Noninvasive Measurements of the Membrane Potential and GABAergic Action in Hippocampal Interneurons

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Neurotransmitters affect the membrane potential ($V_m$) of target cells by modulating the activity of receptor-linked ion channels. The direction and amplitude of the resulting transmembrane current depend on the resting level of $V_m$ and the gradient across the membrane of permeant ion species. $V_m$, in addition, governs the activation state of voltage-gated channels. Knowledge of the exact level of $V_m$ is therefore crucial to evaluate the nature of the neurotransmitter effect. However, the traditional methods to measure $V_m$, with microelectrodes or the whole-cell current-clamp technique, have the drawback that the recording pipette is in contact with the cytoplasm, and dialysis with the pipette solution alters the ionic composition of the interior of the cell. Here we describe a novel technique to determine the $V_m$ of an intact cell from the reversal potential of K+ currents through a cell-attached patch. Applying the method to interneurons in hippocampal brain slices yielded more negative values for $V_m$ than subsequent whole-cell current-clamp measurements from the same cell, presumably reflecting the development of a Donnan potential between cytoplasm and pipette solution in the whole-cell mode. Cell-attached $V_m$ measurements were used to study GABAergic actions in intact CA1 interneurons. In 1- to 3-week-old rats, bath-applied GABA inhibited these cells by stabilizing $V_m$, at a level depending on contributions from both GABA$_A$ and GABA$_B$ components. In contrast, in 1- to 4-d-old animals, only GABA$_A$ receptors were activated resulting in a depolarizing GABA response.

Key words: hippocampus; interneuron; potassium channels; cell-attached patch-clamp; membrane potential; GABA

GABA is the principal inhibitory neurotransmitter in the mammalian brain. Chloride-permeable channels, with a substantial permeability also for HCO$_3^\text{-}$ (Bormann et al., 1987), open when GABA binds to GABA$_A$ receptors (Ozawa and Yuzaki, 1984; Gray and Johnston, 1985), whereas binding to GABA$_B$ receptors leads to G-protein-mediated K+ channel activation (Osis et al., 1993). The resulting transmembrane currents either depolarize or hyperpolarize a postsynaptic cell, depending on the equilibrium potentials for these ions and the membrane potential ($V_m$) of the cell. Measuring the GABA response reversal potential ($E_{GABA}$) and $V_m$ with classical methods is surprisingly difficult. Penetration with sharp microelectrodes makes a hole in the cell membrane and introduces a significant leak conductance (Spruston and Johnston, 1992), whereas whole-cell patch-clamp electrodes dialyze the cell, imposing the pipette ion concentrations on the recorded cell. Furthermore, whole-cell measurements probably underestimate $V_m$ because an undefined Donnan potential exists between cytoplasm and pipette solution (Marty and Neher, 1995).

These problems may be avoided using a noninvasive approach, based on the reversal potential of K+ currents through cell-attached patches, to measure $V_m$ (Verheugen et al., 1995). When the K+ concentration in the pipette is equal to the intracellular level, the equilibrium potential for K+ across the membrane patch is zero. Voltage-gated K+ currents [K(V)], elicited via the patch electrode, will reverse direction when the pipette potential ($-V_k$) equals $V_m$. Repetitive measurements of K(V) reversal in the cell-attached mode may then be used to follow fluctuations in $V_m$. Furthermore, changes in K(V) reversal during exposure to GABA and selective agonists of GABA$_A$ and GABA$_B$ receptors can provide estimates of respectively $E_{GABA}$, $E_{GABA-A}$, and $E_{GABA-B}$, or at least (in the case other conductances still significantly contribute in setting $V_m$) of their polarity with respect to the resting $V_m$.

The relation between $E_{GABA}$ and $V_m$ of hippocampal interneurons is of particular interest. GABA-mediated membrane currents in hippocampal neurons may be depolarizing in young animals (Mueller et al., 1984; Ben-Ari et al., 1989) and under certain conditions in adult animals (Alger and Nicoll, 1979; Thompson and Gähwiler, 1989; Michelson and Wong, 1991). GABA-mediated depolarizing postsynaptic potentials (Kaila et al., 1997) could function as a source of positive feedback and so synchronize discharge in interneuron networks (Michelson and Wong, 1991). Interneuron synchronization has been implicated in the generation of neonatal hippocampal oscillations (Strata et al., 1997) and of the 40 Hz gamma rhythm in adult hippocampus and cortex (Whittington et al., 1995). We therefore studied the GABA actions on intact interneurons from rats at different stages of postnatal development using the reversal potential of cell-attached K(V) currents as monitor of $V_m$.

**MATERIALS AND METHODS**

**Hippocampal slice preparation.** Rats aged between 1 and 21 d were anesthetized by intraperitoneal injection of a ketamine–chloral hydrate solution (5 and 18%, respectively; 1 ml/200 gm). Under deep anesthesia, the vascular system was perfused through the heart with an ice-cold low Ca$^{2+}$ solution containing (in mm): 130 NaCl, 2.7 KCl, 20 NaHCO$_3$, 0.4 CaCl$_2$, 1 MgCl$_2$, 1.3 Na$_2$HPO$_4$, and 25 glucose, equilibrated with 5% CO$_2$ and 95% O$_2$. After perfusion, the brain was removed, and sagittal slices of 200–300 μm were cut from the middle third of the hippocampus.
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of CA1 interneurons. A voltage steps, and B shows the response to a voltage ramp applied to the same cell, with a pipette solution containing 155 mm K\(^+\). A. The inward K\(^+\) current caused the generation of action currents in this cell (Lynch and Barry, 1989). At more depolarized potentials the current became independent of the relative contributions of the transient and sustained component. B. A voltage ramp stimulation gave a similar \(I-V\) profile, but no action currents were generated because of a reduced charge flux. With symmetrical K\(^+\), the current reversal at \(V_h = +64\) mV indicated a membrane potential of \(-64\) mV. C. With a low K\(^+\) pipette solution, in which 135 mm K\(^+\) was replaced with the impermeant cation N-methyl-D-glucamine, the voltage-gated current was outward for all voltages beyond the activation threshold, demonstrating its K\(^+\) selectivity.

Figure 1. Voltage-gated K\(^+\) currents measured in cell-attached patches of CA1 interneurons. A shows currents activated by applying depolarizing voltage steps, and B shows the response to a voltage ramp applied to the same cell, with a pipette solution containing 155 mm K\(^+\). A. The inward K\(^+\) current caused the generation of action currents in this cell (Lynch and Barry, 1989). At more depolarized potentials the current became independent of the relative contributions of the transient and sustained component. B. A voltage ramp stimulation gave a similar \(I-V\) profile, but no action currents were generated because of a reduced charge flux. With symmetrical K\(^+\), the current reversal at \(V_h = +64\) mV indicated a membrane potential of \(-64\) mV. C. With a low K\(^+\) pipette solution, in which 135 mm K\(^+\) was replaced with the impermeant cation N-methyl-D-glucamine, the voltage-gated current was outward for all voltages beyond the activation threshold, demonstrating its K\(^+\) selectivity.
Figure 2. Differences between cell-attached and whole-cell $V_m$ measurements. 

A, Determination of $K^+$ current reversal, and hence membrane potential, from a CA1 interneuron after exposure to high external [K$^+$] and to GABA (50 µM). This cell discharged spontaneously, giving rise to action currents visible in the cell-attached record (arrows). For each condition, five consecutive current traces recorded at 2 sec intervals are shown. Bottom panels show the inward K(V) and its reversal in greater detail.

B, Plot of $V_m$ determinations (circles; for each condition average $V_m$ and SD (Figure legend continues).
reversal potential of voltage-gated K$^+$ currents activated by command potentials applied via a cell-attached pipette (Fig. 1). This technique permits control of the timing of measurements and is more precise than estimates from changes in amplitude of spontaneous K$^+$ channel open events (Zhang and Jackson, 1993; Soltész and Mody, 1994; Verheugen et al., 1995). Voltage steps applied to somatic patches of interneurons elicited K$^+$ currents [I$_{K(V)}$] with a transient (inactivation time constant, 12.9 ± 4.0 msec) and a sustained component (Fig. 1A) corresponding to the I$_A$ and delayed rectifier components of whole-cell K$^+$ currents (Zhang and McBain, 1995). With 155 mM K$^+$ in the pipette, K(V) currents were activated between -10 and +50 mV (relative to V$_m$) and were initially inward reaching a maximum amplitude of 32 ± 35 pA. They reversed at potentials between +55 and +90 mV, and outward currents of 279 ± 204 pA were attained at +140 mV.

Voltage ramp stimulation produced similar I-V relations to those derived from responses to current steps (Fig. 1B). The currents were selective for K$^+$ because they were outward over the entire voltage range with pipettes containing low K$^+$ (20 mM; Fig. 1C). Furthermore, there were no differences in depolarization-evoked currents when the pipette solution contained Cl$^-$ or gluconate as the main anion (p > 0.1; data not shown).

Ramp stimuli were routinely used to determine the K(V) current reversal potential and hence V$_m$. A correction was made for the linear leak current evident at potentials below the K(V) current threshold (Fig. 1, dotted lines). Short duration (15-20 msec) ramps were preferred because they minimized transmembrane charge flux while still producing close to maximal activation of the K(V) current (Figs. 2-5). Ramp stimuli could be repeated at frequencies up to 1 Hz without significant accumulation of use-dependent K$^+$ current inactivation. The mean resting V$_m$, obtained immediately after the membrane was broken and did not depend on the composition of the pipette solution. This point was confirmed by examining the time course of changes in membrane potential and firing after break-in during GABA application, in experiments with pipette solutions containing either a high or a low Cl$^-$ concentration. As expected, GABA excited cells recorded in the whole-cell mode with a high Cl$^-$ pipette solution (Fig. 2C, right panel), whereas it had an inhibitory action when cells were recorded with a low Cl$^-$ solution (data not shown). However, these actions did not occur immediately but had a slow onset that reached a steady-state 0.5-2 min after break-in as expected if they resulted from intracellular perfusion. These results therefore suggest that the establishment of whole-cell recording alone can modify neuronal excitability, possibly by changing the activation state of voltage-gated channels.

Origin of the difference in cell-attached and whole-cell V$_m$ estimates

Two possible explanations were considered to explain the difference in cell-attached and whole-cell estimates for V$_m$. First, it could result from an artifactual shift in V$_m$ induced by the ramp stimulation in cell-attached recordings. Determinations of V$_m$ made using a second independent method based on spontaneous single K$^+$ channel openings seem to exclude this explanation.

In some patches, spontaneous openings of non-voltage-activated K$^+$ channels were apparent in addition to the K(V) current gated by membrane depolarization. Figure 3A shows
spontaneous activity of a channel with conductance and inward rectification properties similar to those of an ATP-sensitive K\(_\text{1}\) channel described in cortical cells (Ohno-Shosaku and Yamamoto, 1992; Sakura et al., 1995). These current records provide two independent means to measure \(V_m\). Dividing the single-channel amplitude of K\(_{\text{ATP}}\) by its conductance, calculated from open states during ramp stimuli (see Fig. 3, legend), gives the driving force for current flowing through the channel. The reversal potential was identical for both channels, indicating that they are equally K\(_\text{1}\) selective, and the driving force is therefore the difference between \(E_K\) and the patch potential. In the records shown in Figure 3, the single-channel amplitude at \(V_h = -60\) mV and the single K\(_{\text{ATP}}\) channel conductance calculated from the open levels during the voltage ramp stimulation, the driving force for K\(_+\) flux (\(V_{\text{patch}} - E_K\); with \(V_{\text{patch}} = V_h + V_m\) and \(E_K \approx 0\) mV) amounts to 10.8 pA/84 pS = 128.6 mV at \(V_h = -60\) mV, yielding an estimated \(V_m\) of \(-68.6\) mV. The value determined for \(V_m\) from reversal of the macroscopic K(V) current was \(-69.2\) mV. The close agreement between these two values indicates that the ramp stimulation does not significantly affect \(V_m\). Subsequent whole-cell current-clamp measurement in the same cell gave a value of \(-53 \pm 3\) mV for \(V_m\). Evidence for a change in Donnan junction potential with time after the establishment of whole-cell recordings in the same cell as A and B. Whole-cell K(V) currents activated by ramp stimuli showed a gradual hyperpolarizing shift in voltage dependence. C2, Both the activation threshold (open circles) and the voltage corresponding to the half-maximal current (filled circles) shifted by approximately \(-15\) mV during the first 4–8 min after break-in. Current traces were normalized to compensate for a partial rundown of the K(V) current (C1, inset). The slice was from a 15-d-old rat.

*Figure 3.* The Donnan potential between cytoplasm and pipette in the whole-cell mode can account for the difference in cell-attached and current-clamp \(V_m\) estimates. A, Three superimposed cell-attached current traces from a cell with spontaneous single-channel activity (K\(_{\text{ATP}}\)). Two independent estimates for \(V_m\) can be obtained from these records. With the single-channel amplitude at \(V_h = -60\) mV and the single K\(_{\text{ATP}}\) channel conductance calculated from the slope of the open levels during the voltage ramp stimulation, the driving force for K\(_+\) flux (\(V_{\text{patch}} - E_K\); with \(V_{\text{patch}} = V_h + V_m\) and \(E_K \approx 0\) mV) amounts to 10.8 pA/84 pS = 128.6 mV at \(V_h = -60\) mV, yielding an estimated \(V_m\) of \(-68.6\) mV. The value determined for \(V_m\) from reversal of the macroscopic K(V) current was \(-69.2\) mV. The close agreement between these two values indicates that the ramp stimulation does not significantly affect \(V_m\). B, Subsequent whole-cell current-clamp measurement in the same cell gave a value of \(-53 \pm 3\) mV for \(V_m\). C1, Evidence for a change in Donnan junction potential with time after the establishment of whole-cell recordings in the same cell as A and B. Whole-cell K(V) currents activated by ramp stimuli showed a gradual hyperpolarizing shift in voltage dependence. C2, Both the activation threshold (open circles) and the voltage corresponding to the half-maximal current (filled circles) shifted by approximately \(-15\) mV during the first 4–8 min after break-in. Current traces were normalized to compensate for a partial rundown of the K(V) current (C1, inset). The slice was from a 15-d-old rat.
The difference between values for $V_m$ determined using the cell-attached and whole-cell techniques was approximately $-15$ mV. In the first 20 min after the onset of whole-cell recording, the $K(V)$ current–voltage relation shifted by the same order of magnitude (Fig. 3C). The mean shift in the $K(V)$ activation threshold, in the period of 5–20 min after break-in was $-16 \pm 10$ mV ($n = 5$ cells; range, 3–27 mV), which corresponded to $80 \pm 30\%$ of the difference in $V_m$ estimates. In the absence of TTX, which was
During experiments to examine Donnan shifts in voltage dependence of K⁺ currents, a partial rundown of the peak current was usually apparent (Fig. 4C). In contrast, the K(V) current never ran down in the cell-attached mode. This difference presumably reflects the better conservation of the cytoplasmic environment in cell-attached recordings. Cell-attached measurements of $V_m$ might therefore offer significant advantages in studies on neurotransmitters whose actions depend critically on the cytoplasmic contents. One such neurotransmitter is GABA, which opens Cl⁻/HCO₃⁻-permeable channels and activates a G-protein-mediated K⁺ conductance, by activation of GABA$_A$- and GABA$_B$-linked ion channels, respectively. We therefore used the cell-attached approach to study the effects of GABA on CA1 interneurons.

Exogenous GABA (50 μM) and the selective GABA$_A$ and GABA$_B$ agonists muscimol (50 μM) and baclofen (50 μM) were applied by bath perfusion while monitoring $V_m$ from the reversal of cell-attached K⁺ currents (Fig. 4). Because GABAergic actions on pyramidal cells are reported to change with age (in rabbit: Mueller et al., 1984; in rat: Ben-Ari et al., 1989; Zhang et al., 1991; Chen et al., 1996; Owens et al., 1996), slices were used from animals at various periods after birth. Indeed, there were significant differences in responses to GABA of interneurons from young [postnatal day 1–4 (P1–P4)] and older (P7–P21) animals.

In the older age group, GABA hyperpolarized a small minority of the interneurons (5%; $n = 39$). More cells (26%) depolarized in the presence of GABA (Fig. 4B). However, in the vast majority of the cells (69%), no significant change in the average level of $V_m$ occurred (Fig. 4A). These qualitatively different responses to GABA could be observed in interneurons derived from the same animal (Fig. 4C). Irrespective of the change in average $V_m$, GABA tended to stabilize interneuron membrane potential (Fig. 4A,B; see also Fig. 2). This effect was particularly striking in cells with strong $V_m$ fluctuations and spontaneous firing activity, which invariably ceased in the presence of GABA (Figs. 2, 4C).

As described above, these membrane potential fluctuations were usually associated with action current generation and, as expected, were considerably reduced in the presence of a 0.5 μM concentration of the Na(V) channel blocker TTX (Fig. 4A). It seems probable that action potential generation by these interneurons was largely dependent on excitatory synaptic inputs because exposure to the excitatory amino acid antagonists NBQX (20 μM) and APV (100 μM) also significantly reduced membrane potential fluctuations (Fig. 4B). These data suggest therefore that the primary effect of simultaneous strong activation of both GABA$_A$ and GABA$_B$ receptors is to stabilize $V_m$ of interneurons from P7–P21 rats, presumably by a shunting action.

In contrast to the relatively small effects on the average level of $V_m$ in older animals, GABA invariably depolarized interneurons from animals aged between 1 and 4 d (Figs. 4C, 5C). In young animals its effect in stabilizing fluctuations in $V_m$ was much less pronounced (Fig. 4C). However, in young animals, as in old, depolarizations induced by GABA rarely caused an excitation. Only in one of seven P1–P4 cells, the depolarization induced by GABA was sufficient to evoke APs.

The contribution of different subtypes of GABA receptors was examined by comparing the effects on $V_m$ of GABA (50 μM), muscimol (50 μM) to activate GABA$_A$ receptors, and baclofen (50 μM) to activate GABA$_B$ receptors (Fig. 5). These experiments revealed that responses to GABA depended on the balance between contributions of the GABA$_A$-activated Cl⁻/HCO₃⁻ conductance and the GABA$_B$-activated K⁺ conductance. Thus, in interneurons from P7–P21 rats, the level at which $V_m$ stabilized in the presence of GABA ($−69 ± 12$ mV from a resting level of $−78 ± 9$ mV; $n = 15$) was between the hyperpolarized level reached in the presence of baclofen ($−89 ± 8$ mV) and the depolarized response to muscimol ($−54 ± 8$ mV; Fig. 5A,B).
the GABA<sub>µ</sub>- and GABA<sub>α</sub>-induced changes in V<sub>m</sub> relaxed during prolonged receptor activation, presumably because of redistribution of permeant ions (Kaila, 1994). In contrast, GABA responses remained usually stable during maintained applications.

Whereas GABA and muscimol had different effects on V<sub>m</sub> in P7–P21 rats, in interneurons from young animals these two agonists caused similar depolarizations, to −54 ± 8 mV and −55 ± 8, respectively, from a resting level of −74 ± 14 mV (n = 5). This difference may be explained by an absence of functional GABA<sub>µ</sub> receptors in neurons from P1–P4 animals because baclofen had no effect on V<sub>m</sub> (Fig. 5C). Thus, in these cells, exogenous GABA only increases the Cl<sup>−</sup>/HCO<sub>3</sub>− permeability of the membrane, resulting in a depolarization. Of note, the level of V<sub>m</sub> reached in the presence of muscimol was the same for both age groups. Like the depolarizing GABA response in young animals, the depolarization induced by muscimol in both young and older animals was rarely excitatory. Action currents were generated by the muscimol-induced depolarization in one of five P1–P4 cells and in two of 15 P7–P21 cells.

**DISCUSSION**

Measurements of neuronal membrane potential and neurotransmitter actions are more difficult than commonly admitted. We have shown that the reversal of voltage-gated K<sup>+</sup> currents elicited in cell-attached patches can provide a noninvasive way to determine these parameters. This technique has several advantages: (1) it provides a local measure of V<sub>m</sub>, at the site of the patch; (2) it does not disrupt the cytoplasmic environment; and (3) it avoids Donnan junction potential problems. In this study we validated the technique and used it to examine GABA actions on hippocampal interneurons.

**Validation of the technique**

Variations in amplitude of cell-attached currents passing through single potassium channels have previously been used to infer changes in V<sub>m</sub> in neurons in response to GABA (Zhang and Jackson, 1993; Soltesz and Mody, 1994) and in T lymphocytes during Ca<sup>2+</sup> signaling (Verheugen and Vijverberg, 1995). This technique has the disadvantage that measurements are limited to periods when channels are open. For instance, the Ca<sup>2+</sup>-activated K<sup>+</sup> channels used in these studies open only when [Ca<sup>2+</sup>]<sub>i</sub> is higher than normal, a state that is usually associated with membrane hyperpolarizations as a consequence of this increase in K<sup>+</sup> conductance (Verheugen and Korn, 1997). In contrast, the voltage-gated K<sup>+</sup> channels used to determine the K<sup>+</sup> current reversal potential in the present study could be opened by voltage steps applied to the patch at will, independent of intracellular conditions. Furthermore, while changes in single-channel amplitude provide relative estimates of V<sub>m</sub>, an absolute value of V<sub>m</sub> is obtained from the K<sup>+</sup> current reversal (Verheugen and Vijverberg, 1995).

One potential problem is that the ion current through the K(V) channels could result in a change of V<sub>m</sub> (Fig. 1A). However, because the patch current is, by definition, zero at the reversal potential, this point should be accurate. The use of fast voltage ramps to determine K(V) reversal further reduces transmembrane charge fluxes. Similar values for V<sub>m</sub> estimates from macroscopic and single-channel currents (Fig. 3A) provide additional evidence that under the present conditions the cell-attached currents had little influence on V<sub>m</sub>.

Another potential source of error might be a mismatch between the [K<sup>+</sup>]<sub>i</sub> used in our pipette solution and the effective cytoplasmic [K<sup>+</sup>]. Deviations from symmetrical K<sup>+</sup> would result in an E<sub>K</sub> across the patch different from 0 mV. However, based on the Nernst equation we calculate that an error of for instance 15 mV in the choice of pipette [K<sup>+</sup>] would result in a systematic error of only 3 mV in our determinations of V<sub>m</sub>.

The V<sub>m</sub> values obtained from the cell-attached K(V) reversal were on average 15 mV more negative than those measured subsequently in whole-cell current-clamp recordings in the same neuron. The existence of a Donnan potential between the cytoplasm and pipette solution immediately after break-in to the whole-cell mode probably accounts for this difference. During the first 5–20 min after the whole-cell configuration was established, the voltage dependence of voltage-gated currents shifted by a similar value (Fig. 3C) most likely corresponding to equilibration between the cytoplasm and the recording pipette and consequent dissipation of the Donnan potential (Marty and Neher, 1995). Therefore, the more negative cell-attached V<sub>m</sub> estimates seem likely to be more accurate than the values derived from whole-cell records.

Another advantage of this technique is that the cytoplasmic environment is preserved. In contrast, in the whole-cell technique the pipette solution controls over time the intracellular content, disturbing physiological ion gradients and diluting intracellular factors. The perforated patch technique, which uses antibiotics to render cell membranes permeant to monovalent ions (e.g., nystatin; Horn and Marty, 1988) or selectively to small cations (gramicidin; Eibihara et al., 1995, Kyrozis and Reichling, 1995), thus making electrical contact with the cell interior, avoids these problems to a large extent. Nevertheless, cytoplasmic isolation is not complete, and problems such as an imperfect space-clamp (Müller and Lux, 1993) persist. In contrast, V<sub>m</sub> measurements based on cell-attached K<sup>+</sup> current reversal concerns the local potential at the site of the patch, whereas V<sub>m</sub> of the attached cell is not clamped and able to show unrestrained physiological fluctuations. The point nature of this type of measurement may eventually prove useful to study possible regional variations in V<sub>m</sub> over the neuronal membrane.

**Measurements of GABA actions on hippocampal interneurons**

Actions of the neurotransmitter GABA depend crucially on the intracellular activities of Cl<sup>−</sup>, HCO<sub>3</sub>−, and K<sup>+</sup> ions. For example, concentration shifts of these ions are thought to occur during prolonged GABAergic stimulation (see below). Because it does not perturb the cytoplasm, a cell-attached approach to determine V<sub>m</sub> is particularly well suited to explore GABA actions. Although the time resolution of this technique (limited to ~1 Hz) did not permit individual synaptic events to be resolved, the effects on V<sub>m</sub> of exogenous applied GABA or selective agonists for GABA<sub>µ</sub> and GABA<sub>α</sub> receptors are easily detected.

We found that bath-applied GABA stabilized the V<sub>m</sub> of interneurons at a level similar to its control value in slices from animals aged 1–3 weeks. Dissection of this response using selective agonists suggested that the membrane stabilization represented a balance between a hyperpolarizing action via GABA<sub>µ</sub> receptors and a depolarization mediated by GABA<sub>α</sub> receptor activation. In contrast, in animals aged 1–4 d, the GABA<sub>µ</sub> receptor agonist baclofen had no effect, as observed also in CA3 pyramidal cells at a similar developmental stage (Strata and Cherubini, 1994; Gaieska et al., 1995), and the depolarizing GABA<sub>α</sub>-mediated response predominated. Even in P1–P4 animals the GABA<sub>α</sub>-dependent depolarization rarely induced cell firing, as judged by
an absence of action currents from cell-attached records. The stabilization of $V_m$ induced by GABA in neurons from older animals, and to a lesser extent also young animals, presumably reflects the shunting of the cell membrane by the GABA-activated conductances (Staley and Mody, 1992; Zhang and Jackson, 1993).

The origin of depolarizing responses to GABA$_A$ receptor activation remains to be completely understood. In this study, muscimol depolarized CA1 interneurons to similar membrane potentials, close to −55 mV, in animals from all ages that we examined (Fig. 5). There are numerous reports of biphasic GABA$_A$ responses generated in pyramidal cells, either by repetitive synaptic stimulation or by applying exogenous GABA. These responses consist of a short initial hyperpolarization, followed by a prolonged depolarization (Alger and Nicoll, 1979; Staley et al., 1995; Kaila et al., 1997). It seems probable that the time resolution of our method was not sufficient to capture the initial hyperpolarization and that we largely recorded the depolarizing component of the response. This component may result from an activity-dependent collapse of the Cl$^-$/gradient after which the HCO$_3^-$ current through the GABA$_A$ channel becomes dominant (Staley et al., 1995) analogous to the mechanism in crayfish muscle (Kaila et al., 1989). An additional depolarization may result from an increase in extracellular K$^+$ ($K_e$) (Barolet and Morris, 1991) because of the activity in other interneurons (Kaila et al., 1997), although our results showing that GABA did not enhance cell firing might argue against this.

The observation that muscimol depolarizes $V_m$ of CA1 interneurons in young and old animals to exactly the same level could suggest that the Cl$^-$ reversal potential is the same for both age groups, in contrast to what has been suggested for hippocampal pyramidal cells (Mueller et al., 1984; Ben-Ari et al., 1989), and that in interneurons age-related changes in GABAergic action arise primarily from a change in functional receptor repertoire. However, the possible ionic shifts associated with the prolonged GABAergic stimulation in the present experiments precludes a reliable estimation of resting levels of intracellular ions, and other experiments in which ion shifts are somehow controlled are needed to specifically address the question of developmental changes in Cl$^-$ homeostasis.

In this study we assessed the effects of GABA receptor activation on $V_m$ from the reversal of somatic K$^+$ fluxes. This approach yields a point potential at the somatic patch, which may differ from that at other membrane surface sites (Spruston and Johnston, 1992). It might be especially interesting to use this technique to look for differences between somatic and dendritic responses to GABA receptor activation (Misgeld et al., 1986; Staley et al., 1995; Kaila et al., 1997).

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