Voltage-Dependence of Glycine-Activated Cl^- Channels: A Potentiometer for Inhibition?

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Single- and double-electrode voltage-clamp techniques have been employed *in situ* to analyze Mauthner cell inhibitory synaptic responses produced both by activation of the recurrent collateral network and by direct intracellular stimulation of single presynaptic interneurons. The results demonstrate that the synaptically evoked glycinergic postsynaptic currents exhibit a striking voltage sensitivity. Specifically, the time constant of the decay of the synaptic conductance is increased by depolarization and decreased by hyperpolarization. This parameter is exponentially related to membrane potential, changing e -fold for a 45 mV potential shift, regardless of the degree of intracellular chloride loading or the magnitude of the underlying synaptic conductance. In addition, the amplitude of this inhibitory conductance change is decreased by membrane hyperpolarizations of 15 mV or more. Computer modeling demonstrates that the voltage dependence of the kinetics of the synaptic response may serve to enhance the magnitude and duration of inhibitory responses appreciably in the face of increased excitation.

Synaptic transmission in the vertebrate CNS involves a balance between excitation and inhibition. The latter is generally due to the activation of Cl^- -dependent channels by the transmitters glycine and GABA. However, in contrast with the wealth of information on the characteristics of ACh-liganded channels, little is known about the microscopic events underlying inhibitory synaptic currents, especially for adult neurons *in situ*. Rather, most related biophysical investigations necessarily utilized *in vitro* preparations or cultured embryonic tissue, and diverse channel properties have been observed (Gold and Martin, 1983; Hamill et al., 1983; Collingridge et al., 1984; Gundarsen et al., 1984; Segal and Barker, 1984a, b). It is likely that differences in channel behavior are partly the consequences of the required experimental and environmental manipulations. These potential complications can be avoided with the teleost Mauthner (M-) cell, which is subjected to a powerful glycinergic inhibition and has already been used for estimating the stoichiometry of functional glycine and GABA binding (Diamond and Roper, 1973; Mazliah and Werman, 1974) and for establishing that the exponential decay of IPSPs reflects mean channel lifetime (Faber

and Korn, 1980, 1982). Voltage-clamp analysis of the same responses now reveals an as yet unreported steep voltage dependence of the Cl^- currents, which may exist in other central neurons in their native state and have functional significance.

Materials and Methods

Experiments were performed on goldfish anesthetized with MS-222 and paralyzed with Flaxedil (1 $\mu\text{g}/\text{gm}$ body weight). The preparation and basic electrophysiological techniques (illustrated in Fig. 1A) were similar to those described previously (Korn and Faber, 1975, 1976). The M-cell soma was penetrated with KCl- or KAc-filled microelectrodes (3 or 4 M, respectively), and single (chopping frequency, 16–33 KHz) or double electrode voltage-clamp techniques were used (Axoclamp, Axon Instruments). Synaptic currents were evoked either by activation of the whole recurrent collateral network following an antidromic M-axon stimulus or by intracellular stimulation of a single inhibitory interneuron, identified as previously (Korn and Faber, 1975, 1976). In case of simultaneous recordings, the electrodes were coated with a conducting silver paint and appropriately shielded. To avoid possible changes in Cl^- ion distributions due to shifts in membrane potential, (1) the M-cell was clamped at resting level and (2) inhibitory postsynaptic currents (IPSCs) were evoked during brief (20–40 msec) command pulses of varying amplitude, at frequencies less than 1 Hz.

Data were stored on tape for subsequent computer analysis of the evoked IPSCs, which were calculated as follows. At each membrane potential studied, voltage command pulses were applied with and without activation of the inhibitory input. In the first case, both leakage and synaptic currents were recorded, while in the absence of stimulation only the leakage current was obtained. Thus, the IPSC equaled the difference between the responses obtained in these 2 conditions. In all instances, averaged currents were obtained ($n \geq 4$). Finally, decay time constants were measured graphically or with an exponential curve-fitting program.

Results

As shown in Figure 1B1, when the recurrent inhibitory network was activated, the collateral IPSP, due to a conductance change of about 6×10^{-6} S (Faber and Korn, 1982), was clamped quite well, although voltage control was less complete during the more rapid antidromic spike. In this example, which is from a cell with only a slightly elevated internal Cl^- concentration, the IPSP was about 5 mV in amplitude, and, as predicted previously (Faber and Korn, 1980) on the basis of the M-cell's short membrane time constant (~ 500 μsec ; see Furshpan and Furukawa, 1962), its time course paralleled that of the underlying inward current recorded at resting potential. Furthermore, since the IPSC magnitude is the product of both the activated inhibitory conductance, g_{IPSC} , and the driving force on Cl^- ions (the difference between membrane potential, V_m , and E_{Cl} , the Cl^- equilibrium potential), the synaptic current should increase during hyperpolarization. The records in Figure 1B2 illustrate that was the case in this cell when the driving force was increased by 20

Received May 20, 1986; revised Aug. 25, 1986; accepted Aug. 26, 1986.

We thank Dr. F. Sachs for assistance with the computer modeling and Dr. C. Lewis for helpful discussions. Supported in part by NIH Grants NS15335 and 21848 and by INSERM.

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Figure 1. Voltage dependence of inhibitory synaptic currents. **A**, Schematic of the experimental set-up and the recurrent collateral network of the Mauthner (M-) cell, with microelectrodes in the pre- and post-synaptic elements, for activation of single interneurons and voltage clamping, respectively, and a bipolar stimulating (*Stim*) electrode on the M-axon for evoking a maximal inhibitory response. **B1**, Typical M-cell antidromic spike and subsequent IPSP recorded in a Cl⁻-loaded cell (*upper trace*) and corresponding clamped voltage (*middle trace*) and inward current (*lower trace*), at resting potential. **B2**, The net synaptic current (*lower trace*) at different membrane potential levels (here, 20 mV beyond resting level, $\Delta V = -20$ mV) was determined by subtraction of the leak current produced by the command pulse (*upper trace*, with and without evoked responses). **C**, Depolarizing IPSP large enough to trigger an action potential, and associated inward current recorded at resting level for another cell (*upper two traces*). Below is a family of IPSCs recorded at 10 mV increments, from -82 to -112 mV. Note that current amplitude increases progressively and the largest one decays most rapidly. (All traces are averages of 4 or more responses; chopping frequency as noted.)

mV; indeed, a plot of IPSC magnitude versus V_m was linear in the immediate vicinity of the resting potential, and E_{Cl} was calculated to be about 10 mV in the depolarizing direction. That is, the magnitude of the collateral IPSP was about half the driving force in this and other experiments (mean ratio, 0.52 ± 0.057 ; $n = 13$). This value, comparable to that estimated earlier with indirect means (Faber and Korn, 1982), validates the equations used to calculate the number of channels opened by a single quantum of transmitter (Korn et al., 1981, 1982).

The 2 major observations addressed in this report are apparent in the family of currents shown in Figure 1C. First, the peak synaptic conductance often decreased during hyperpolarization, as indicated by smaller increments in synaptic current or by actual reductions in the IPSC magnitude. The second nonlinearity, which was more consistent, was that the rate of IPSC decay was slowed during depolarizations and accelerated during hyperpolarizations (Fig. 1, *B2* vs *B1*, and *C*).

These phenomena occurred in neurons where the IPSC equilibrium potential varied greatly, i.e., from 5 to 48 mV in the depolarizing direction, owing to differing degrees of Cl⁻ loading, and to confirm their independence from elevated internal Cl⁻ concentrations (Gold and Martin, 1983), the experiments were

Figure 2. Evidence that IPSC amplitude is a nonlinear function of membrane potential. **A**, Net collateral synaptic currents recorded at the indicated membrane potentials with a KAc electrode in an M-cell whose resting potential was -83 mV. Note that the magnitude of outward current increases as the cell is depolarized more but that the inward current does not grow in proportion to the level of hyperpolarization. **B**, Plot of the synaptic current amplitudes (*ordinate*) versus the membrane potentials (V_m , *ordinate*) at which they were evoked. The relationship was linear over the range of $V_m = -83$ to -58 mV, yielding an inhibitory conductance, $g_{coll} = 2.6 \mu S$. Note that at more hyperpolarized potentials, the slope of the current-voltage plot decreased and then became negative, indicating a voltage-dependent decrease in synaptic conductance. *SEVC*, single electrode voltage clamp, employed at the indicated chopping frequency.

repeated with KAc electrodes. The inactivation of the peak synaptic conductance during hyperpolarization is clearly illustrated in the records and plot of Figure 2, *A* and *B*. In this experiment, the resting membrane potential was -83 mV, and, at that level, there was at most a small inward inhibitory current (Fig. 2A). When the driving force on Cl⁻ ions was made positive by depolarizing the membrane, there was a clear outward current, which increased in proportion to the potential change (compare records at -78 and -68 mV). Similarly, during moderate hyperpolarizations the IPSC was inward going, as expected. However, as the magnitude of the hyperpolarization was increased beyond -103 mV, the IPSC amplitude actually decreased. Also, comparison of the records obtained at -68 and -103 or -108 mV demonstrates that this inactivation was associated with a decrease in the response peak time. The complete current-voltage relation for the IPSC, obtained for potentials ranging from -58 to -118 mV, is shown in Figure 2B. It indicates that the synaptic current was a linear function of membrane potential in the depolarizing region, with the collateral conductance equaling $2.6 \mu S$. The nonlinearity was evident at small hyperpolarizations as a decrease in the slope of the plot, and, as indicated, with the larger hyperpolarizations, the response amplitude actually diminished. This apparent inactivation of the synaptic conductance was observed in most experiments, although it varied in degree and in the voltage level at which it occurred (from within 5 to 40 mV of resting potential).

As depicted in Figure 3A, the rates of decay of the outward and inward currents recorded during de- and hyperpolarization, respectively, were also markedly voltage dependent when KAc electrodes were used. Semilogarithmic plots of the normalized currents versus time demonstrated that, for all values of membrane potential above and below the resting level, this decay was exponential, with the time constant, τ_D , being maximal

Figure 3. Variations of time constant of IPSC decay with membrane potential. *A*, Outward (upper) and inward (lower) currents recorded at the indicated potentials with a KAc electrode in a neuron whose resting and Cl^- equilibrium potentials were -74 and -71 mV, respectively. Note the differences in response kinetics, including the rates of return to baseline (dashed lines). *B*, Semilogarithmic plots of the falling phases of the synaptic currents evoked at membrane potentials ranging from -67 to -89 mV. Currents (ordinates) were normalized with respect to the individual peaks ($I_{\max}$). For each average response ($n \geq 4$), the data points were fit by a straight line, indicating exponential decay according to the relation: $I_{\text{normalized}} = I_{\max} \exp(-t/\tau_D)$ ($0.97 \leq r^2 \leq 1.0$). The listed values of τ_D , the decay time constant, decreased progressively with hyperpolarization (symbols pertain to those fit by corresponding curves).

during the largest depolarization and decreasing consistently as a function of membrane polarization (Fig. 3*B*). It can be stressed that in this typical example, τ_D varied 2-fold for only a 22 mV shift in V_m , i.e., from 5.8 msec at -89 mV to 11.8 msec at -67 mV.

This strong voltage sensitivity of the exponential decays was essentially the same for both collateral and unitary responses recorded in Cl^- -loaded cells, as is apparent in the plot of Figure 4. It should be reiterated that the degree of Cl^- loading in these experiments was quite variable, with the IPSC driving force ranging from 5 to 48 mV. Furthermore, since there is an equivalence between τ_D and mean Cl^- channel lifetime for the smaller IPSCs (Faber and Korn, 1980, 1982), it follows that the same must apply for the full collateral IPSC, even though it is brought about by transmitter release from a large population of interneurons. The data were fit according to the relation $\tau_D = \tau_0 \exp(V_m/B)$, where τ_0 is the decay time constant at 0 mV, V_m is the absolute membrane potential, and B defines the potential shift necessary for an e -fold change in τ_D (Magleby and Stevens, 1972). The regression line calculated for the responses obtained in the Cl^- -loaded cells could not be distinguished from that

Figure 4. Evidence that a single steep exponential relates τ_D to membrane potential, regardless of internal Cl^- concentration and expected effects on IPSC time course. Semilogarithmic plot of the time constant (τ_D) of IPSC decay (ordinate) versus V_m (abscissa); data points were obtained, as indicated by the symbols, under different experimental conditions, IPSCs being evoked by activation of the collateral (Coll., 8 experiments) network and of single interneurons (Unit., 4 experiments) in Cl^- -loaded cells and of the former in the absence of loading (KAc electrodes, 3 experiments). Line fitting the data points according to the expression $\tau_D = \tau_0 \exp(V_m/B)$ was derived from all of them ($n = 74$, $r^2 = 0.75$, $p < 0.001$).

shown in Figure 4 and includes values measured with KAc electrodes as well. Specifically, τ_0 varied between 40.5 and 44.3 msec and B from 45.6 to 50.3 mV for data obtained with ($n = 17$, 3 experiments) and without ($n = 57$, 12 experiments) KAc. When pooled, these values yielded a voltage sensitivity of an e -fold change per 45.9 mV, which is appreciably greater than that reported thus far for transmitter-activated channels.

Discussion

The present results demonstrate that inhibitory synaptic currents of the goldfish M-cell *in vivo* exhibit 2 forms of voltage sensitivity. One phenomenon, a decrease of the peak synaptic conductance with hyperpolarization, was variable in degree, although it almost always occurred. It presumably does not have appreciable functional importance, as the M-cell is unlikely to be hyperpolarized significantly beyond its resting potential in normal physiological conditions. The other effect, characterized by an increase in τ_D during depolarization, occurred consistently under diverse recording conditions and may well influence M-cell postsynaptic responsiveness.

Possible sources of error have been considered, and we conclude that, in fact, the actual voltage sensitivity of τ_D may be greater than estimated here. (1) Large currents were necessary to clamp the collateral responses, but data from unitary IPSCs, which are an order of magnitude smaller (Faber and Korn, 1982; Korn et al., 1982), were the same, as were those with the 2-electrode voltage clamp. (2) A voltage-dependent component of the M-cell's leakage conductance, characterized by a reduction of up to 40% during hyperpolarizations (Faber and Korn, 1985), might have modified the spatial quality of the clamp, but it only occurred at the extreme end of the potential range

utilized. (3) Inhibitory synapses more than 100 μm from a somatic current electrode (Korn et al., 1982) might have been exposed to potential steps smaller than those at the source. That is, since the soma and lateral dendrite have a space constant of about 175–250 μm (Diamond, 1968; Faber et al., 1981), clamp control at distal synaptic sites may be reduced slightly. However, most of the inhibitory synapses are within 100 μm of the soma (Triller and Korn, 1982) and are electrotonically close to a somatic electrode, as indicated by evidence that quantal size is the same for all synaptic boutons (Korn et al., 1982). Nevertheless, if the potential change at the distal contacts is less than at the recording site, the overall voltage sensitivity of τ_D may be underestimated.

It is important that the extreme voltage-sensitive features of IPSCs described here were found *in situ*. Both have been reported before in isolated preparations and in cultured neurons, where, however, the relation between τ_D and V_m was much less pronounced. For instance, a decrease in the peak conductance during hyperpolarization occurs at human glycine receptors “transplanted” into *Xenopus* oocytes (Gundarsen et al., 1984), at invertebrate stretch receptors and muscles (Dudel et al., 1980; Adams et al., 1981; Cull-Candy and Miledi, 1981), and at GABA-sensitive mammalian hippocampal neurons (Collingridge et al., 1984; Segal and Barker, 1984a); with the same preparations, an e -fold change in τ_D required between 100 and 226 mV. Furthermore, none of these properties, which are common to most liganded channels, has been found for glycine responses of cultured spinal or hippocampal neurons (Barker et al., 1982; Segal and Barker, 1984a) or of Lamprey supraspinal cells (Gold and Martin, 1983). A patch-clamp analysis of the spinal cells also apparently did not reveal voltage-sensitive kinetics, although single-channel currents did exhibit a predictable outward rectification (Hamill et al., 1983). Finally, the voltage sensitivity of τ_D reported here is also appreciably greater than that found for other synaptically activated conductances. For example, at the frog end plate, an e -fold change in τ_D typically occurs for about a 100 mV shift in V_m , with reported values ranging from 56 to 300 mV (Anderson and Stevens, 1972; Auerbach et al., 1983; Lewis, 1985). Similarly, in the case of the cholinergic response at the rabbit sinoatrial node, limited data suggest a value of about 58 mV (Noma et al., 1979).

Although the precise mechanisms of voltage dependence of agonist-operated channels is unresolved (Stevens, 1979; Adams, 1981), any conformational change that produces a molecular rearrangement and a change in the dipole moment of the molecule should be sensitive to imposed electrical fields. In this context, the above mentioned variations may not be surprising, given the number of intrinsic and extrinsic factors that could conceivably affect channel properties. Indeed, it has been established for the vertebrate neuromuscular junction that τ_D , and its voltage sensitivity can be altered by ionic substitutions (Gage and VanHelden, 1979; Fiekers and Henderson, 1982), by imposed clustering of the receptors (Young and Poo, 1983), and by molecular events that are associated with maturation (Sakmann and Brenner, 1978; Fischbach and Schuetze, 1980) of channels. Other environmental constraints, such as the membrane lipids and their effects of fluidity or surface charge density (Sakmann and Boheim, 1979; Cahalan and Hall, 1982), and the nature and/or integrity of neural connections (Marshall, 1985) should not be underestimated. It should also be noted that direct iontophoretic effects of the imposed electrical field on the binding of glycine to its receptor probably do not account for the

Figure 5. Voltage dependence of IPSC decay enhances inhibitions evoked during depolarization. Plotted are time courses of simulated hyperpolarizing IPSPs evoked from an arbitrary membrane potential of -60 mV (arrow) and reaching a peak at -75 mV. The three solid lines illustrate the effects on IPSP return to baseline of varying τ (middle trace), from an initial rapid rate equal to that for the more negative potential to that reached at -60 mV (upper and lower curves, respectively). The specific parameters used were: $g_0 = G_m = 6 \times 10^{-6} \text{S}$, $E_{\text{Cl}} = -90$, $V_m = -60$ at $t = 0$, and $\tau_0 = 44$ msec, $B = 45.9$ mV, for calculating $\tau(V_m)$. For further comparison, the dashed line illustrates exponential decay if the voltage was proportional to the conductance change, with $\tau_D = \tau(-75)$: This situation corresponds to responses of small amplitude relative to that produced by activation of the whole collateral network (see text for equations used).

observed voltage dependence, since depolarization would retard, not augment, binding of this positively charged agonist.

Voltage dependence of liganded channels may not have a unique function, if any. On the other hand, conditions that result in an imbalance in excitatory and inhibitory influences in the CNS, such as genetic deficits in glycine receptors (Heller and Hallett, 1982; White and Heller, 1982), can have profound neurological consequences. In the case of central cells, it is reasonable to postulate that an increase in τ_D during depolarization would enhance the efficacy of synaptically driven inhibition. We have evaluated this postulate using the known M-cell properties to solve for IPSP amplitude and time course, given the kinetics and voltage sensitivities of the synaptic conductance.

Three simultaneous equations were solved: (1) $\tau_D = \tau_0 \exp(V_m/B)$; (2) the inhibitory conductance decays as $g = g_0 \exp(-t/\tau_D)$ where g_0 is the peak value; and (3) membrane potential during the IPSP, $V_{\text{IPSP}} = (E_{\text{Cl}} - V_m) [g/(g + G_m)]$, where G_m is the resting input conductance of the M-cell (Faber and Korn, 1982). It was found that IPSP time course parallels that of the conductance change when the latter is relatively small, and that it varies in strict accordance with τ_D , for example, increasing by 55% for a shift from a resting level at -80 to -60 mV. Since there is the same proportional increase in the time integral of the inhibitory conductance, the effective magnitude of inhibition in this case is thus increased appreciably when the M-cell is depolarized close to threshold by excitatory inputs. The model results illustrated in Figure 5 indicate that with larger conductance changes, such as during a collateral IPSP, the increase in τ_D that would occur in the presence of depolarizing excitatory inputs cannot account for the enhanced response duration alone. Effects of nonlinear summation of voltage versus conductance (Martin, 1955) also contribute significantly. In Figure 5, the initial mem-

brane potential was taken to be -60 mV and the peak inhibitory conductance change, g_o , to equal G_m , which is the condition pertaining during the collateral response (Faber and Korn, 1982). Inspection of the simulated curves for fixed and variable τ_D s demonstrates that the IPSP decays more slowly than would occur with the simple exponential expected for a smaller conductance change. Furthermore, because of the variable τ_D there is an additional compensatory prolongation of the IPSP as the conductance falls. Finally, it should be stressed that in the case of synaptic inhibition the shunt produced by the underlying conductance change is often more important than the actual IPSP amplitude (Fukami et al., 1965). In this context, the increase in τ_D during depolarization may provide an important inhibitory safety factor that would compensate for any reequilibration of Cl^- ions and diminution of the IPSP driving force. Thus, in the M-cell, a clear mechanism exists to enhance inhibitory responses in the face of increased excitation. This effect can be contrasted with the weaker voltage dependence reported for excitatory junctions (Magleby and Stevens, 1972; and references in Lewis, 1985), which nevertheless also decreases excitability during depolarization.

References

- Adams, P. R. (1981) Acetylcholine receptor kinetics. *J. Membr. Biol.* 58: 161–174.
- Adams, P. R., A. Constanti, and F. W. Banks (1981) Voltage clamp analysis of inhibitory synaptic action in crayfish stretch receptor neurons. *Fed. Proc.* 40: 2637–2641.
- Anderson, C. R., and C. F. Stevens (1973) Voltage clamp analysis of acetylcholine produced endplate current fluctuations at frog neuromuscular junction. *J. Physiol. (Lond.)* 235: 655–691.
- Auerbach, A. J., J. DelCastillo, P. C. Specht, and M. Titmus (1983) Correlation of agonist structure with acetylcholine receptor kinetics: Studies on the frog and end-plate and on chick embryo muscle. *J. Physiol. (Lond.)* 343: 551–568.
- Barker, J. L., R. N. McBurney, and J. F. Macdonald (1982) Fluctuation analysis of neutral amino acid responses in cultured mouse spinal neurones. *J. Physiol. (Lond.)* 322: 365–387.
- Cahalan, M. D., and J. Hall (1982) Alamethicin channels incorporated into frog node of Ranvier. Calcium-induced inactivation and membrane surface charges. *J. Gen. Physiol.* 79: 411–436.
- Collingridge, G. L., P. W. Gage, and B. Robertson (1984) Inhibitory postsynaptic currents in rat hippocampal CA1 neurones. *J. Physiol. (Lond.)* 350: 551–564.
- Cull-Candy, S. G., and R. Miledi (1981) Junctional and extrajunctional membrane channels activated by GABA in locust muscle fibres. *Proc. R. Soc. London [Biol.]* 211: 527–535.
- Diamond, J. (1968) The activation and distribution of GABA and L-glutamate receptors on goldfish Mauthner neurones: An analysis of dendritic remote inhibition. *J. Physiol. (Lond.)* 194: 669–723.
- Diamond, J., and S. Roper (1973) Analysis of Mauthner cell responses to iontophoretically delivered pulses of GABA, glycine and L-glutamate. *J. Physiol. (Lond.)* 232: 113–128.
- Dudel, J., W. Finger, and H. Stettmeier (1980) Inhibitory synaptic channels activated by γ -aminobutyric acid (GABA) in crayfish muscle. *Pfluegers Arch.* 387: 143–151.
- Faber, D. S., and H. Korn (1980) Single shot channel activation accounts for duration of inhibitory postsynaptic potentials in a central neuron. *Science* 208: 612–615.
- Faber, D. S., and H. Korn (1982) Transmission at a central inhibitory synapse. I. Magnitude of the unitary postsynaptic conductance change and kinetics of channel activation. *J. Neurophysiol.* 48: 654–678.
- Faber, D. S., and H. Korn (1985) Voltage dependent synaptic and non-synaptic conductances in the Mauthner cell. *Soc. Neurosci. Abstr.* 11: 465.
- Faber, D. S., C. Kaars, and S. J. Zottoli (1981) Dual transmission at morphologically mixed synapses: Evidence from postsynaptic cobalt injections. *Neuroscience* 5: 433–440.
- Fiekers, J. F., and E. G. Henderson (1982) Voltage clamp analysis of the effect of cationic substitution on the conductance of end-plate channels. *Pfluegers Arch. Eur. J. Physiol.* 394: 38–47.
- Fischbach, G. D., and S. M. Schuetze (1980) A post-natal decrease in acetylcholine channel open time at rat end-plates. *J. Physiol. (Lond.)* 303: 125–137.
- Fukami, Y., T. Furukawa, and Y. Asada (1965) Excitability changes of the Mauthner cell during collateral inhibition. *J. Gen. Physiol.* 48: 581–600.
- Furshpan, E. J., and T. Furukawa (1962) Intracellular and extracellular responses of the several regions of the Mauthner cell of the goldfish. *J. Neurophysiol.* 25: 732–771.
- Gage, P. W., and D. VanHelden (1979) Effects of permeant monovalent cations on end-plate channels. *J. Physiol. (Lond.)* 288: 509–528.
- Gold, M. R., and A. R. Martin (1983) Analysis of glycine-activated inhibitory post-synaptic channels in brain-stem neurones of the lamprey. *J. Physiol. (Lond.)* 342: 99–117.
- Gundarsen, C. B., R. Miledi, and I. Parker (1984) Properties of human brain glycine receptors expressed in *Xenopus* oocytes. *Proc. R. Soc. London [Biol.]* 221: 235–244.
- Hamill, O. P., J. Bormann, and B. Sakmann (1983) Activation of multiple-conductance state chloride channels in spinal neurones by glycine and GABA. *Nature* 305: 805–808.
- Heller, A. H., and M. Hallett (1982) Electrophysiological studies with the spastic mutant mouse. *Brain Res.* 234: 299–308.
- Korn, H., and D. S. Faber (1975) An electrically mediated inhibition in the goldfish medulla. *J. Neurophysiol.* 38: 452–471.
- Korn, H., and D. S. Faber (1976) Vertebrate central nervous system: Same neurons mediate both electrical and chemical inhibitions. *Science* 194: 1166–1169.
- Korn, H., A. Triller, A. Mallet, and D. S. Faber (1981) Fluctuating responses at a central synapse: n of binomial fit predicts number of stained presynaptic boutons. *Science* 213: 898–901.
- Korn, H., A. Mallet, A. Triller, and D. S. Faber (1982) Transmission at a central inhibitory synapse. II. Quantal description of release, with a physical correlate for the binomial n . *J. Neurophysiol.* 48: 679–707.
- Lewis, C. A. (1985) Deuterium oxide and temperature effects on the properties of endplate channels at the frog neuromuscular junction. *J. Gen. Physiol.* 85: 137–156.
- Magleby, K. L., and C. F. Stevens (1972) The effects of voltage on the time-course of end-plate currents. *J. Physiol. (Lond.)* 223: 151–171.
- Marshall, L. M. (1985) Presynaptic control of synaptic channel kinetics in sympathetic neurones. *Nature* 317: 621–623.
- Martin, A. R. (1955) A further study of the statistical composition of the end plate potential. *J. Physiol. (Lond.)* 130: 114–122.
- Mazliyah, Y., and R. Werman (1974) The actions of glycine and GABA compared on the same cells in vertebrates. *Isr. J. Med. Sci.* 10: 566.
- Noma, A., K. Peper, and W. Trautwein (1979) Acetylcholine-induced potassium current fluctuations in the rabbit sino-atrial node. *Pfluegers Arch.* 381: 255–262.
- Sakmann, B., and G. Boheim (1979) Alamethicin-induced single channel conductance fluctuations in biological membranes. *Nature* 282: 336–339.
- Sakmann, B., and H. R. Brenner (1978) Change in synaptic channel gating during neuromuscular development. *Nature* 276: 401–402.
- Segal, M., and J. L. Barker (1984a) Rat hippocampal neurons in culture: Properties of GABA-activated Cl^- ion conductance. *J. Neurophysiol.* 51: 500–515.
- Segal, M., and J. L. Barker (1984b) Rat hippocampal neurons in culture: Voltage-clamp analysis of inhibitory synaptic connections. *J. Neurophysiol.* 52: 469–487.
- Stevens, C. F. (1979) Acetylcholine concentration into voltage. Receptor-mediated transduction. In *Membrane Transduction Mechanisms*, R. A. Cone and J. E. Dowling, eds., pp. 79–89, Raven, New York.
- Triller, A., and H. Korn (1982) Transmission at a central inhibitory synapse. III. Ultrastructure of physiologically identified and stained terminals. *J. Neurophysiol.* 48: 708–736.
- White, W. F., and A. H. Heller (1982) Glycine receptor alteration in the mutant mouse *spastic*. *Nature* 298: 655–657.
- Young, S. H., and M.-M. Poo (1983) Topographical rearrangement of acetylcholine receptors alter channel kinetics. *Nature* 304: 161–163.