Inhibitory Glutamate Receptor Channels in Cultured Lobster Stomatogastric Neurons

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Cleland, Thomas A. and Allen I. Selverston. Inhibitory glutamate receptor channels in cultured lobster stomatogastric neurons. J. Neurophysiol. 79: 3189–3196, 1998. Inhibitory glutamate receptor channels (IGluRs) are ligand-gated ionotropic receptors related to ionotropic \( \gamma \)-aminobutyric acid (GABA) and glycine receptors and expressed in neural and muscular tissues. In the crustacean stomatogastric ganglion (STG), IGluRs mediate recurrent synaptic inhibition central to the rhythmic capabilities of its embedded neural circuits. IGluRs expressed in cultured spiny lobster STG neurons exhibited an \( EC_{50} \) of 1.2 mM and a Hill coefficient of 1.4. They were neither cross-activated nor cross-desensitized by GABA, although a distinct GABA-gated chloride current was observed. Glycine did not evoke any current from STG neurons. The IGluR was weakly blocked by the chloride channel blocker furosemide and the excitatory glutamate receptor antagonist 6-cyano-7-nitroquinoline-2,3-dione (CNQX), but was not inhibited by bicuculline methiodide, strychnine, kynurenic acid, \( \gamma \)-D-glutamylglycine, or aspartate. Outside-out patch-clamp recordings were analyzed using the mean-variance histogram technique. Under excised-patch conditions, the receptor exhibited only a single open state with an estimated unitary conductance of \( 80 \pm 8.6 \) (SD) pS. The distinct GABA receptor also displayed a single open state with a conductance of \( 72 \pm 10 \) pS.

INTRODUCTION

Inhibitory glutamate receptors (IGluRs) are ligand-gated ion channels, closely related to ionotropic glycine and \( \gamma \)-aminobutyric acid receptors (GABA\(_ R \)) and primarily permeable to chloride ions. IGluR channels have been cloned from Caenorhabditis elegans and Drosophila melanogaster (Cully et al. 1994, 1996); the sequences of these IGluRs confirm that they are not closely related to any of the excitatory glutamate receptor families. IGluR orthologs are found in diverse species, in both neural and muscle tissues, and can be expressed both synaptically and extrasynaptically (reviewed by Cleland 1996). Among the 27–32 (King 1976) identified neurons of the stomatogastric ganglion (STG) of the Pacific spiny lobster, Panulirus interruptus, IGluRs are responsible for the majority of the intraganglionic synaptic inhibition that underlies burst phase regulation and rhythogenesis in this well-defined motor pattern generator (Bidaut 1980; Harris-Warrick et al. 1992; Marder and Paupardin-Tritsch 1978). The chemical modulation of glutamatergic synaptic efficacy contributes to the dramatic reconfiguration of the motor output patterns generated by the STG (Johnson and Harris-Warrick 1997; Johnson et al. 1995); direct modulation of the IGluR itself contributes to some, but not all, of these modulatory effects on glutamatergic synaptic efficacy (Cleland and Selverston 1997). These results establish that functional IGluR modulation can be mediated by multiple effectors; other known STG modulatory pathways show similar convergence and divergence properties (Kiehn and Harris-Warrick 1992a,b; Zhang and Harris-Warrick 1994).

The voltage- and ligand-gated membrane currents of crustacean stomatogastric neurons have recently begun to be investigated in neurons isolated in primary culture (Cleland and Selverston 1995, 1997; Turrigiano et al. 1994, 1995; Turrigiano and Marder 1993). Neurons cultured with short neuritic stumps (Fig. 1A) are electrotonically compact, such that even currents normally expressed in the distal neuropil can be studied quantitatively under voltage clamp or excited in membrane patches. As postsynaptic receptors, IGluRs in the intact STG are expressed at neuritic sites electrically distant from electrodes implanted into the somata; however, after 2–3 days in primary culture, many stomatogastric neurons will express functional IGluRs in the somatic membrane (Fig. 1B). Furthermore, neurons in culture are unambiguously isolated from the influences of other neurons in the network, and their modulatory chemical environment can be more closely controlled. The advantages of these studies complement research efforts in the intact pattern generator, in which the constancy of cell identity is more assured and in which the spatial and contextual information destroyed by the culturing process remains relatively intact.

The original mapping of the STG circuit laid the groundwork for the discovery of neuromodulatory circuit reconfiguration. Subsequent studies of the effects of these modulators on STG intrinsic rhythmicity and motor output have demonstrated that the regulation of the pattern generator circuit is highly distributed in organization. A similarly concerted effort to map several of these distributed elements (receptors, effectors, modulators, and signal pathways) among the identified neurons of the STG has the potential to guide us to an understanding of the fundamental principles by which they are integrated to form robust, adaptive behaviors.

Some of these data were previously published in abstract form (Cleland and Selverston 1996).

METHODS

Cell preparation and primary culture

Adult spiny lobsters, Panulirus interruptus, were captured and maintained in running seawater tanks until use. Lobsters were chilled under ice for anesthesia; the stomatogastric ganglion was removed and cultured as described previously (Cleland and Selverston 1995). Briefly, ganglia were incubated in 2 mg/ml subtilisin for 1 h and washed for \( \approx 2 \) h; neurons were individually isolated by suction and plated into Falcon Primaria culture dishes with sterile Panulirus maintenance medium. Recordings were made...
filled with a solution of 0.6 M K₂SO₄ and 20 mM KCl. In some recordings (Figs. 3 and 5, C and D), the voltage-recording (passive) electrode contained 3 M KCl. Two-electrode voltage-clamp recordings were made with an Axoclamp-2A (Axon Instruments, Flinn, 2002).

**Media**

Panulirus saline was prepared as described in Mulloney and Selverston (1974). Panulirus maintenance medium for cultured neurons (PMM) was prepared as described in Cleland and Selverston (1995); briefly, it consisted of sterile, salt-balanced L-15 medium with 2–3 mg/ml glucose, 100 U/ml penicillin, and 100 μg/ml streptomycin added.

Whole cell recordings were performed in Panulirus saline with or without added pharmacological agents. In outside-out patch-clamp recordings, the bath was Panulirus saline, while the “extracellular” face of the patch was bathed in a constant flow of a medium composed of Panulirus saline with 50 μM Ni²⁺, 200 μM Ca²⁺ (to block low-voltage–activated and high-voltage–activated calcium channels, respectively), and 0.1% Fast Green (for visualization of the stream) added. The “intracellular” (patch pipette) medium was composed of the following (in mM): 53.8 NaCl, 450 CsCl, 0.5 CaCl₂, 5.0 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 5.0 maleic acid, 11.0 Trizma base, and 5.0 Mg-ATP, pH 7.45 with NaOH. Predicted free intracellular calcium was 14 nM (due primarily to chelation by EGTA); predicted free intracellular magnesium was ~600 μM (Schoenmakers et al. 1992). Chloride concentrations were symmetrical across the membrane patch (E_Cl = –1 mV).

**Two-electrode voltage-clamp recording**

Two-electrode voltage-clamp recordings were performed as described previously (Cleland and Selverston 1995). Briefly, electrodes of 8–15 MΩ were pulled from Sutter BF100-78-10 thin-walled borosilicate glass (Sutter Instrument, Novato, CA) and after 3–6 days of incubation at 15°C. Experiments were performed in Panulirus saline using two-electrode voltage-clamp or outside-out patch-clamp techniques.

**FIG. 1.** A: spiny lobster stomatogastric neuron in culture. Absence of extensive neuritic arborization improves the clamping of the membrane potential at the locus of all expressed membrane currents, even those normally expressed in the distal neuropil. Scale bar: 50 μm. B: response of a cultured stomatogastric neuron to short puffs of glutamate, applied with a picospritzer. The application of 1 mM L-glutamate to the neuron resulted in a cessation of spiking activity and a transient hyperpolarization. In the depicted cell, this hyperpolarization has also brought the membrane potential down from its stable plateau state into its lower stable state, close to ~60 mV, before the wash out of the agonist allowed the neuron to regain its plateau potential. Some identified stomatogastric neurons in situ are known to “rest” in their depolarized, constitutively spiking plateau state when the ganglion is chemically unmodulated.

**FIG. 2.** A: dose-response curve for L-glutamate, applied so as to immerse the entire cell in agonist. Data were gathered from several neurons (n = 5); each data point was normalized with respect to the application of 1 mM glutamate to the same cell. After all data were collected, the values were normalized again to the glutamate concentration evoking the maximum membrane current. The power of submaximal agonist concentration to which the current response is proportional is termed the Hill coefficient, and represents the minimum number of agonist molecules that must bind to a receptor for it to be activated (Colquhoun 1975; Hill 1909; Werman 1969). The Hill coefficient of this relationship was calculated by fitting the data to the equation Y = 1/[1 + (EC₅₀/[Glu])ⁿ] using the Levenberg-Marquardt algorithm, where Y is the ratio of the current to its maximum value, [Glu] is the glutamate concentration, EC₅₀ is the glutamate concentration yielding half-maximal effect, and n is the Hill coefficient. EC₅₀ and n were free parameters. The EC₅₀ of this receptor for glutamate was 1.2 mM, whereas the Hill coefficient was estimated at 1.4. B: same dose-response curve plotted in double logarithmic coordinates, demonstrating that the Hill coefficient of the GluR is a constant (i.e., linear) for submaximal glutamate concentrations. The Hill coefficient is the maximum limiting slope of a dose-response curve plotted in double logarithmic coordinates. The line depicted to highlight linearity was fit to data derived from submaximal glutamate concentrations using the equation Y = axᵇ (Levenberg-Marquardt algorithm).
Foster City, CA). If fast sodium spike currents were present, they were blocked with a pulse of tetrodotoxin (TTX; 1 µM) before the experiment began. In most experiments, agonists were applied with a hydrostatic pulse delivery system as described previously (Cleland and Selverston 1995), and contained 0.05% Fast Green for visualization of the agonist stream. Where specially noted, agonists were delivered by picospritzer (Fig. 3A; General Valve, Fairfield, NJ). Note that the hydrostatic method immersed the entire cell in the agonist stream ("concentration clamp"), whereas the picospritzer applied agonist to a localized area of the membrane.

Data were low-pass filtered at 1 kHz, digitized to disk at 2 kHz using Data-Pac II 4.0 software (RUN Technologies, Laguna Hills, CA), and analyzed by computer.

Patch-clamp recording and analysis

Patch electrodes were pulled from WPI 1B150F-4 aluminosilicate glass, coated with wax to reduce tip capacitance, and filled with intracellular medium. Outside-out membrane patches (2–3 pF capacitance) were pulled from the somatic membrane of unidentified cultured STG neurons in a bath of Panulirus saline. The extracellular faces of excised patches were superfused with a Biologic RSC-100 focal superfusion switcher (Molecular Kinetics, Pullman, WA); agonist-free and agonist-containing extracellular media were delivered to the bath in adjacent parallel streams, which were rapidly (10 ms) switched to apply or remove agonists to the patch. The constant-flow environment minimized pressure waves associated with agonist application, thus mitigating potential artifacts due to pressure-sensitive membrane channels. The concentration clamp time constant using this method was substantially faster than that for the hydrostatic method employed for two-electrode voltage-clamp (whole cell) recordings.

Data were recorded with an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA), low-pass filtered at 2 kHz, digitized to disk at 10 kHz using pClamp 6.0 software (Axon Instruments, Foster City, CA), and analyzed using pClamp and mvMachine software (J. B. Patlak, University of Vermont), with which mean-variance histogram analyses were performed. Single-channel conductances were estimated with MGVMachine software (J. B. Patlak, University of Vermont), with which mean-variance histogram analyses were performed. Single-channel conductances were estimated with

![FIG. 3.](image)

**FIG. 3.** γ-Aminobutyric acid (GABA) and glycine do not cross-activate the inhibitory glutamate receptor channel (IGluR) in spiny lobsters. A: response of a given cell to a brief puff of 1 mM L-glutamate, applied with a picospritzer, differed in shape from its response to a similar puff of 1 mM GABA (Vi = −70). B: a high bath concentration of GABA (1 mM) did not alter the current evoked by hydrostatic whole cell superfusion of 1 mM L-glutamate in either amplitude or waveform, indicating that GABA neither appreciably activates the IGluR nor competes for the glutamate binding site on the receptor. GABA (1 mM) was superfused along with L-glutamate to maintain the extracellular GABA concentration during glutamate superfusion (Vi = −40 mV; n = 3). C: glycine (1 mM) did not evoke any current from cells that responded to 1 mM glutamate both before and after glycine application (Vi = −40 mV; n = 2).
comprising variance within these Markov states from data points that incorporate transitions between states. The means ± SD of the Gaussian functions can be calculated in each dimension (current and current variance); these values represent the mean holding current and mean current noise of the respective Markov states of the channel. Subtracting the values calculated for each open state from those calculated for the closed state yield quantitative estimates of the mean unitary current and open-channel noise characteristic of that open state. Notably, these estimates include error terms in each dimension (i.e., the SD of the fitted Gaussian function). The choice of window width is important for dwell time analyses, but not for estimation of unit conductance and noise. Consequently, in this study, various window widths (from 5 to 50 data samples) yielded similar results; all results herein are reported using a window width of 20 data samples.

**Suppliers**

L-glutamic acid, GABA, glycine, aspartic acid, furosemide, bicuculline methiodide, kynurenic acid, γ-D-glutamylglycine (γ-DGG), and strychnine were purchased from Sigma (St. Louis, MO). 6-Cyano-7-nitroquinoxaline-2,3-dione [CNQX, in 2-hydroxypropyl-β-cyclodextrin (HBC) complex] was purchased from Research Biochemicals (Natick, MA). TTX was purchased from Calbiochem (San Diego, CA). All drugs were mixed fresh from dry stocks on the day of the experiment, except for TTX, which was kept in frozen aliquots.

**RESULTS**

**Dose-response relationship**

In two-electrode voltage-clamp recordings, the IGluRs of lobster stomatogastric ganglion neurons exhibited an EC50 of 1.2 mM to L-glutamate (Fig. 2A; n = 5), an agonist sensitivity comparable with that observed in orthologous neuronal IGluRs (Cully et al. 1994; Ikemoto et al. 1988; King and Carpenter 1989). Such a relatively low sensitivity is associated with postsynaptic, as opposed to extrajunctional, IGluRs (Cleland 1996). The dose-response relationship had a Hill coefficient of 1.4 (Fig. 2B), implying that more than one glutamate binding event was required to open the channel, or that the binding of multiple glutamate molecules cooperatively enhanced the gating of the IGluR channel. Because receptor desensitization will cause Hill coefficients to be underestimated, the slope of 1.4 should be considered as a minimum value. Hill coefficients of 1.6–2.5 have been reported for neuronal IGluRs in other species (Arena et al. 1992; Ikemoto et al. 1988; King and Carpenter 1989; Sawada et al. 1984; Wafford and Sattelle 1989); these values are also similar to those exhibited by many postsynaptic GABA Rs (King and Carpenter 1989; Smart and Constanti 1986; Takeuchi and Takeuchi 1967).

**Agonist pharmacology**

In several species, GABA and L-glutamate cross-activate and/or cross-desensitize the same receptor channels (see Cleland 1996 for review). In the crustacean STG, it is known that superfusion of GABA can suppress the ganglion’s intrinsic rhythmicity (Cazalets et al. 1987), and that both L-glutamate and GABA evoke chloride and potassium currents in STG neurons in situ (Bidaut 1980; Eisen and Marder 1982; Elson and Selverston 1995; Marder and Paupardin-Tritsch 1978). Both L-glutamate and GABA evoke chloride currents in stomatogastric neurons in culture, although the shape of the whole cell response to brief puffs of the two agonists differed (Fig. 3A). Direct efforts to cross-activate or cross-desensitize the IGluR with high concentrations of GABA failed (Fig. 3B; n = 3), indicating that L-glutamate and GABA activate distinct receptors in Panulirus STG neurons.

Although IGluR pharmacology most closely resembles that of GABA Rs, cloned IGluRs (from Caenorhabditis eleg-

**FIG. 5.** A: response of an outside-out patch to continuous application of 1 mM L-glutamate. Currents at −60, −30, and +30 mV are each averages of 2 traces that are depicted separately in B. Offset currents are arbitrarily set for visibility (ECl = −1 mV). B: same data as depicted in A, separated into individual current traces and expanded in the time dimension. Offset currents are arbitrary. C: 2-electrode voltage-clamp recording from a cell in which the 2-component response to glutamate application is distinct. The time constant of desensitization of the fast component, although contaminated with the rise of the slower component, appears similar to that recorded from the macropatch in A. The time bases in A, C, and D are identical (VH = −60 mV). D: pair of more typical voltage-clamp recordings, in which the observed distinction between 2 components is diminished or absent (VH = −60 mV).
The crustacean stomatogastric IGluR is known to be inhibited by picrotoxin, a broad antagonist of ionotropic GABA-gated chloride channels, and the fenamate niflumic acid, a chloride channel blocker (Bidaut 1980; Cleland and Selverston 1995; Marder and Paupardin-Tritsch 1978). It was less powerfully inhibited by furosemide (1 mM; n = 4; Fig. 4), another chloride channel blocker, and by the excitatory glutamate receptor antagonist CNQX (200 μM; n = 3). The IGluR was not substantially inhibited by the vertebrate GABAA antagonist bicuculline methiodide (either at 100 μM or 1 mM; n = 3) or the glycine receptor antagonist strychnine (100 μM; n = 2). It was also insensitive to the excitatory glutamate receptor antagonists kynurenic acid (1 mM; n = 2), and γ-DGG (1 mM; n = 3). Finally, the crustacean IGluR was not competitively inhibited by 1 mM aspartate (n = 2), which activates IGluRs in pulmonate molluscs and several insect species and interacts with the IGluR in Aplysia californica neurons (reviewed in Cleland 1996).

**Macropatch versus whole cell recording**

When IGluRs were isolated in outside-out macropatches (containing on the order of tens of IGluR channels), fast superfusion of 1 mM L-glutamate evoked a fully desensitizing current reversing at $E_{Cl}$ (Fig. 5, A and B). The time constant of receptor desensitization in the cell depicted in Fig. 5 was 65 ms at a membrane potential of −60 mV (increasing to 85 ms at −30 mV and 155 ms at +30 mV), appearing superficially similar to that estimated for the distinct fast component sometimes seen in whole cell recordings (Fig. 5C). The apparent voltage dependence of desensitization may reflect the decreased desensitization at depolarized potentials that was previously observed in whole cell IGluR conductance measurements (i.e., differences between peak and offset conductances) (Cleland and Selverston 1995). In most whole cell voltage-clamp recordings, the evoked current components were less discrete, appearing as a partial desensitization (Fig. 5D), although this response may be the sum of a fully desensitizing and a nondesensitizing component (Cleland and Selverston 1995). A reduced extracellular calcium concentration may decrease the rate of desensitization (cf. Fig. 9 in Cleland and Selverston 1995), as was reported for the IGluR-like multiantagonist receptor of Austropotamobius (crayfish) muscle (Zufall et al. 1988).

**Single-channel recordings and mean-variance histogram analysis**

Single IGluR channels were recorded from four excised, outside-out patches from different neurons stimulated with continuous superfusion of 10 μM L-glutamate, a nondesensitizing concentration (Fig. 6). Ninety seconds of continuous data were analyzed by the mean-variance histogram method, as outlined in the METHODS and described in detail by Patlak (1993). The channel appeared to exhibit simple gating, with one main conductance state of $80 \pm 8.6$ (SD) pS (measured at −50 mV). No significant subconductance states were observed in our recordings (Fig. 7). The subsequent application of 10 μM GABA to the same patch depicted in Fig. 6 gated a channel with a unitary conductance of $72 \pm 10$ pS (measured at −50 mV; statistically indistinguishable from the IGluR conductance estimate, $P > 0.05$), and also with no prominent subconductance states. However, a rare putative subconductance state was anecdotally observed during GABA superfusion (Fig. 6, arrow), which may have led to a slight underestimate of the main open state conductance of the GABA$_{R}$.

**DISCUSSION**

**Comparative pharmacology**

IGluRs exhibit some pharmacological variation among orthologs (reviewed in Cleland 1996); in particular, some...
crustacean IGluRs are cross-activated by GABA. If GABA interacted with the stomatogastric IGluR, it would have specific implications for the roles of known GABAergic inputs into the STG (Cazalets et al. 1987; Christie et al. 1995; Nagy et al. 1994). While a GABA-gated current was present in cultured STG neurons, GABA did not gate, inhibit, or desensitize the IGluR in these cells (Fig. 3B).

Upon confirming that the IGluRs and GABA$_A$S in lobster stomatogastric neurons constituted separate populations, one of our goals was to find a pharmacological agent capable of separating them; to date, studies that depend on blocking glutamatergic synapses in lobsters using picrotoxin have concurrently blocked GABA$_A$S as well (Bidaut 1980; Cazalets et al. 1987), although the GABA-gated chloride channel in the crab...
Cancer pagurus is picrotoxin insensitive (Marder and Paupardin-Trisch 1978). In cockroach neurons, the excitatory glutamate receptor antagonist γ-DGG (100 μM) has been shown to block IGluRs but not GABA-Rs expressed in the same neurons (Wafford and Sattelle 1989). In lobster neurons, however, γ-DGG failed to block the IGluR, even at 1 mM concentration (Fig. 4). To date, no pharmacological antagonist has been discovered that can clearly distinguish between these two receptor populations in lobsters. If and when one such is discovered, it will be a useful tool.

Single-channel analysis

Very few inhibitory glutamate receptors have been studied at the single-channel level to date. Single channels from extrajunctional “H-receptors” of locust muscle (Schistocerca gregaria) were patch clamped by Dudel et al. (1989), revealing a 25-pS channel. A unique extrajunctional receptor from crayfish muscle (Austropotamobius torrentium), gated by multiple agonists including glutamate and exhibiting IGluR-like pharmacology, was investigated by Franke et al. (1986). It exhibited three open states (22, 43, and 68 pS), with glutamate preferentially gating the 22-pS state and GABA primarily evoking the 43-pS state.

To our knowledge, the lobster stomatogastric IGluR is the first neuronal, putatively synaptic IGluR to be isolated in excised patches. The 80 ± 8.6 pS estimated conductance of this channel is substantially larger than that of the two muscle receptors; furthermore, both IGluRs and GABAgs in these neurons displayed a higher unitary conductance than most ionotropic GABAgs channels. Neuronal, putatively postsynaptic IGluRs also typically possess a lower sensitivity to glutamate than extrajunctional IGluRs expressed in muscle tissues (Cleland 1996). All patches with IGluR activity were pulled from cell somata and contained on the order of tens of active channels. Furthermore, the large majority of successful patches exhibited no channel activity of any kind, implying both that unwanted currents had been successfully blocked and that IGluRs were expressed in a patchy distribution on the somatic membrane.

We observed no subconductance states in our recordings from IGluR channels, and rarely if at all from GABAgs channels. If most of the putative subconductance events were very short, however, they would be underemphasized by mean-variance histogram representation, due to the internal integration of dwell time data implicit in this analysis (Patlak 1993). Among IGluRs, the Schistocerca H-receptor displayed no subconductance states, whereas the Austropotamobius multiagonist receptor demonstrated multiple conductance states preferentially activated by different agonists. GABAgs and glycine receptors often possess multiple conductance states (Bormann et al. 1987). Two rare subconductance states were reported for a GABA receptor from cultured lobster (Homarus gammarus) thoracic neurons that exhibited a main open state of 56 ± 2.9 pS (Jackel et al. 1994). However, another invertebrate GABAgs excised from crayfish muscle (Astacus astacus), with a low agonist sensitivity suggestive of a postsynaptic receptor, showed only a single conductance state of 35 pS (Adelsberger et al. 1994).

IGluR channel properties in excised patch recordings could differ from the properties of the receptor in vivo due to the extraction of neurons into primary culture, although IGluRs in cultured Aplysia californica neurons exhibited similar properties to those studied in situ (King and Carpenter 1987). Channel properties could also be affected by the excision of the membrane patch from the intact neuron; such effects have been demonstrated for NMDA receptors in rat hippocampal and cerebellar granule neurons, in which the effect was attributed to cytoskeletal breakdown due to calcium-dependent actin depolymerization (Clark et al. 1997; Rosenmund and Westbrook 1993). Finally, the whole cell slope conductance of the Panulirus IGluR is known to be modulable by dopamine (Cleland and Selverston 1997); the single-channel biophysics could certainly be affected by the modulatory state of the receptor, presumably mediated by the occupancy of specific regulatory sites.

Mean-variance histogram analysis

The analysis of mean-variance histograms is appropriate when investigating novel channels, particularly those that may exhibit unknown subconductance states or low signal-to-noise ratios. The mean-variance histogram (see METHODS) is an unbiased representation of raw single-channel data; it presents a visual summary of these data without dependence on preselected state amplitudes chosen by the experimenter, such as are required for current idealizations pursuant to half-amplitude analysis. It is also independent of the underlying kinetic model, except for the fundamental Markov process assumptions that channels spend the great majority of their time in discrete stable states, with fast transitions between them (the minimum window widths used must be long enough to satisfy this assumption). Also, as previously mentioned, very fast components will be deemphasized during the integration step of the mean-variance algorithm, and may consequently be missed. A thorough discussion of the assumptions and limitations of this method is provided by Patlak (1993).

Mean-variance histogram analysis is a robust means of estimating basic channel parameters such as unit conductance, subconductance states, and current noise, and quantifies the error of these estimates. Dwell-time estimates can also be performed using mean-variance techniques; however, these analyses do not provide estimates of error and were omitted from the present study. More sophisticated analyses (e.g., involving burst durations or interdependencies between states) are not addressed by this method, although use of the mean-variance histogram can optimize the choice of model under which to idealize current data pursuant to these analyses. We omitted these further analyses in this study, because they require data from isolated single channels; all of our patches contained multiple channels.

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