptors and glycine receptors (Dionne and Stevens 1975; Gill et al. 2006), however, show some weak voltage dependence in their activity. Thus, the recording voltage cannot be chosen at random. Recordings at potentials up to 100 mV can last tens of minutes in good conditions. More extreme potentials tend to dramatically shorten patch lifetime, probably because of voltage-dependent “creeping” deformation (Suchyna et al. 2009).

PREPARING THE SETUP

The luxury of building a single-channel setup from scratch is less common than adapting an existing rig. Most equipment is standard for any patch-clamp electrophysiology, but some attention should be paid to the choice of manipulator, to ensure mechanically stable, low-noise recordings. Hydraulic manipulators are naturally electrically silent but do seem to suffer from drift more than their motorized counterparts. Mechanical drift is as problematic for cell-attached recording as it is for whole-cell patch clamp. In severe cases, patch excision will remove this constraint. Scientifica manipulators have admirable stability and apparently no noise. Stepper-motor-driven manipulators can also work well, but most designs require that current flows to keep the manipulator still. In the popular Sutter manipulators, the stepper holding current can be adjusted (reduced) by using a secret menu at the expense of slightly more drift (contact Sutter for details). In our hands, Luigs & Neumann manipulators, although having incredible mechanical stability, are not optimized for low electrical noise. These manipulators, and some others, can be switched off after a recording begins, without mechanical disturbance.

Electrical noise in the patch-clamp record has numerous sources, both those intrinsic to the amplifier and those extrinsic (i.e., from the environment) (Benndorf 1995). Noise adds in quadrature: In practice, the largest source of noise swamps the rest. Thus, after removing a major source of noise, all previously ineffective manipulations should be repeated. The practical process is illustrated in Table 1. Recently, inexpensive digital oscilloscopes became available that can generate noise spectra on the fly. This feature can be useful for detecting major sources of noise.

The first assumption should be that every electrical device is a major source of noise until proven otherwise. Typical troublemakers are the often inexpensive power supplies provided for cameras and illuminators. Video monitors, mobile phones, refrigerators, and centrifuges are further familiar culprits. Unfortunately, some microscopes were not manufactured with low electrical noise in mind. In our hands, the IX series from Olympus can be grounded entirely from one point at the back corner of the movable stage.

Solution lines that are not needed should be removed—especially those that run outside the Faraday cage. In any case, the best noise conditions are obtained if the patch is held near the top of the bath, so that the immersion of the pipette is minimized. For cell-attached recordings, perfusion can be removed altogether, as long as bathing solution is exchanged regularly by hand. Perfusion of excised patches brings noise. The major source is microphonic pickup of acoustic noise by the pipette as solution is sucked from the bath. We avoid this problem by removing solution from the bath only between episodes and otherwise stopping the suction using a pinch valve under computer control. The slow increase in the level of the bath alters capacitance of the pipette, and thus brings noise pickup, but

TABLE 1. Hypothetical noise troubleshooting

Noise source	Amplifier limit ^a	Manip	Lamp	Camera	Earth loop	Dirty holder	Total	Fraction in round (%)
Round #1								
Setup	50	50	100	300	400	800	951	
Switch off manipulator	50		100	300	400	800	950	100
Insulate lamp cable	50	50		300	400	800	946	99
Turn off camera	50	50	100		400	800	903	95
Rewire	50	50	100	300		800	863	91
Clean the holder	50	50	100	300	400		515	54
Conclusion: Removing stray salt and dirt from the pipette holder reduces noise by ~50%.								
Round #2								
Setup	50	50	100	300	400		515	
Switch off manipulator	50		100	300	400		512	100
Insulate lamp cable	50	50		300	400		505	98
Turn off camera	50	50	100		400		418	81
Rewire	50	50	100	300			324	63
Conclusion: Removing an earth loop, caused by too many grounding cables, reduces noise by ~40%.								
Round #3								
Setup	50	50	100	300			324	
Switch off manipulator	50		100	300			320	99
Insulate lamp cable	50	50		300			308	95
Turn off camera	50	50	100				122	38
Conclusion: Turning off the camera after finding the cell reduces noise by 60%.								
Round #4								
Setup	50	50	100				122	
Switch off manipulator	50		100				112	91
Insulate lamp cable	50	50					71	58
Conclusion: Insulating the lamp cable from the tabletop reduces noise by 40%.								

Typical noise contributions (in fA RMS) in the 5-kHz band are indicated. Four successive rounds of searching for the major source of noise return a major reduction from a new source each time. For example, turning off the camera has no effect in round #1 but has a major effect in round #3. Sources of noise in each row (σ_i , corresponding to each component) add in quadrature to give the total:

$$\sigma_{\text{total}} = \sqrt{\sigma_1^2 + \sigma_2^2 + \dots}$$

The boxed value is the lowest for each round.

Manip, micromanipulator.

^aAmplifier limit cannot be changed or removed.

this effect is preferable to continuous suction noise. If required, the suction line from the bath can be internally grounded with a long length of silver wire.

A key principle is to avoid ground loops by limiting the number of grounding connections and by testing all of them for electrical integrity. Major optimizations can be done in a few hours simply by cleaning out the setup. Start with a minimal setup and only add back essential items. To achieve very low noise levels is extremely challenging but a little careful work at this stage can produce major dividends. Once the setup is adjusted, it will not necessarily be trivial to switch between different recording configurations. Maddeningly, counterintuitive modifications (such as disconnecting unique earth leads) may improve noise.

For excluding noise whose source cannot be throttled, structural shielding of the prep with wire mesh or metallic fabric (e.g., Shieldex Bremen) is a useful adjunct to the Faraday cage. Metallic fabric can act as a very effective full curtain at the front of a cage as well.

Once the noise levels are minimized at the setup, the main source of noise will become the seal itself—tight, multi-gigaohm seals are indispensable for low-noise recording.

DATA ANALYSIS

A common question is: How much data do I need? Conductance measurements require relatively few isolated openings. For fitting of kinetics, it is necessary to collect 5000–10,000 or more events in each

recording. However, what can be collected depends on channel activity. Ideally, measuring events with a mean lifetime of 10 msec, there will be 100 events per second, which means recording for a couple of minutes will yield enough. This goal is not trivially achieved.

It is crucial to examine the digitized record by eye to confirm that the recording is stable. Glitches, other channels, or noise and drift will play havoc with even the simplest measurements of conductance. Baseline drifts can be corrected by hand or, in some software packages, automatically. But no automated procedure can assure a clean record without checking.

To measure kinetic information seriously, the digitized record must be idealized into a list of events, with their durations, amplitudes, and other properties. The fastest events that can be seen depend on the relation between the single-channel conductance and the background noise, because more noise means a more closed (and thus slower) filter.

Several packages are available to evaluate putative kinetic models against idealized data, but this approach is beyond this scope of this introduction. To make meaningful inferences at this stage, it is essential to reliably identify stretches of the record where only one channel is active. There may be a degree of iteration required to identify the right recording conditions to get meaningful information. In low-activity conditions, we expect bursts (groups of openings) to be well spread out. However, the degree to which bursts should be separated to be well defined can be surprisingly large—about 100-fold longer than the mean duration of the next shortest shut interval (Lape et al. 2012).

APPLICATIONS

Most single-channel studies are done in human embryonic kidney (HEK) 293 cells or from patches excised from *Xenopus* oocytes. (For methods to record from HEK cells, see Protocol: **Single-Channel Recording of Glycine Receptors in Human Embryonic Kidney (HEK) Cells** [Plested and Baranovic

FIGURE 2. The single-channel recording landscape. The larger the conductance, or the slower the kinetics, the easier it is to obtain recordings, because the signal-to-noise ratio is larger at a given filter setting. The size of each circle corresponds to the approximate number of single-channel papers for the channel in question in the PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed/>) in 2014. The range is from ~5 (HCN) to ~600 (muscle nicotinic). Abbreviations: VGSC, voltage-gated sodium channel; VGPC, voltage-gated proton channel; VGCC, voltage-gated calcium channel; HCN, hyperpolarization-gated cation channel; CNG, cyclic nucleotide-gated channel.

2016].) Recording single channels from neurons and other native cells has somewhat fallen out of vogue, mainly because so many different channel variants are expressed at somatic membranes, and there are few ways to control expression. However, there is great value in this approach if channels can be “fingerprinted” from their conductance or kinetics. In recent years, there have been considerable advances in the recording of intracellular channels or channels in specialized cells such as sperm. These approaches are often key for answering the question of whether a given protein is a channel or a transporter. When choosing a topic of study, the simple relation plotted in Figure 2 shows what is likely to be achievable. This figure shows the single-channel research landscape—how many papers were found in PubMed for each type of channel plotted against conductance and open time. It is notable that publishing on small or fast channels is comparatively much more difficult, or less popular, than large-conductance channels such as the BK potassium channels. But the lesser-studied channels remain important for physiology and represent understudied targets that may still yield fascinating insights.

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