Development of Glycinergic Synaptic Transmission to Rat Brain Stem Motoneurons

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Singer, Joshua H., Edmund M. Talley, Douglas A. Bayliss, and Albert J. Berger. Development of glycinergic synaptic transmission to rat brain stem motoneurons. J. Neurophysiol. 80: 2608–2620, 1998. Using an in vitro rat brain stem slice preparation, we examined the postnatal changes in glycinergic inhibitory postsynaptic currents (IPSCs) and passive membrane properties that underlie a developmental change in inhibitory postsynaptic potentials (IPSPs) recorded in hypoglossal motoneurons (HMs). Motoneurons were placed in three age groups: neonate (P0–3), intermediate (P5–8), and juvenile (P10–18). During the first two postnatal weeks, the decay time course of both unitary evoked IPSCs [mean decay time constant, $\tau_{\text{decay}} = 17.0 \pm 1.6$ (SE) ms in neonates and 5.5 ± 0.4 ms in juveniles] and spontaneous miniature IPSCs ($\tau_{\text{decay}} = 14.2 \pm 2.4$ ms in neonates and 6.3 ± 0.7 ms in juveniles) became faster. As glycine uptake does not influence IPSC time course at any postnatal age, this change most likely results from a developmental alteration in glycine receptor (GlyR) subunit composition. We found that expression of fetal ($\alpha_2$) GlyR subunit mRNA decreased, whereas expression of adult ($\alpha_1$) GlyR subunit mRNA increased postnatally. Single GlyR-channels recorded in outside-out patches excised from neonate motoneurons had longer mean burst durations than those from juveniles (18.3 vs. 11.1 ms). Concurrently, HM input resistance ($R_i$) and membrane time constant ($\tau_m$) decreased ($R_i$ from 153 ± 12 MΩ to 63 ± 7 MΩ and $\tau_m$ from 21.5 ± 2.7 ms to 9.1 ± 1.0 ms, neonates and juveniles, respectively), and the time course of unitary evoked IPSPs also became faster ($\tau_{\text{decay}} = 22.4 \pm 1.8$ and 7.7 ± 0.9 ms, neonates vs. juveniles, respectively). Simulated synaptic currents were used to probe more closely the interaction between IPSC time course and $\tau_m$, and these simulations demonstrated that IPSP duration was reduced as a consequence of postnatal changes in both the kinetics of the underlying GlyR channel and the membrane properties that transform the IPSC into a postsynaptic potential. Additionally, gramicidin perforated-patch recordings of glycine-evoked currents reveal a postnatal change in reversal potential, which is shifted from $-37$ to $-73$ mV during this same period. Glycinergic PSPs are therefore depolarizing and prolonged in neonate HMs and become faster and hyperpolarizing during the first two postnatal weeks.

INTRODUCTION

Functional maturation of ligand-gated receptors underlying fast synaptic transmission in the mammalian CNS results from postnatal changes in their subunit composition as illustrated by studies of nicotinic acetylcholine receptors (Mishina et al. 1986; Sakmann and Brenner 1978), $\gamma$-aminobutyric acid-A (GABA$_A$) (Tia et al. 1996), glycine (Takahashi et al. 1992), and N-methyl-D-aspartate (NMDA) receptors (Carmignoto and Vicini 1992; Flint et al. 1997; Hestrin 1992). In all of these systems, the decay time course of postsynaptic currents (PSCs) becomes faster with postnatal development in concert with an increase in the underlying channel deactivation rate.

Factors other than receptor-channel deactivation, however, can influence the time course of fast synaptic transmission: for example, both the temporal profile of transmitter concentration in the synaptic cleft and the rate of transmitter clearance from the synapse can contribute to shaping synaptic currents (reviewed in Clements 1996; Edmonds et al. 1995; Jonas and Spruston 1994). Further, the duration of the postsynaptic potentials (PSPs), which ultimately influence a neuron’s behavior may be governed by factors other than the duration of the postsynaptic conductance change, including the electrotonic location of the activated synapses and the passive electrical properties of the postsynaptic cell (Jack and Redman 1971; Jack et al. 1971; Rall 1967; Rall et al. 1967).

Glycine mediates the majority of inhibitory neurotransmission in the mature mammalian spinal cord and brain stem (Werman et al. 1967) via ligand-gated receptor-channels (GlyRs). The native GlyR is a pentamer (Langosch et al. 1988) that can contain one or more of four subunits: $\alpha_1$, $\alpha_2$, $\alpha_3$, and $\beta$ (Akagi et al. 1991; Greentnloeh et al. 1987, 1990; Kuhse et al. 1990, 1991). Expression of these subunits throughout the CNS is thought to be developmentally regulated such that $\alpha_2$ is the fetal form, predominant at birth, and $\alpha_1$ and $\alpha_3$ are adult subunits that replace it during the first weeks of postnatal life (Akagi and Miledi 1988; Akagi et al. 1991; Becker et al. 1988; Malosio et al. 1991). The strength of glycine-mediated inhibition governs the rhythmic output of mammalian motor systems (Bracci et al. 1996), including the medullary respiratory network (Paton and Richter 1995), and GlyR deficits are linked to human pathologies (i.e., hereditary hyperekplexia or startle syndrome) (Rajendra et al. 1994; Rees et al. 1994; Shiang et al. 1993). Factors governing the time course of glycinergic inhibitory postsynaptic potentials (IPSPs) therefore could have a strong influence on motor control.

Hypoglossal motoneurons (HMs) innervate the tongue musculature and subserve a number of motor functions including respiration, vocalization, and deglutition (Low 1980). Activation of afferent sensory inputs to the hypoglossal motor nucleus (n. XII) elicits both excitatory and inhibi-
tory potentials in HMs (Kubin et al. 1993; Lowe 1978; Sumino and Nakamura 1974; Withington-Wray et al. 1988), which are thought to coordinate tongue movements during complex behaviors such as mastication. Tongue muscle tone, particularly that of the extrinsic genioglossus and styloglossus muscles, also is modulated throughout the respiratory cycle to maintain upper airway patency (reviewed in Lowe 1980), and loss of inspiration-related activity in these muscles is thought to underlie respiratory pathologies like obstructive sleep apnea (Remmers et al. 1978). Thus glycine-ergic synaptic transmission to HMs may be of particular importance because it allows the tongue to perform a variety of voluntary motor functions while receiving rhythmic, respiratory input.

We therefore examined postnatal development of glycine-ergic synaptic transmission to HMs with two questions in mind. First, how are developmental alterations in GlyR mRNA expression correlated with the time course of glycine-ergic inhibitory postsynaptic currents (IPSCs) in HMs? Second, because the morphology (Nunez-Abades and Cameron 1995; Nunez-Abades et al. 1994) and passive membrane properties (Viana et al. 1994) of HMs are modified postnatally, we sought to understand how the transformation of a synaptic current into a postsynaptic potential changes with development. We found that neonatal motoneurons exhibit relatively slow glycine channel kinetics and membrane time constants that prolong the time course of glycine-ergic PSPs. These PSPs were shown to be excitatory to HMs during the first days of postnatal life. During the first two weeks of postnatal life, glycine-ergic PSPs become faster and hyperpolarizing, possibly to enable precise coordination of increasingly varied motor behaviors.

**METHODS**

**In situ hybridization**

Sprague-Dawley rats of either sex (P0–18) were decapitated rapidly; tissue was prepared and in situ hybridization performed as previously described (Bayliss et al. 1994). Two 33-base oligonucleotide probes were generated from published sequences (Akagi et al. 1991; Grenningloh et al. 1987, 1990; Kuhse et al. 1990; Malosio et al. 1991) for each of the four rat glycine receptor subunit cDNAs. α1 probes (Gene Bank Accession No. D0083): gTTggCACCCTTgACAgAgATgCCATCCCTTggC (nts 1310–1342) and CTTgggTCAgCgGgATcGCCTCgTACgCCTgTTT (nts 254–286). α2 probes (Gene Bank Accession No. X57281): gCgggCaAgtgTTggCcAgAgATgCcACgCgTACgCCTgTTT (nts 2335–2367) and CaAggTCTgCgaAggATtgTTGCCACACTgAg (nts 1266–1298). α3 probes (Gene Bank Accession No. M55250): gAgCcGCgACTCTCCCTCACCCTCACCCTAgCTgGt (nts 1510–1542) and CTTgggCACCCTCgACgCTgTTgCCTTgCT (nts 487–519). β probes: gCgggCaAgtgTTggCcAgAgATgCcACgCgTACgCCTgTTTgCt (nts 337–369) and ACTTCAATgggTTTTCCCCATAgCgTCATgAgg (nts 1522–1554).

Oligonucleotides were labeled using terminal deoxynucleotidyl transferase (Bethesda Research Labs) and α-[^32P]dATP (New England Nuclear), ethanol precipitated, and resuspended in hybridization buffer. In preliminary experiments, each of the two probes to the same GlyR subunit labeled identically serial brain sections (not shown), and the CNS distributions of the four subunits were unique and identical to previously published findings (Malosio et al. 1991). We therefore combined the two probes for each receptor subunit in the hybridization buffer to enhance specific signals.

Following hybridization, the sections were washed and air-dried (see Bayliss et al. 1994) and exposed to Hyperfilm β-max X-ray film (Amersham) in cassettes for 5–6 days. After exposure to film, slides were dipped in liquid emulsion (Ilford K5-B), air-dried, and exposed for 10–12 days at 4°C. The emulsion was developed in D19 (Kodak) and fixed. Sections were counterstained with Toluidine blue (0.25%). A computerized image analysis system (MCID; Imaging Research) was used for quantitation of film autoradiograms. Sections (6 for each subunit in each animal) were scanned, and the average relative optical density (O.D.) in the hypoglossal nucleus (n. XII) was determined. O.D. measurements from each of the six sections from an animal were averaged and treated as a single data point for subsequent analysis. The O.D. measurements from three animals in each age group were averaged, and statistical differences between means (significance accepted as P < 0.05) were determined by analysis of variance (ANOVA). Darkfield images of silver grains were photographed using a camera mounted on a Leitz Diaplan microscope.

**Electrophysiology**

**BRAIN STEM SLICE PREPARATION.** Experiments were performed using brain stem slices from Sprague-Dawley rats (P0–18). Animals were anesthetized by either hypothermia (P0–3) or intramuscular injection of a ketamine/xylazine mixture (200 and 10 mg/kg, respectively) and decapitated. The brain stem was isolated and cut in 250–300 μm transverse sections with a vibratome (DSK or Pella) in an ice-cold Ringer solution containing (in mM) 130 NaCl, 26 NaHCO3, 1.25 NaH2PO4, 3 KCl, 10 glucose, 1 CaCl2, and 5 MgCl2. Slices were incubated at 37°C for 1 h in this Ringer solution and then maintained at room temperature in the same solution, except with 2 CaCl2 and 2 MgCl2. Solutions were continuously bubbled with a 95%O2-5%CO2 gas mixture.

Whole cell and outside-out patch recordings were obtained from visualized HMs in brain stem slices at room temperature. Slices were submerged in a chamber mounted on a microscope (Carl Zeiss) equipped with Nomarski optics and a ×40 water-immersion objective, and the recording chamber was perfused at ~3 ml/min. Slices were illuminated with near-infrared light (750–790 nm), and HMs were visualized using an infrared-sensitive CCD video camera (Hamamatsu) connected to a video monitor (Sony). HMs were identified by their location within the hypoglossal nucleus (n. XII) and by their size and shape.

**Solutions**

Voltage-clamp recordings were made using borosilicate glass pipettes (Clark Electromedical) containing (in mM) 130 CsCl, 10 NaCl, 4 MgCl2, 10 N-2-hydroxyethyl-piperazine-N’-2-ethanesulfonic acid (HEPES), 10 ethylene glycol-bis-(β-aminoethy)ether-N,N,N’,N’-tetraacetic acid (EGTA), 3 ATP-Mg, and 0.3 GTP-tris (hydroxymethyl)aminomethane (Tris) for whole cell recording (in some recordings, Cs-glucamine was substituted for CsCl) or 140 N-methyl-D-glucamine Cl, 5 HEPES, 1 EGTA, 3 ATP-Mg, and 0.3 GTP-Tris for patch recording. pH was adjusted to 7.3 with CsOH or NaOH (whole cell and patch solutions, respectively) and osmolality to 305 mosm with sucrose. Gramicidin-perforated whole cell recordings were made using an internal solution of (in mM) 130 CsCl, 10 N-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium bromide (QX-314, RBI), 10 HEPES, and 0.2 EGTA (pH was adjusted to 7.3 with CsOH and osmolality to 305 mosm with sucrose), into which a stock solution of gramicidin (Sigma, 10 mg/ml in methanol) was diluted to a final concentration of 100 μg/ml just before use. The diluted gramicidin solution was effective for several hours. The pipette solution for whole cell current-clamp recordings contained (in mM) 122.5 or 20 K-
MeSO\textsubscript{4}, 17.5 or 120 KCl, 9 NaCl, 10 HEPES, 0.2 EGTA, 3 ATP-Mg\textsubscript{2}, and 0.3 GTP-Tris.

The extracellular solutions for whole cell recording were composed of (in mM) 140 NaCl, 3 KCl, 10 HEPES, 10 glucose, 2 CaCl\textsubscript{2}, and 2 MgCl\textsubscript{2} or 130 NaCl, 26 NaHCO\textsubscript{3}, 1.25 NaHPO\textsubscript{4}, 3 KCl, 10 glucose, 2 CaCl\textsubscript{2}, and 2 MgCl\textsubscript{2}. pH was adjusted to 7.4 with NaOH and osmolality to 315 mOsm by addition of sucrose. Bicuculline methiodide (5–10 \mu\text{M}, Sigma), 6,7-dinitro-quinoxalineidine (D(−)-2-amino-5-phosphono-pentanoic acid (APV, 25 \mu\text{M}, Research Biochemicals), and D(−)-2-amino-5-phosphono-pentanoic acid, and N-methyl-D-aspartate (NMDA)-receptor-mediated currents, respectively. Tetrodotoxin (TTX, 0.5–1.0 \mu\text{M},Calbiochem) and cadmium (100–200 \mu\text{M}) were added to block action potential- and calcium channel-dependent synaptic transmission while recording mIPSCs and glycine-evoked currents. Addition of Cd\textsuperscript{2+} to the superfusate had no effect on GlyR channel kinetics (data not shown).

In situ hybridization techniques were used to document cord recordings, the extracellular solution contained (in mM) 132 NaCl, 26 NaHCO\textsubscript{3}, 1.25 NaHPO\textsubscript{4}, 3 KCl, 10 glucose, 2 CaCl\textsubscript{2}, and 2 MgCl\textsubscript{2}. pH was adjusted to 7.4 with NaOH and osmolality to 315 mOsm by addition of sucrose. We found that the substitution of NMDGCl for NaCl greatly improved patch stability and had no effect on GlyR channels (data not shown).

**Electrophysiological recording**

Recordings were made with an Axopatch 200B amplifier (Axon Instruments). Series resistance in the whole cell configuration was <10 M\Omega and was compensated 70–98%; experiments were terminated if the series resistance increased by >25%. For perforated-patch recording, experiments commenced when the series resistance reached a stable level of ~50 M\Omega, usually after ~60 min. Voltage errors resulting from this series resistance were compensated off-line; corrected voltages are presented here.

Holding potential was −70 mV for whole cell voltage-clamp current recordings and −60 mV for single-channel recording. For current-clamp recording, only HMs with stable membrane potentials hyperpolarized to −60 mV that were capable of generating action potentials throughout a 10-s injection of depolarizing current were selected for analysis. To ensure consistency between recordings, baseline membrane potential was set to −70 mV by current injection. Voltage-clamp recordings (both whole cell and perforated-patch) were not corrected for small liquid junction potentials; current-clamp recordings were compensated for larger (~10 mV) liquid junction potentials created by substitution of MeSO\textsubscript{4} for Cl\textsuperscript{−} in the pipette solution.

Unitary glycocnergic synaptic currents were evoked by extracellular stimulation of single, visualized interneurons in the Nucleus of Roller, just ventrolateral to n. XII, as described previously (Umemiya and Berger 1995). Compound IPSCs were evoked by extracellular stimulation in the Nucleus of Roller. Illustrated current and voltage traces represent the average of five or more trials.

**Data acquisition and analysis**

Data were filtered at 1–10 kHz and digitized at 2–20 kHz (pCLAMP, Axon Instruments; WCP, Strathclyde Electrophyysiology Software) or recorded onto video tape at 80 kHz (Neuro-Net, NeuroData Instruments). Whole cell current and voltage decays were fit by a Chebychev algorithm (pCLAMP). Evoked and spontaneous miniature IPSC decay phases were best fit by two exponentials, and the mean time constant, \( \tau \), was calculated from these time constants and their relative amplitudes: \( \tau = \tau_1 + \tau_2 \). IPSC amplitudes were fit by a single exponential.

Single-channel amplitude distributions (all points histogram, binwidth = 0.2 pA) were fit by Gaussian functions (Levenberg-Marquard method). Channel openings were identified by pCLAMP using a 50% threshold-crossing detector, and the data were idealized for kinetic analysis. The dead time of the system was 100 \mu\text{s}, and no correction was made for missed events. Channel bursts were defined as openings or groups of openings separated by a relatively long closed time. The interburst interval (i.e., the minimum value of a closed time separating bursts) was determined using an algorithm that increased a test interburst interval until the number of openings in a burst became relatively insensitive to further changes in this interval (Sigurdson et al. 1987).

In this interval was 5–10 ms for all patches, which is quite similar to values reported previously (Twyman and MacDonald 1991). Burst durations were binned logarithmically (10 bins/decade), and a square-root transformation of the histogram ordinate was used to keep errors constant throughout the plot (Sigworth and Sine 1987). The binned data were fit by a third-order exponential function, the minimum number of statistically significant exponentials describing the distribution. Increasing the number of exponentials (>3) in the fit did not improve the fit in a statistically significant way (F test, \( P < 0.05 \)). The mean single-channel burst duration \( (\tau_{\text{mean}}) \) was calculated from the time constants, and their relative amplitudes of the \( \chi^2 \) fit: \( \tau_{\text{mean}} = \tau_1a_1 + \tau_2a_2 + \tau_3a_3 \).

Data are presented as means ± SE unless otherwise noted. Statistical significance was determined using ANOVA for between-group comparisons or a two-tailed t-test for within-cell comparisons. Miniature IPSC amplitude distributions and burst duration distributions were compared with a Kolmogorov-Smirnov test. Changes were considered significant if \( P < 0.05 \).

**RESULTS**

We examined glycocnergic neurotransmission to HMs in rats aged P0–18. Motoneurons have acquired adult-like electrophysiological, morphological, and biochemical properties by approximately P16 (Berger et al. 1996; Kalb and Hockfield 1994). For simplicity, animals were placed in three age groups: neonate (P0–3), intermediate (P5–8), and juvenile (P10–18).

**In situ hybrdization**

We used in situ hybridization techniques to document postnatal changes in GlyR subunit mRNA expression. To examine GlyR subunit mRNA expression in HMs, two oligonucleotide probes for each GlyR subunit were hybridized simultaneously to brain stem sections prepared from rats aged P0–18.

At birth (P0), the predominant GlyR \( \alpha \) subunit in HMs was \( \alpha_2 \), and its expression decreased to near background levels by P18 (Fig. 1A, \( P < 0.05 \), P0 vs. P18). In contrast, only low levels of the \( \alpha_1 \) GlyR subunit were present at birth, and \( \alpha_1 \) expression increased dramatically during the first 2 wk of postnatal life (\( P < 0.05 \), P0 vs. P18). GlyR subunit \( \beta \) was expressed at high levels throughout this time, whereas the \( \alpha_3 \) subunit was not expressed to any significant extent in HMs. The pattern of GlyR subunit expression during the first 18 days of postnatal life is summarized in Fig. 1B. These data suggest that GlyR in neonate HMs are heteromultimers composed primarily of \( \alpha_2 \) and \( \beta \) subunits and that during the first 2 wk of postnatal life, HM GlyR switch subunit composition, becoming \( \alpha_1/\beta \) heteromultimers.

**Single-channel recording: steady-state glycine application**

We next studied single channel currents in outside-out patches excised from neonate and juvenile motoneurons to
FIG. 1. Subunit switch of glycine receptor mRNAs in developing hypoglossal motoneurons. A: at P0, the \( \alpha_2 \) subunit predominates, and its expression decreases to background levels by P18. \( \alpha_1 \) expression is very low at birth and increases dramatically by P18. Images are darkfield photomicrographs of hypoglossal motor nucleus (n. XII) from emulsion dipped slides. Scale bar is 200 \( \mu \)m. B: summary of postnatal changes in glycine receptor (GlyR) subunit expression plotted as optical density (O.D.) within n. XII normalized to background. \( \alpha_1 \) expression is increased with postnatal development, whereas \( \alpha_2 \) expression is decreased. \( \alpha_3 \) subunit is not expressed to any significant degree by hypoglossal motoneurons (HMs), and the \( \beta \) subunit is expressed at high levels throughout development.

determine if the observed changes in GlyR subunit mRNA expression are correlated with altered GlyR channel behavior. Channel openings were elicited by steady-state application of glycine (5–10 \( \mu \)M) to the patch and were blocked by strychnine (1 \( \mu \)M, data not shown). Channel openings primarily were to a 30-pS conductance level, and the predominant single-channel conductance did not change with postnatal development (slope conductance = 30 pS in both neonates and juveniles, \( n = 5 \) and 4, respectively, data not shown). In some patches (6/14), we observed multiple subconductance levels (20–80 pS), but as these events were rare, we limited our analysis to the 30-pS openings. These measurements of native GlyR-channel conductance are similar to others (Kaneda et al. 1995; Takahashi and Momiyama 1991; Takahashi et al. 1992; Twyman and MacDonald 1991), and the preponderance of relatively small-conductance (30 pS) events is consistent with the hypothesis that HM GlyRs are heteromultimers composed of \( \alpha \) and \( \beta \) subunits and not homomultimers of \( \alpha \) subunits (Bormann et al. 1993).

Glycine channels opened in bursts on bath application of glycine (Fig. 2A). Burst analysis of single-channel openings at a holding potential of \(-60\) mV revealed a marked decline in burst duration with age. Data from all patches in each age group were pooled, and normalized burst duration histograms for neonate (\( n = 5 \) patches) and juvenile (\( n = 4 \) patches) glycine channels are illustrated in Fig. 2B. In both groups, the histograms were fit by three exponential components with essentially identical time constants (in ms, neonates vs. juveniles, relative amplitude, “a,” of time constant given in parentheses): \( \tau_1 = 0.3 \) (0.46) vs. 0.3 (0.56), \( \tau_2 = 10.0 \) (0.35) vs. 8.4 (0.35), \( \tau_3 = 78.1 \) (0.19) vs. 90.2 (0.09). Mean burst duration, \( \tau_{\text{mean}} = \tau_1 a_1 + \tau_2 a_2 + \tau_3 a_3 \), decreased significantly with age from 18.3 ms (neonate) to 11.1 ms (juvenile) (\( P < 0.05 \)). The most striking difference was the reduction in amplitude of the slowest exponential compo-
nent, $\tau_5$, from 19% in neonate HMs to 9% in juvenile HMs, and the corresponding increase in the fastest exponential component, $\tau_1$, from 46 to 56% (neonate and juvenile, respectively; $P < 0.05$).

**Glycinergic inhibitory postsynaptic currents**

After documenting postnatal changes in GlyR subunit mRNA expression and single-channel kinetics, we next examined the time course of IPSCs throughout early HM development. Unitary IPSCs were evoked by stimulation of single, inhibitory interneurons just ventrolateral to n. XII (Umemiya and Berger 1995), which are involved in reflex inhibition of HMs induced by craniofacial sensory afferent activation (Sumino and Nakamura 1974). Generally, the decay time course of IPSCs was fitted best by two exponentials, and a weighted mean time constant, $\tau_{\text{decay}}$, was calculated: $\tau_{\text{decay}} = \tau_{\text{fast}}(a_{\text{fast}}) + \tau_{\text{slow}}(a_{\text{slow}})$. $\tau_{\text{decay}}$ decreased during the first two postnatal weeks, from $17.0 \pm 1.6$ ms in neonate HMs ($n = 11$) to $8.4 \pm 0.7$ ms in intermediates ($n = 9$) and $5.5 \pm 0.4$ ms in juveniles ($n = 21, P < 0.05$, neonates vs. juveniles, Fig. 3A). The mean peak amplitude of the unitary IPSCs was $-195 \pm 39$ pA in neonates, $-296 \pm 83$ pA in intermediates, and $-268 \pm 37$ pA in juveniles. These differences were not statistically significant ($P = 0.2$, neonates vs. juveniles) and are summarized in Table 1.

The IPSC waveform, however, may be influenced by the electrotonic properties of the postsynaptic cell and/or by asynchronous transmitter release (Diamond and Jahr 1995; Isaacson and Walmsley 1995). To exclude these factors, we examined spontaneous miniature IPSCs (mIPSCs) recorded in the presence of TTX (0.5–1.0 μM) and cadmium (100–200 μM). Miniature IPSCs are thought to reflect postsynaptic responses to individual vesicles of transmitter from single synaptic release sites (Katz 1969).

We studied mIPSCs with fast rise times ($\leq 1$ ms) to exclude events that might be altered significantly by electrotonic filtering (Fig. 3B1). For these mIPSCs, there was no significant correlation between half-width and rise-time ($r^2 \leq 0.1, n = 29$ cells, Fig. 3B2). Like the unitary evoked IPSCs, the decay phase of spontaneous mIPSCs was best fit by a biexponential function, and it became faster with age: $\tau_{\text{decay}} = 14.2 \pm 2.4$ ms in neonates ($n = 11$), $9.1 \pm 1.4$ ms in intermediates ($n = 8$), and $6.3 \pm 0.7$ ms ($n = 10$) in juveniles ($P < 0.05$, neonates vs. juveniles, Fig. 3C). This change was due to a reduction in the slow decay time constant and its relative amplitude: $\tau_{\text{slow}} = 31.3 \pm 7.0$ ms (40±6%) in neonates, $15.6 \pm 3$ ms (41±7%) in intermediates, and $11.9 \pm 1.2$ ms (24±4%) in juveniles. The fast decay time constant did not change significantly with development ($P = 0.1$, neonate vs. juvenile), but its relative amplitude increased significantly: $\tau_{\text{fast}} = 6.0 \pm 0.4$ ms (60±6%) in neonates, $4.9 \pm 0.6$ ms (59±7%) in intermediates, and $4.6 \pm 0.5$ ms (76±4%) in juveniles ($P < 0.05$, neonate vs. juvenile). Mean mIPSC amplitude increased postnatally: amplitude $= -42 \pm 6$ pA in neonates, $-54 \pm 11$ pA in intermediates, and $-78 \pm 11$ pA in juveniles ($P < 0.05$, neonates vs. juveniles). These data are summarized in Table 1. As the $\tau_{\text{decay}}$ of both IPSCs and mIPSCs was virtually identical at each developmental stage, asynchronous trans-
mIPSCs with 10 ± 90% rise times are known to change with postnatal development (Johnston and Davies 1974; Supplisson and Bergman 1997). Specific blockers of GLYT-2 do not exist, but substitution of 80% of the extracellular Na⁺ by Li⁺ can effectively inhibit glycine transport (Titmus et al. 1996). We compared the kinetics of glycineergic IPSCs and the whole cell responses to glycine applied by pressure ejection into the slice before and after transporter blockade by sarcosine (500 μM) or LiCl substitution for perfusate NaCl in neonate and juvenile HMs. Because the effects of sarcosine and Li⁺ were identical, the data from these experiments were pooled.

Blocking glycine uptake with sarcosine or Li⁺ caused an increase in the motoneurons’ integrated current response to a glycine puff (200 μM, 5–20 psi, 5–100 ms) to 115 ± 8% of control in neonates and 207 ± 18% of control in juveniles (n = 7 and 8, respectively, Fig. 4, A and B, right. P < 0.05). A number of factors could account for this marked difference, including developmental changes in glycine transport capacity (Johnston and Davies 1974; Jursky and Nelson 1996). This phenomenon, however, was not explored further. An increase in cell holding current and a decrease in membrane input resistance (Rᵢ), both of which were blocked by strychnine (10 μM), also were observed in both neonate and juvenile HMs after sarcosine or Li⁺ application (holding current increased to 253 ± 94% of baseline).

Glycine uptake and the IPSC

We next sought to determine whether glycine uptake shapes the decay of glycineergic IPSCs, and if so, does it account for some of the observed postnatal change in IPSC time course? This question is of particular importance as patterns of glycine transporter expression and transporter Vₘₐₓ are known to change with postnatal development (Johnston and Davies 1974; Jursky and Nelson 1996).

Two families of sodium-dependent glycine transporters have been identified, GLYT-1 and GLYT-2 (Borowski et al. 1993; Guastella et al. 1992; Liu et al. 1993; Smith et al. 1992). Whereas GLYT-1 transporters are found in glia and GLYT-2 transporters are neuronal, it has been suggested that both operate in concert in regions where they are found together and function to clear glycine from the extracellular space (Jursky and Nelson 1996). Sarcosine (N-methylglycine), a glycine transport system substrate, has been shown to block by >85% GLYT-1-dependent transport at a concentration of 500 μM (Guastella et al. 1992; Supplisson and Bergman 1997). Specific blockers of GLYT-2 do not exist, but substitution of 80% of the extracellular Na⁺ by Li⁺ can effectively inhibit glycine transport (Titmus et al. 1996). Values are means ± SE, n, number of cells; t_decay, exponential fit to waveform decay phase; mIPSC, miniature inhibitory postsynaptic current; eIPSC, evoked inhibitory postsynaptic current; eIPSP, evoked inhibitory postsynaptic potential. * Differences between age groups are significant (P < 0.05); Kolmogorov-Smirnov test for mIPSC amplitude, analysis of variance for t_decay.
control in neonates and 271 ± 49% of control in juveniles; $R_N$ decreased to 92 ± 9% of control in neonates and 89 ± 6% of control in juveniles, $n = 7$ and 8, respectively; $P < 0.05$ for changes in both holding current and $R_N$. These effects most likely result from an accumulation of glycine in the extracellular environment, and consequent activation of GlyRs, as has been reported previously (Titmus et al. 1996).

Although glycine uptake clearly was inhibited by sarcosine and Li+, the decay time course of IPSCs was unaffected ($\tau_{\text{decay}} = 95 ± 7%$ of control in neonates and 104 ± 3% of control in juveniles; $n = 10$ and 17, respectively. Figure 4, A and B, left; $P = 0.89$ for neonates and 0.98 for juveniles). To increase the likelihood of observing some effect of these transport blockers on the IPSC decay, we evoked trains (100 Hz for 100 ms) of compound IPSCs to create conditions under which synaptically released glycine might “spill-over” and activate receptors outside the synapse into which it was released (Isaacson et al. 1993). We found that under no stimulus conditions was the current decay altered by sarcosine or Li+ substitution (Fig. 4C).

Interestingly, blocking glycine transport caused a decrease in the peak amplitude of synaptic currents. As sarcosine and Li+ had no effect on the amplitude of excited patch currents ($n = 2$, data not shown), we, like others (Titmus et al. 1996), attribute this effect to a reduced number of free GlyRs available to bind synaptically released glycine. The reduction of IPSC amplitude tended to be larger in neonates than juveniles, but this difference was not statistically significant (IPSC amplitude was reduced to 77 ± 23% of control in neonates and 93 ± 7% of control in juveniles; $n = 10$ and 17, respectively, $P = 0.38$).

**Postnatal development of passive membrane properties**

Having determined what factors may account for the postnatal development of the glycinergic IPSC waveform, i.e., the change in GlyR channel burst duration, we next examined postnatal changes in HM passive membrane properties. These shape the transformation of IPSCs into IPSPs. Previously, sharp microelectrode recording techniques have been used to demonstrate postnatal changes in HM $R_N$ and membrane time constant ($\tau_m$) (Nunez-Abades et al. 1993; Viana et al. 1994). Because our study used patch electrodes, which yield different estimates of $R_N$ than obtained with sharp electrodes (see, for example, Spruston and Johnston 1992), we determined $R_N$ and $\tau_m$ in neonate and juvenile HMs in our preparation.

Motoneuron $R_N$ was derived from the slope of the relationship of averaged steady-state voltage responses to small 250-ms current pulses. Input resistance decreased by 59% over the first two postnatal weeks (Fig. 5A, $R_N=153 ± 12$ MΩ in neonates and 63 ± 7 MΩ in juveniles, $n = 23$ and 31, respectively, $P < 0.05$). This reduction is similar to that reported by Viana et al. (1994). The mechanism(s) underlying this reduction were not explored further.

HM membrane time constant, $\tau_m$, was calculated using the short current pulse method described by Durand et al. (1983). Brief (1 ms) hyperpolarizing (−400 pA) current pulses were injected into HMs, and these elicited a membrane potential response that decayed biexponentially. The $\tau_m$ was taken as the time constant of the slow component of voltage decay, and $\tau_m$ became shorter with age (Fig. 5B, $\tau_m=21.5 ± 2.7$ ms in neonates and 9.1 ± 1.0 ms in juveniles, a change of 58%. $P < 0.05$, $n = 23$ and 31, respectively). As $\tau_m$ is the result of the product of membrane resistance and membrane capacitance, and the relative changes in $R_N$ and $\tau_m$ with age are almost identical (59 vs. 58%, $R_N$ and $\tau_m$, respectively), we suggest that the postnatal change in $\tau_m$ results almost exclusively from changes in $R_N$ rather than cell capacitance. This assertion is supported by morphometric data (Nunez-Abades and Cameron 1995), which show no change in HM total membrane surface area throughout the first two postnatal weeks (also see Viana et al. 1994).

**Postnatal development of glycinergic IPSPs**

As both glycinergic synaptic currents and the $\tau_m$ of HMs become faster postnatally, glycinergic IPSPs should be of...
shorter duration in juvenile as compared with neonate HMIs. We recorded unitary evoked IPSPs in neonate and juvenile HMIs (PSPs are depolarizing in the whole cell recording configuration, \( E_{rev}(\approx 0 \text{ mV}) \)) and observed a clear postnatal increase in the rate of IPSP decay. Glyceric IPSPs decayed monoexponentially, and the decay time constant, \( \tau_{\text{decay}} \), was reduced from 22.4 ± 1.8 ms in neonates to 7.7 ± 0.9 ms in juveniles (Fig. 6A, \( n = 6 \) and 11, neonates and juveniles, respectively; \( P < 0.05 \)). Glyceric IPSP rise-time also became faster with postnatal development (10–90% rise-time = 4.3 ± 0.6 ms in neonates and 1.8 ± 0.1 ms in juveniles, \( P < 0.05, n = 6 \) and 11). These results are summarized in Table 1.

An important functional consequence of this postnatal change in IPSP decay is revealed by evoking trains of unitary IPSPs. Closely spaced IPSPs summate at much lower frequencies in neonate (between 10 and 30 Hz) as compared with juvenile (between 30 and 50 Hz) HMIs (\( n = 5 \) and 7, neonates and juveniles, respectively). Consequently, neonatal HMIs are more effective integrators of glycinergic synaptic inputs than are juvenile HMIs. This may reflect a changing role for glycinergic neurotransmission with postnatal development (see DISCUSSION).

To examine the relative contributions of changes in IPSC time course and \( \tau_m \) to the postnatal changes in IPSC time course, we examined the membrane potential response (MP) to simulated IPSC waveforms (\( I_{\text{sim}} \)) injected through our whole cell recording pipette. The \( I_{\text{sim}} \) waveform was designed to mimic approximately the unitary evoked IPSC and had a rise-time of 1 ms, an amplitude of 200 pA, and a monoexponential decay with a time constant of either 15 or 5 ms (to reproduce neonate and juvenile IPSCs, respectively). Both neonate and juvenile \( I_{\text{sim}} \) waveforms were injected into neonate and juvenile HMIs, and the resultant MPs are illustrated in Fig. 7.

The MP in an HM resulting from an age-matched current injection (i.e., a neonate \( I_{\text{sim}} \) injected into a neonate HM) was virtually identical to the evoked IPSC waveform recorded in the same cell as illustrated in Fig. 7A4 and summarized in Table 2. Age mismatched current injections produced MPs that did not resemble IPSPs in their waveform: a juvenile \( I_{\text{sim}} \) injected into a neonate HM elicited a MP with a \( \tau_{\text{decay}} = 13.2 ± 1.2 \text{ ms} \) (\( n = 5 \)), which is 41% faster than the neonate evoked IPSP \( \tau_{\text{decay}} = 22.4 ± 1.8 \text{ ms} \). The MP of a juvenile HM in response to injection of a neonate \( I_{\text{sim}} \) was 17.9 ± 0.8 ms (\( n = 8 \)), 129% slower than the evoked IPSP recorded in juvenile HMIs (7.7 ± 0.9 ms). The implications of this simulation will be considered in DISCUSSION.

**Glycine depolarizes neonatal HMIs**

Intracellular Cl\(^-\) concentration is thought to be elevated in neonatal neurons (Owens et al. 1996). As GlyR-channel-mediated currents are generated primarily by Cl\(^-\) flux, the reversal potential of such currents recorded in HMIs is likely to change postnatally. We therefore used the gramicidin perforated-patch recording technique, which does not alter the intracellular Cl\(^-\) concentration (Meyers and Haydon 1972; Rhee et al. 1994) to study glycine-activated currents in neonatal and juvenile HMIs. Glycine (0.1–1 mM) was applied to the surface of neonatal or juvenile brain stem slices by pressure ejection (5–10 psi for 10–100 ms), and glycine-evoked currents were recorded in HMIs at various holding potentials (Fig. 8, A and B). To permit confirmation of the integrity of our perforated-patch recording configuration, we included QX-314 (a Na\(^+\) channel blocker) in our recording pipette solution. Accidental patch rupture resulted in an absence of action currents; these were evoked readily in the perforated-patch configuration (data not shown).

Glycine applied to neonatal HMIs (Fig. 8A1) induced currents that had a reversal potential of −37 mV (\( n = 3 \), Fig. 8B). In juvenile HMIs (Fig. 8A2), glycine-activated currents displayed a reversal potential of −73 mV (\( n = 3 \), Fig. 8B), which is hyperpolarized relative to the resting membrane potential of these neurons (about −70 mV) (Viana et al. 1994). After the establishment of a whole cell recording configuration by intentional patch rupture, the reversal potential of glycine-activated currents recorded in both neonatal and juvenile HMIs shifted toward the calculated reversal potential of Cl\(^-\) (0.2 mV). Thus in neonate HMIs, glycineric PSSPs are depolarizing (Fig. 8C) as well as prolonged relative to those observed in juvenile cells.

We calculate, using the Nernst equation and assuming that the glycine currents are produced by Cl\(^-\) flux exclusively, that the internal Cl\(^-\) concentration in HMIs is reduced from 33 to 8 mM during the first weeks of postnatal development. These values are quite similar to those reported by others in studies of neural development (Owens et al. 1996).
FIG. 6. Glycinergic inhibitory postsynaptic potential (IPSP) waveform changes with postnatal development. A1: unitary, evoked IPSPs recorded in neonate (P1) and juvenile (P16) HMs; \( V_m = -70 \text{ mV} \). Juvenile trace has been scaled to the peak amplitude of the neonate trace; \( \cdot \cdot \cdot \), single exponential fits to the decay phase. PSPs are depolarizing in the whole cell recording configuration. A2 and A3: summary of postnatal changes in IPSP \( \tau_{\text{decay}} \) and 10–90% rise-time: \( \tau_{\text{decay}} = 22.4 \pm 1.8 \text{ ms in neonates to } 7.7 \pm 0.9 \text{ ms in juveniles (} n = 6 \text{ and } 11, \text{ respectively); } 10–90\% \text{ rise-time } = 4.3 \pm 0.6 \text{ ms in neonates and } 1.8 \pm 0.1 \text{ ms in juveniles (} n = 6 \text{ and } 11 \text{).} \bullet \), mean values for the age groups (individual data points are \( \circ \) and are connected by solid lines). B1 and B2: trains (700 ms) of IPSPs evoked at 10 and 30 Hz in neonate and juvenile HMs (same cells as in A1) illustrate IPSP summation at lower frequencies in neonates.

FIG. 7. Simulated IPSCs (\( I_{\text{sim}} \)) and resultant membrane potential changes (MP) illustrate the interaction of synaptic currents with HM passive membrane properties. A1: evoked IPSCs and IPSPs recorded in the same neuron illustrate the relationship between current and voltage decay phases in neonate (P1) and juvenile (P12) motoneurons. Note the difference in both IPSC and IPSP decay time courses between neonate and juvenile cells. A2: MP response to an age-matched \( I_{\text{sim}} \) almost exactly replicates the evoked IPSP, as illustrated in A4. A3: age-mismatched \( I_{\text{sim}} \) elicits a MP that differs from both the age-matched MP and the evoked IPSC. A4: voltage response to an age-matched \( I_{\text{sim}} \) is essentially identical to the evoked IPSP. Vertical scale bar is 5 mV, 125 pA for neonate traces, 3 mV, 150 pA for juvenile traces. B1: illustration of the relative contributions of PSC waveform and \( \tau_m \) to PSP time course. Traces a and b are averages of MP responses, normalized to the same peak amplitude, to the neonate (a) or juvenile (b) \( I_{\text{sim}} \) injected into both neonate and juvenile HMs (corresponding to \( \tau_m \) mismatch in B2). Traces c and d are normalized mean MP responses of neonate (c) and juvenile (d) HMs to both the neonate and juvenile \( I_{\text{sim}} \) (corresponding to \( I_{\text{sim}} \) mismatch in B2). Insets: \( I_{\text{sim}} \) waveforms (not to scale). Note that the difference in decay time course between the 2 traces is much greater in c and d than in a and b, indicating that changes in \( I_{\text{sim}} \) have a more significant impact on MP than do changes in \( \tau_m \). B2: summary and comparison of \( \tau_m \) and \( \tau_{\text{decay}} \) of HM MPs and evoked IPSPs. Note that the differences between MP \( \tau_{\text{decay}} \) are larger when \( I_{\text{sim}} \) is changed within age groups (\( I_{\text{sim}} \) mismatch, thin line, c and d in B1) than when cells with different \( \tau_m \) receive the same \( I_{\text{sim}} \) (\( \tau_m \) mismatch, thick line, a and b in B1).
Factors governing IPSC time course

Many factors can contribute to the decay time course of synaptic currents. These include the intrinsic kinetics of the channels underlying the current as well as the transmitter time course in the synaptic cleft (Clements 1996; Edmonds et al. 1995; Jonas and Spruston 1994). The relationship between GlyR kinetics and glycine-mediated neurotransmission has been explored in Mauthner cells of both zebrafish and goldfish (Faber and Korn 1980; Legendre and Korn 1994). Based on spectral analysis of glycine-induced conductance changes, it was inferred that the relaxation kinetics of activated channels are rate-limiting during the IPSC decay (Faber and Korn 1980). More recently, Legendre (1998) has demonstrated that glycine receptor-channel deactivation rate is the primary determinant of glycine-mediated decay time course in zebrafish hindbrain. The close correlation between mean channel burst duration and mIPSC \( \tau_{\text{decay}} \) we observe, when considered in light of these other studies, indicates that a postnatal change in glycine channel behavior underlies the development of IPSC time course in mammalian motoneurons. It has been observed, however, that glycine channel mean open time increases with glycine concentration (Twyman and MacDonald 1991). Thus it is possible, although we consider it unlikely (also see Takahashi et al. 1992), that the developmental changes in glycine channel burst duration we observe reflect, in part, an altered concentration dependency of channel behavior.

Transmitter uptake shapes the time course of synaptic events at some mature, but not neonate, inhibitory synapses (Draguhn and Heinemann 1996). We find that while blockade of the glycine uptake system with sarcosine or Li\(^+\) substitution enhances HM responses to glycine applied to the slice, it has no effect on the time course of inhibitory synaptic transmission. We attempted to induce synaptic spill-over of glycine with trains of multiunit stimuli, but in no instance, and at no stage of postnatal development, did we find that glycine uptake inhibitors had any effect on the time course of synaptic glycine currents. We conclude, then, that the postnatal change in IPSC time course does not result from enhanced glycine uptake.

Transformation of synaptic currents into postsynaptic potentials

Using whole cell recording techniques, we observed that the proportional changes in motoneuron \( R_N \) and \( \tau_m \) with postnatal development are quite similar to those determined by sharp-electrode recordings (Nunez-Abades et al. 1993; Viana et al. 1994). Given the postnatal change in motoneuron \( \tau_m \), what impact does the developmental alteration of GlyR kinetics and IPSC decay rate have on IPSP time

![Figure 8](http://jn.physiology.org/DownloadedFrom/.../J308-8/9k2e$$no48 10-26-98 22:41:54 neupa LP-Neurophys.png)

**FIG. 8.** Reversal potential (\( E_{\text{rev}} \)) of glycine-activated currents becomes more negative with postnatal development. **A1:** current responses elicited by brief applications of glycine (1 mM) to neonate (P2) and juvenile (P15) HMs at various holding potentials (values indicated in mV), recorded using the gramicidin perforated-patch technique. These illustrate a clear development change in glycine \( E_{\text{rev}} \). Note the difference in \( E_{\text{rev}} \) after establishment of a whole cell recording configuration in A2. **B:** summary of current-voltage relationship of neonate (\( n = 3 \)) and juvenile (\( n = 3 \)) glycine-evoked responses. \( E_{\text{rev}} = -37 \) mV in neonates and \(-73 \) mV in juveniles. **C:** spontaneous glycine-activated PSPs recorded in a neonate (P1) HM are depolarizing when recorded at \( V_m = -70 \) mV using the gramicidin perforated-patch technique.
course? By simulating synaptic currents with somatic current injections and comparing the resultant membrane potential waveforms to evoked IPSPs, we demonstrated that the synaptic current is the primary determinant of PSP waveform.

When we compared the MP waveforms resulting from neonatal \( I_{\text{sim}} \) injections into neonatal and juvenile motoneurons (\( \tau_m \) is mismatched: Fig. 7B, I and 2, Table 2), we found that their \( \tau_{\text{decay}} \) was similar: \( \sim 21 \text{ ms} \) in neonates and \( \sim 18 \text{ ms} \) in juveniles, a ratio of 1.21 (neonate to juvenile). The disparity between MPs elicited by injection of both neonate and juvenile \( I_{\text{sim}} \) into neonate HMs alone was much greater: \( \sim 21 \text{ ms} \) versus \( \sim 13 \text{ ms} \), or 1.61 (neonate \( I_{\text{sim}} \) to juvenile \( I_{\text{sim}} \)). Similar discrepancies were seen with juvenile motoneurons. The ratio of the MP responses to a juvenile \( I_{\text{sim}} \) injected into neonate and juvenile HMs was 1.5:1 (\( \sim 13 \text{ ms} \) vs. \( \sim 9 \text{ ms} \), neonate vs. juvenile); the \( \tau_{\text{decay}} \) of the juvenile motoneurons’ MP response to a juvenile \( I_{\text{sim}} \) was \( \sim 9 \text{ and} \sim 18 \text{ ms} \) for a neonate \( I_{\text{sim}} \) (2:1, neonate \( I_{\text{sim}} \) vs. juvenile \( I_{\text{sim}} \)). Thus the relative difference between neonate and juvenile MP \( \tau_{\text{decay}} \) when \( I_{\text{sim}} \) time course is mismatched is 33% greater than when \( \tau_m \) is mismatched (see Fig. 7B, I and 2), indicating that changes in PSC time course have a more profound effect on PSP decay than do alterations of \( \tau_m \).

Synaptic current time course, then, may be matched to the motoneurons’ passive membrane properties; a similar hypothesis has been proposed for the developing neuromuscular junction (Jaramillo et al. 1988). This ensures that IPSPs are slow and summate at low frequencies in neonate motoneurons and become faster with postnatal development, as n. XII-mediated motor behaviors become more complex and likely more dependent on the timing of inhibition.

Role for depolarizing glycinergic inputs to neonatal HMs

Glycine depolarizes neonate HMs, as demonstrated by our gramicidin perforated-patch recordings. Depolarization in neonatal neurons induced by inhibitory neurotransmitters (both glycine and GABA) has been observed previously in the spinal cord (Fulton et al. 1980; Nishimaru et al. 1996; Reichling et al. 1994; Takahashi 1984; Wu et al. 1992), the brain stem (Bakus et al. 1998; Kandler and Friauf 1995), the hippocampus (Ben-Ari et al. 1989; Ito and Cherubini 1991), the hypothalamus (Chen et al. 1996), and the neocortex (Flint et al. 1998; Owens et al. 1996). While the role of such an action is uncertain, it may be related to activity-dependent strengthening of immature inhibitory synapses. Indeed, studies have demonstrated that activity of inhibitory inputs can influence the development of neuronal circuitry (Sanes and Hafidi 1996; Sanes and Takacs 1993).

Glycine-evoked depolarization can allow Ca\(^{2+}\) influx into immature neurons through voltage-gated calcium channels (Flint et al. 1998; Lo et al. 1998; Reichling et al. 1994), and calcium entry into neurons is thought to be important in the regulation of various transcription factors that may be involved in synapse development (Goodman and Shatz 1993). Additionally, this depolarization may facilitate activation of NMDA receptors (Ben-Ari et al. 1997; Kotak and Sanes 1996), through which activity-dependent increases in synaptic strength most likely are mediated (Constantine-Paton et al. 1990; Goodman and Shatz 1993). Neonatal HMs exhibit a significant low-voltage-activated (T type) Ca\(^{2+}\) current with a half-activation potential of approximately \( -30 \text{ mV} \) (Umemiya and Berger 1994), near \( E_{\text{Ca}} \). This T current comprises \( \sim 35\% \) of the total Ca\(^{2+}\) current in neonate HMs (measured at 0 mV), and its contribution to total Ca\(^{2+}\) current decreases with postnatal development (Umemiya and Berger 1994). Glycine-induced membrane depolarization is likely, therefore, to elicit Ca\(^{2+}\) entry into HMs through partial activation of a T-type Ca\(^{2+}\) channel. Additionally, as NMDA receptors play a significant role in mediating excitatory synaptic transmission to neonate HMs at membrane potentials as hyperpolarized as \( -50 \text{ mV} \) (Singer et al. 1996) it is possible that glycine-induced depolarization partially relieves the Mg\(^{2+}\)-block of NMDA receptors and permits glycineergic and glutamatergic inputs to exert synergistic excitatory effects on these motoneurons.

We suggest that the relatively slow GlyR channel kinetics and resultant IPSC waveform observed in neonate motoneurons allow long glycine-induced PSPs to activate T-type Ca\(^{2+}\) channels and/or facilitate activation of NMDA receptors, thereby encouraging activity-dependent strengthening of developing inhibitory synapses. Later in postnatal life, as neural circuits mature, glycine-induced PSPs become faster and hyperpolarizing to allow for precise coordination of complex motor output patterns.

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