INTRODUCTION

Since the classic work of Liley and North (1953) at the neuromuscular junction (NMJ), the responses of synapses to repetitive stimulation has been studied intensively because they provide clues to the basic mechanisms that control transmitter release (Magleby 1987; Zucker 1989, 1999). There has been a great deal of recent work on short-term depression and facilitation of synaptic transmission in mammalian central synapses, particularly using in vitro techniques applied to junctions where the presynaptic elements are accessible for direct electrical and/or optical recordings. A wealth of data has become available from such studies that indicate the existence of multiple mechanisms that can both depress and enhance synaptic transmission (see reviews in MacDermott et al. 1999; Zucker 1989, 1999). These studies suggest ways in which transmission at less accessible synaptic systems can be examined.

Group Ia muscle spindle afferents make monosynaptic excitatory connections to alpha motoneurons in the spinal cord, providing a functionally defined synaptic system that has been used for decades as a model system to study synaptic transmission in the mammalian CNS (Burke and Rudomin 1977; Eccles 1964). Mears and Frank (1997) have shown that this system is present in the neonatal mouse spinal cord. Using the isolated spinal cord of neonatal rats in vitro, Lev-Tov and Pinco (Lev-Tov and Pinco 1992; Pinco and Lev-Tov 1993a) have shown that monosynaptic excitatory postsynaptic potentials (EPSPs) produced by dorsal root stimulation in spinal motoneurons exhibit profound synaptic depression at low frequencies. We have been interested in studying the maturation of this synaptic system with postnatal age in the mouse because of the growing importance of this animal for studies of neurological mutants.

The present paper provides data on monosynaptic EPSPs in the neonatal mouse spinal cord at 2–4 days of postnatal age (P2–P4) over a stimulus frequency range within which the monosynaptic EPSPs display relative facilitation as well as profound depression. We have examined the effects of changes in extracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_o$) and temperature on paired-pulse as well as steady-state depression, using short (10 pulse) trains at 0.125–8 Hz as well as EPSPs generated by irregular pulse patterns. These data have been compared with expectations from six empirical models of increasing complexity.

The model that best fit all of the observations suggests the co-existence of two separate mechanisms that enhance transmitter release, superimposed on depression due to depletion of two independent, presynaptic compartments. The models are described in some detail because they are likely to be useful in

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**References**


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codifying differences during postnatal development. Preliminary results have appeared in abstract form (Burke and Li 2000; Li and Burke 1999, 2000).

**METHODS**

**Preparation**

Experiments were performed on the isolated spinal cords of neonatal Swiss-Webster mice at 2–4 days postnatal (P2–P4). Animal care and use procedures were in accord with the “Principles of Laboratory Animal Care” (National Institute of Health Publication 86-23) and were approved by the National Institute of Neurological Disorders and Stroke Committee on Animal Care and Use.

Neonatal mouse pups were anesthetized by inhalation of methoxyflurane (Metofane) in a small chamber and then quickly decapitated, eviscerated, and transferred into a dissection chamber circulated with cold (4°C) artificial cerebrospinal fluid (ACSF) bubbled with 95% O2-5% CO2. The composition of the normal ACSF was (in mM) 128 NaCl, 4 KCl, 2 CaCl2, 1 MgSO4, 1 NaH2PO4, 25 NaHCO3, and 30 glucose, bubbled with 95% O2-5% CO2 (pH 7.3). After ventral laminectomy, the spinal cord with intact dorsal (DR) and ventral roots (VR) was dissected free from DR ganglia. The dura and pia mater were removed carefully, and the spinal cord was hemisected longitudinally from T6 to the sacral segments with a tungsten needle. One hemiscord was placed into a silicon elastomer (Sylgard)-based recording chamber that was continuously superfused with oxygenated ACSF flowing at 10–14 ml/min.

For testing the effects of low (0.8 mM) or high (4.0 mM) Ca2+ ACSF, only Ca2+ concentration was changed while Mg2+ remained constant at 1 mM. The temperature of bath solution was monitored by a thermistor and controlled by passing inflowing ACSF through a servo-controlled heater (TC-324B, Warner Instrument). The bathing solution was recirculated at all times except when drugs were added or washed out.

Dorsal and ventral rootlets of the L4 and L5 segments were drawn into the polyethylene suction electrodes for either stimulation or recording. Micropipettes for intracellular recording were made from 1.2-mm filament glass (WPI) drawn to produce DC electrode resistance between 40 and 90 MΩ (Sutter Instruments Model P-87). Micropipettes were filled with 2 M K-acetate and 100 mM QX-314 (Alamone Labs, Jerusalem, Israel) to suppress Na+ action potentials (Frazier et al. 1970). In some experiments, the electrode solution also contained 2% biocytin to label the recorded cells. Motoneurons were impaled from the lateral aspect of the hemisected cord and identified by antidromic invasion after VR stimulation. In some experiments, extracellular recordings in the ventral horn were obtained with broken-tip electrode filled with 2 M NaCl (DC resistance, 5–7 MΩ).

**Stimulation and recordings**

Mono- and polysynaptic EPSPs were produced by stimulating a DR filament with trains of 10 pulses (duration, 0.5 ms) at eight equally spaced frequencies from 16 Hz down to 0.125 Hz (intervals of 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, and 8.0 s). Trains were separated by 2-min intervals (Fig. 1), which were sufficiently long such that the first EPSPs in each train remained constant, indicating full recovery of evoked synaptic release (see Lev-Tov and Pinco 1992). Changing the ordering of train frequencies produced no changes in response patterns. Stimulus amplitudes were adjusted to be just supramaximal for monosynaptic responses that were temporally regular (e.g., Fig. 2C). The stability of DR-evoked reflexes and motoneuron population responses recorded from VRs served as indices of preparation viability. Most preparations remained stable for 8–12 h.

**Data acquisition and measurements**

Signals from suction electrodes on DRs and VRs were amplified with Cyberamp 380 amplifiers (Axon Instruments; band-pass 10–10 kHz). Intracellular potentials were amplified with an AXOCLAMP-2A (Axon Instruments) in current-clamp (bridge) mode. Intracellular signals were low-pass filtered at 10 kHz and digitized at 10 kHz (16-bit resolution) by a multichannel A/D converter (National Instruments NBI-16). Custom-designed software (LabVIEW programming language; National Instruments, Austin, TX) was used to acquire and save the data in a Power Macintosh computer. Intracellular potentials and responses in VRs were also continuously recorded on a digital videotape record (VR-100 B, Instrutech, Great Neck, NY). Data analysis was done off-line using commercial software packages.

Electrotonic potentials generated by DR afferent volleys and recorded by a suction electrode on a DR filament immediately adjacent to the stimulated rootlet were monitored to ensure that the afferent volleys produced by each stimulus pulse in the train was constant (Fig. 3C, top). The amplitudes of monosynaptic EPSPs were measured before the inflections that signaled the onset of polysynaptic evoked PSPs (Fig. 3A, middle, arrows). Reflex responses in the VR (Fig. 3A, bottom) were rectified and integrated for analysis. These usually exhibited parallel changes with monosynaptic EPSPs but will not be considered further in this paper.

**Pharmacological substances**

Drugs were introduced into the ACSF bathing solution via a gravity-fed line (flow rate: 10–14 ml/min) to a recording chamber with total volume of 7.0 ml for minimum of 10 min before new tests were made, to allow equilibration. Drugs used were: (+)-2-amino-5-phosphonovaleric acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), cyclothiazide (CTZ) obtained from RBI (Natick, MA), bicuculline methiodide and 2-hydroxy-saclofen obtained from Sigma (St. Louis, MO), and EGTA-tetra(2-amino-5-phosphonovaleric acid) (EGTA-AM) and BAPTA-tetra(acetoxyethyl ester) (BAPTA-AM) from Molecular Probes (Eugene, OR). AP5 and CNQX disodium were prepared as stock solutions of 30 and 10 mM, respectively, in distilled water, stored at 4°C. Bicuculline and 2-hydroxy-saclofen were freshly made in distilled water. CTZ was prepared as a 60 mM stock solution in methanol and frozen until use. EGTA-AM and BAPTA-AM were prepared as 100 and 10 mM stock solutions, respectively, in dimethyl sulfoxide. Solvent concentrations were <0.1% in the final ACSF.

**Computer modeling**

A variety of assumptions that might “explain” various aspects of the experimental observations formed the basis for equations that were embodied in spreadsheet programs (Microsoft Excel) that permitted comparisons between calculated and actual responses. The behavior of the response profiles with different parameter combinations were heuristically useful, but as the number of free parameters increased, it became impossible to fit the data by hand. Therefore a computer program was written in PASCAL (CodeWarrior, Metrowerks; Macintosh G3 computer) to determine the set of parameters that minimized the error between the calculated responses and experimental depression curves (e.g., Fig. 8). The organization of the
program is discussed in the APPENDIX. Despite the large number of iterations, a complete parameter search with $10^5$ iterations took about 10 s on a G3 Macintosh computer. The source code is available on request.

RESULTS

This description is based on observations in a total of 87 motoneurons recorded in the isolated, hemisected spinal cord of neonatal Swiss-Webster mice at postnatal ages of 2–4 days (P2–P4). Cells accepted for analysis had stable membrane potentials less than $-250$ mV for the 30 min period required for data collection (see METHODS). Some cells had stable membrane potentials for 2 h.

Suppression of background activity

When recorded in normal ACSF at room temperature (23–24°C), the isolated spinal cord of P2–P4 mice usually exhibited considerable spontaneous background activity with paroxysmal depolarizations in motoneuron membrane potential and associated VR discharges. DR stimulation was set at strengths slightly larger than required to produce the largest intracellularly recorded EPSPs (e.g., Figs. 4A and 5A). These stimuli produced additional long-lasting depolarizations superimposed on the falling phase of monosynaptic EPSPs and often outlasted the stimulus trains (Fig. 1A). Preliminary experiments showed that addition of mephenesin (1 mM) (see Lev-Tov and Pinco 1992) largely blocked this activity but also reduced monosynaptic EPSP amplitudes (see also Seebach and Mendell 1996; Ziskind-Conhaim 1990). However, the addition of the N-methyl-D-aspartate (NMDA) receptor blocker AP5 (100 μM) to the ACSF eliminated the both spontaneous activity and long-lasting stimulus-evoked polysynaptic depolarizations in almost all preparations (Fig. 1B) (see also Pinco and Lev-Tov 1993a). Accordingly, all of the synaptic depression data discussed in the following text were obtained in the presence of 100 μM AP5. The peak amplitudes of the monosynaptic components were reduced by an average of about 9% when studied in the same motoneurons before and after addition of AP5, irrespective of their amplitudes (Fig. 2). An additional advantage of using AP5 is that it removes a potential source of nonlinear amplification that could distort the peak amplitudes of large DR EPSPs.
Removing Ca\textsuperscript{2+} from the ACSF blocked the synaptic field potentials, revealing that the terminal potentials were superimposable, demonstrating that fiber activation was reliable at both stimulus frequencies used (see also Kudo and Yamada 1985; Seebach and Mendell 1996). On the other hand, the terminal potentials in the ventral horn were different at the two frequencies, apparently because they were distorted by the onset of extracellular synaptic potentials at \textsim 4 ms after the stimulus onset (Fig. 3, A and C, VH) (see also Lev-Tov and Pinco 1992). Removing Ca\textsuperscript{2+} from the ACSF blocked the synaptic field potentials, revealing that the terminal potentials were in fact the same at 0.125 and 1 Hz (Fig. 3B). The records in Fig. 3C show the traces in Fig. 3A on an expanded time base to emphasize that the VR record contains an electrotonically conducted representation of the earliest EPSP in the motoneurons (arrow) coincident with the terminal potential distortion (Fig. 3A).

The latency changes in DR volleys and EPSP onsets in the same motoneurons at room temperature and after raising bath temperature to 32°C are shown in Fig. 4. In the example shown (Fig. 4A), increased temperature shortened the delay of first reversal for the DR volley by \textsim 0.6 ms but produced even more dramatic shortening of the delay between the afferent volleys and EPSP onsets (from 3.2 to 2.0 ms). The population data for EPSP latencies measured from DR stimulus onset are given in the histogram in Fig. 4B. All of these observations support the conclusion that the earliest DR EPSPs are in fact monosynaptic (Mears and Frank 1997).

Short-duration, low-frequency synaptic depression

The records in Fig. 5 illustrate an example of the EPSP depression found in this work. Figure 5, A–D, shows single sweep intracellular records of the first (R1), second (R2), and the average of the final three EPSPs in the sequence of 10 (“tail” response) during 10 pulse trains at 8 (A and B), 2 (C), and 0.125 Hz (D). The R2 response data represent paired-pulse depression, and the later tail responses provide an estimate of the steady-state depression at a constant input frequency. Figure 5A, inset, shows that the incoming afferent volleys recorded from an adjacent DR were unchanged during the 8-Hz train; DR volleys in the other trains were essentially identical (not shown). Figure 5B shows that the latency of R2 and tail responses were, despite their appearance in A, the same as R1 after adjusting them to match R1. The EPSP amplitudes were measured at the time denoted by the vertical dashed line, prior to the inflections that signal the onset of polysynaptic components (Mears and Frank 1997; Pinco and Lev-Tov 1993b; Ziskind-Conhaim 1990). Despite the presence of some polysynaptic components, the potential returned to the baseline between stimuli in the presence of AP5.

Figure 5E illustrates the complete curves of EPSP amplitudes for the 10 pulses in each frequency trial, normalized by the amplitude of R1 in each train. Randomizing the order to frequency presentation did not affect the depression curves. The tail average represented a stable plateau of depression for each frequency (see also Pinco and Lev-Tov 1992; von Gersdorff et al. 1998), while the amplitudes of R2 and R3 captured the shapes of the initial depression curves. A semi-logarithmic plot of the three normalized response values against stimulus frequency (Fig. 5F) provided a convenient summary of the curves for different stimulation frequencies. The curve for the tail responses exhibited a relatively smooth decline with increasing frequency while those for the R2 and R3 were more irregular, presumably because they were from single measurements.

The three EPSP depression curves for individual motoneurons for P2–P4 mice exhibited relatively narrow ranges of variation, consistent with the relatively narrow ranges in postsynaptic motoneuron properties found in this age range in the rat (Seebach and Mendell 1996). Therefore we averaged the normalized EPSP data for 29 cells collected under the baseline conditions ([Ca\textsuperscript{2+}])\textsubscript{o} = 2.0 mM; temperature 23 to 24°C). These averaged curves in Fig. 6 show that R2, R3, and tail responses all declined similarly as stimulation frequency increased but R2 diverged upward at frequencies >1 Hz. In all cases, the afferent volleys were constant across frequencies (not shown; see Fig. 3), indicating that changes in the numbers of afferents stimulated could not account for these observations. Data from trials at 16 Hz (0.0625-s intervals; see METHODS) were sometimes contaminated by residual polysynaptic PSPs evoked by successive stimuli and were therefore not included in the following analysis.

Effect of altered [Ca\textsuperscript{2+}]\textsubscript{o}

We examined the effect of changing the Ca\textsuperscript{2+} concentration in the bathing solution, [Ca\textsuperscript{2+}]\textsubscript{o}, on the amplitude of monosynaptic DR EPSPs and on the depression curves. Decreasing [Ca\textsuperscript{2+}]\textsubscript{o} from the normal of 2.0 to 0.8 mM without changing...
The onset of the distorting VH synaptic field (right dashed line; rats (Seebach and Mendell 1996). Comparable in magnitude with observations in P1–P3 neonatal rats (n = 8) from the average of 12.7 ± 5.3 mV (n = 29) at 2.0 mM. When compared with the EPSPs at normal Ca\textsuperscript{2+}\textsubscript{o} in the same motoneurons (n = 8), lowering [Ca\textsuperscript{2+}]\textsubscript{o} to 0.8 mM reduced their amplitude by ∼50%. In contrast, increasing [Ca\textsuperscript{2+}]\textsubscript{o} to 4.0 mM increased the average EPSP amplitude to 15.3 ± 6.3 mV (n = 8) or ∼19% in the individual cells examined. The averages were significantly different only for the comparison of low with normal and high [Ca\textsuperscript{2+}]\textsubscript{o}. [Ca\textsuperscript{2+}]\textsubscript{o} at 1 and 0.125 Hz. C: records as in A on faster time base to illustrate the timing of the first reversal of the incoming afferent volley (top, left dashed line) and the onset of the terminal potentials (middle, middle dashed line) in relation to the apparent onset of the distorting VH synaptic field (right dashed line; dotted record is from B at 0 Ca\textsuperscript{2+} and of the electrophysiologically conducted EPSP signal in the VR (bottom; high gain) during 1 Hz (thin traces) and 0.125 Hz (thick gray traces) stimulus trains. The apparent synaptic delay from both estimates was ∼1 ms.

Effect of increased temperature

Increasing bath temperature from 24 to 32°C produced no significant change the average amplitude of monosynaptic EPSPs (average at 32°C =10.7 ± 2.6 mV, n = 12; P > 0.1, 2-tailed t-test). It proved to be difficult to retain acceptable intracellular penetrations in the same cell as the bath temperature was increased but when this was accomplished, the EPSPs were on average 8% smaller compared with EPSPs at 24°C (n = 5). However, the average depression curves at 32°C were quite different from those at 24°C (Fig. 7, C and D), and this was also true when comparing the curves in the few individual neurons that were satisfactory at both temperatures. There was less depression of R2 responses than at 24°C for frequencies <0.2 Hz but at higher frequencies the curves

\[\text{Effect of increased temperature}\]
converged. In particular, the region in which the R2 response began to show relative facilitation was shifted toward higher frequencies than at 24°C. In contrast, the tail responses showed less depression at all frequencies. All of these curve differences were significant by two-way ANOVA ($P < 0.01$).

**Effect of GABAergic synaptic blockade**

Because DR stimulation was used to elicit monosynaptic EPSPs, it is possible that activation of presynaptic inhibitory pathways could affect the observed synaptic depression curves. In the neonatal mouse cord, we found that addition of even small concentrations of the GABAA receptor blocker bicuculline (1–2 μM) to the ACSF with 100 μM AP-5 produced marked increases in spontaneous polysynaptic activity that prevented data collection (Fig. 1A) (see also Seebach and Mendell 1996). However, although we had elected not to use mephenesin for most of these experiments (see preceding text), ACSF containing AP-5 (100 μM) and mephenesin (1 mM) blocked paroxysmal discharges and allowed addition of bicuculline (10–20 μM) to the bath. Bicuculline under these conditions did not alter the depression curves ($n = 5$ cells in 2 animals; not shown). Furthermore, the addition of the GABA$_B$ blocker 2-hydroxyaclofen (100 μM) also produced no changes in depression curves ($n = 6$ cells in 3 animals). We conclude that GABA-related presynaptic inhibition played little or no role in producing the observed EPSP depression curves.

Attempts to use CTZ to test whether AMPA glutamate receptor (GluR) desensitization (Raman and Trussell 1995) might be involved in the present observations were inconclusive because bath application of conventional doses (50–100 μM) produced marked paroxysmal background activity even in the presence of 100 μM AP5. A maximum dose of 15 μM CTZ produced no changes in the depression curves in three cells tested.

**Effect of EPSP amplitude on depression curves**

There was a fivefold range in the amplitude of initial EPSPs (i.e., R1) in different motoneurons in all of the conditions reported above. However, a two-way ANOVA showed that there was no relation between the shape or magnitude of the depression curves in relation to R1 amplitudes. As expected from earlier work (Jahr and Yoshioka 1986; Pinco and Lev-Tov 1993b), the DR EPSPs in P2–P4 mice were quite sensitive to blockade by the AMPA-receptor blocker, CNQX. We added graded doses of CNQX (1–8 μM) to the ACSF to reduce the amplitude of DR EPSPs and found that the depression curves in individual neurons were essentially unchanged when R1 amplitudes were varied over a four- to fivefold range ($n = 8$ cells; not shown). This suggests that voltage-sensitive postsynaptic factors are probably not important in producing the observed synaptic depression curves.
Modeling short-term depression

The systematic changes in synaptic depression with stimulation frequency are difficult to interpret without a consideration of possible underlying mechanisms. For example, the relative facilitation of R2 and R3 EPSPs observed at higher frequencies (Fig. 6) cannot be explained by a simple depletion model (Liley and North 1953; see also Magleby 1987) but requires at least one superimposed process that enhances transmitter output (e.g., Dittman and Regehr 1996; Dittman et al. 2000; Varela et al. 1997; Weis et al. 1999).

We assumed that composite monosynaptic EPSPs in the neonatal mouse motoneurons are produced by multiple group Ia synaptic contacts with wide postsynaptic dispersion, as in the adult cat (e.g., Burke and Glenn 1996). We treated the observed composite EPSPs as if produced by a lumped “virtual synapse” that is reliably activated by each afferent impulse. The latter assumption seems reasonable in view of the constancy of terminal field potentials recorded in the VH (Fig. 3).

Because of the relative inaccessibility of this synaptic system, we explored a series of six phenomenological models of increasing complexity. The equations that define the system, Eqs. A1–A9, are given in the APPENDIX. These recurrence relations were used to define the state of the system at selected time points, $\Delta t$, to calculate the normalized amplitude of each response in pulse trains at different frequencies. Three models use a single compartment, called $N$, representing a pool (perhaps readily releasable synaptic vesicles) that is depleted by transmitter release, while the other three models used two depleting compartments, $N$ and $S$ (where $S$ is perhaps a pool of activation-ready synaptic release sites).

After activation, the $N$ and $S$ pools are refilled by exponential processes with time constants $\tau_{N}$ and $\tau_{S}$, respectively. One- or two-compartment models had no additional processes (simple depletion); a process $P$ that modulates the proportion of release-ready elements that release transmitter; or process $P$ plus a second process $M$ that alters the rate at which pool $N$ is refilled. At each activation process $P$ receives a constant increment, $\Delta P$, that, when positive, increases transmitter release.

FIG. 5. An example of short-term synaptic depression of monosynaptic EPSPs during 10 pulse stimulus trains at different frequencies (P3 mouse; normal ACSF at 24°C). A: single sweep records of the 1st (R1), 2nd (R2), and tail (average of the 8th, 9th, and 10th) EPSPs with 8-Hz stimulation (125-ms intervals). Inset: 10 superimposed records of adjacent DR potential, all essentially identical. B: monosynaptic components of R1, R2, and tail responses from A normalized to the same peak amplitudes to show that all had the same latency. C: R1, R2, and tail responses as in A but with 2-Hz stimulus train. Note that R2 was smaller than R2 at 8 Hz (→). D: as in C but with 0.125-Hz stimulus train. Time base for A–D was measured from the start of the DR stimulus pulse. E: complete depression curves at different stimulation intervals, with EPSP amplitudes (measured at - - - - in A, C, and D) normalized by that of R1 in each train. Note that R2 was larger in the 8 (●) and 4-Hz (○) trains than in the 2-Hz train (□). F: normalized amplitudes of R2, R3, and tail responses in this cell, plotted against logarithm of stimulus frequency.
Averaged R2, R3 and Tail Responses

P2-4, 2 mM Ca²⁺, 24°C

N = 29 Motoneurons

Model assessment: baseline data

A parameter search program was developed that minimized the error between simulated and experimental responses for each of the six models under test (see the 2nd section in the APPENDIX). The relative amplitudes of simulated second (R2), third (R3), and average plateau (tail) EPSPs were compared with the averaged, normalized responses obtained from 29 motoneurons and recorded at 24°C with [Ca²⁺]₀ = 2.0 mM (Fig. 6). The inclusion of R3 in the fitting procedures proved to be essential to produce simulated full depression curves that matched observed records (i.e., Fig. 5E).

**FIG. 6.** Semilogarithmic plot as in Fig. 5C of R2, R3, and tail responses averaged for 29 motoneurons in P2–P4 mice obtained in normal ACSF at 24°C. Error bars denote SDs for R2 (upward) and tail curves (downward). The averaged R2 EPSPs were more variable than the tail responses, which already were averages of three EPSPs. SDs for R3 are not shown for clarity but were similar to those for R2.

**FIG. 7.** Effect of changing [Ca²⁺]₀ (A and B; both at 24°C) or temperature (C and D; both at [Ca²⁺]₀ = 2.0 mM) on R2 and tail depression curves. Error bars denote SDs. A: lowering [Ca²⁺]₀ (dashed line) reduced depression of R2 and raising [Ca²⁺]₀ (thicker shaded line) slightly increased depression at all frequencies. B: [Ca²⁺]₀ effects on tail responses were in the same directions but much smaller. C: increasing bath temperature with [Ca²⁺]₀ = 2.0 mM resulted in markedly less depression of R2 responses at frequencies <0.2 Hz but increased depression at 4 and 8 Hz, producing a rightward shift in the frequency at which upward inflection of the depression curve occurred. D: increased temperature decreased synaptic depression of tail responses at all stimulation frequencies.
When matched against the baseline data set, the best fitting simple depletion model with one compartment (i.e., $R_i = N_i \cdot f$; 2 free parameters: $\tau_N$ and $f$; see Eq. A6 in the APPENDIX) produced a large RMS error (6.5%; Table 1). The two-compartment simple depletion model ($R_i = N_i \cdot S_i \cdot f$; 3 free parameters: $\tau_N$, $\tau_S$, and $f$) produced a smaller RMS error (4.8%; Table 1). Neither model reproduced the observed relative facilitation of R2 and R3 at higher frequencies (Fig. 6), which requires a process to enhance transmitter output at relatively high frequencies.

We therefore assumed a process, $P$, in which each activation produces an increment, $\Delta P$, that enhances transmitter release and decays exponentially with time constant $\tau_p$ (Eq. A7). This effect is presumably due to residual effect of Ca$^{2+}$ entry (Dittman and Regehr 1996; Dittman et al. 2000; reviews in Magleby 1987; Zucker 1989), Data fit with one- and two-compartment models that included $P$ process (4 and 5 free parameters, respectively) exhibited the relative facilitation of R2 but were still not satisfactory (Table 1). Using double-exponential time courses for the decay of the $P$ process actually worsened these fits.

It appeared that a process qualitatively different from release facilitation was needed. We therefore added a second process, $M$, that modulates the rate of restoration of the $N$ compartment ($\tau_N$) because of evidence that repetitive synaptic activation can speed the reformation of releasable transmitter (Stevens and Wessling 1998; Wang and Kaczmarek 1998; Weis et al. 1999). Each activation generates an increment, $\Delta M$, that decays exponentially with time (time constant $\tau_M$; Eqs. A8 and A9). One- and two-compartment models that included both $P$ and $M$ processes (6 and 7 free parameters, respectively) markedly improved the fit, especially with the two-compartment model (Fig. 8, Table 1). The parameter extraction program continued to exhibit robust convergence despite the additional free parameters. It should be noted that $\tau_S$ is not modified by the $M$ process, which enables the program to distinguish its effects on simulated EPSPs from those produced by $\tau_N$. The two-compartment model including both $P$ and $M$ processes, referred to below as the full model, was taken as the best fit to the baseline experimental observations. This model includes elements similar to those in other recent formulations (Dittman and Regehr 1996; Dittman et al. 2000; reviews in Magleby 1987; Zucker 1989, 1999). Data fit with one- and two-compartment models that included both $P$ and $M$ processes in [Ca$^{2+}$]o, Table 2 gives the results when the parameter extraction program was run with each [Ca$^{2+}$]o data set under two conditions: with all parameters free to vary (“free” columns) and with all time constants fixed to their values found for [Ca$^{2+}$]o = 2.0 mM (“constr.” columns). The last column in Table 2 gives the relative reliability of the parameter values extracted by the program with noisy input data (see APPENDIX).

Effect of [Ca$^{2+}$]o on model parameters

Because it is likely that the fractional release, $f$, and the increments $\Delta P$ and $\Delta M$ are dependent on Ca$^{2+}$ entry during synaptic activation (see DISCUSSION), we predicted that these parameters would exhibit the largest alterations with changes in [Ca$^{2+}$]o. Table 2 gives the results when the parameter extraction program was run with each [Ca$^{2+}$]o data set under two conditions: with all parameters free to vary (“free” columns) and with all time constants fixed to their values found for [Ca$^{2+}$]o = 2.0 mM (“constr.” columns). The last column in Table 2 gives the relative reliability of the parameter values extracted by the program with noisy input data (see APPENDIX).

The data from both low and high [Ca$^{2+}$]o conditions yielded parameter sets that differed from the baseline set (Table 2). However, except for $\Delta M$ and $\tau_M$ under the low [Ca$^{2+}$]o condition, which are least reliable in the presence of input noise (“sensitivity” column in Table 2; see APPENDIX), most of the time constants extracted from the two data sets changed little in comparison to the values for $f$, $\Delta P$, and $\Delta M$ extracted when all parameters were free to vary. When all four time constants were fixed to their baseline values (Table 2, “constr.”; Fig. 9),

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Best fits to excitatory postsynaptic potential (EPSP) data at 24°C and [Ca$^{2+}$]o = 2.0 mM.
the extracted values for $f$, $\Delta P$, and $\Delta M$ changed only slightly (with the exception of $\Delta M$ in the low $[Ca^{2+}]_o$ condition) and all were decreased in low $[Ca^{2+}]_o$ and increased in high $[Ca^{2+}]_o$ (Fig. 11A).

**Effect of temperature on model parameters**

We expected that increased bath temperature should mainly affect the time constants of the renewal processes, $\tau_N$ and $\tau_S$ and possibly those of the $P$ and $M$ processes ($\tau_P$ and $\tau_M$). When all parameters were free to find their best fit values, the system recovery time constants, $\tau_N$ and $\tau_S$, showed marked decreases as compared with the 24°C values, while $f$ was approximately the same, again except for the problematic $\tau_M$ (Table 3, “free”). We therefore examined the time constants that best fit the data when the values of $f$, $\Delta P$, and $\Delta M$ were fixed to the values found at 24°C (Fig. 11A).

![FIG. 9. Full model fits to average depression curves with low (A) and high $[Ca^{2+}]_o$ (B). Same format as in Fig. 8. A: fit to data for average data from 9 cells after equilibration with ACSF containing $[Ca^{2+}]_o = 0.8$ mM (Fig. 7, A and B), with all 4 time constants fixed to values obtained with $[Ca^{2+}]_o = 2.0$ mM (see APPENDIX). The fitting parameters are given in the inset (see also Table 2). B: fit as in A but with $[Ca^{2+}]_o = 4.0$ mM. Time constants fixed as in preceding text (see text for full discussion).](image)

### Table 3. Parameters of fits to data obtained at 32°C with $[Ca^{2+}]_o = 2.0$ mM

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<th>Parameter</th>
<th>Free</th>
<th>Constrained*</th>
<th>Baseline</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td>32.0</td>
<td>32.0</td>
<td>24.0</td>
<td></td>
</tr>
<tr>
<td>$\tau_N$, s</td>
<td>3.4</td>
<td>1.1</td>
<td>5.9</td>
<td>1.5</td>
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<tr>
<td>$\tau_S$, s</td>
<td>0.8</td>
<td>2.0</td>
<td>6.1</td>
<td>1.5</td>
</tr>
<tr>
<td>$f$</td>
<td>0.58 [0.55]</td>
<td>0.55</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>$\Delta P$</td>
<td>4.0 [2.1]</td>
<td>2.1</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>$\tau_P$, s</td>
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<td>0.14</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>$\Delta M$</td>
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<td>0.48</td>
<td>24.4</td>
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</tr>
<tr>
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<tr>
<td>RMS err, %</td>
<td>2.0</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* The values enclosed in square brackets in the constrained column are the parameters fixed to their values with temperature 24°C and $[Ca^{2+}]_o = 2.0$.

### Fitting EPSPs produced by pseudorandom interval sequences

There has been considerable recent interest in examining short-term synaptic modulation during random interval activation sequences (Abbott et al. 1997; Dittman and Regehr 1998; Dobrunz and Stevens 1999; Markram et al. 1998). As a final test of the present model, we recorded EPSP amplitudes during activation sequences in which 17 pseudorandom intervals (Fig. 12A) were repeated for six cycles. This approach allows averaging of EPSPs that follow identical stimulus intervals to improve signal to noise ratios. Such data sets were collected from five motoneurons in two P3 mice with $[Ca^{2+}]_o = 2.0$ mM and temperature 24°C. The first cycle of 17 EPSPs, during which the system stabilized, were discarded except for the initial EPSP, which was used to normalize the later responses. The normalized EPSPs in the subsequent five cycles were averaged for individual animals, and these averages were combined to give the normalized EPSPs shown in Fig. 12B (± SE).

The parameter extraction program was modified to accommodate the irregular stimulation data to assess whether the parameters of the full model could be extracted from this short sequence of responses. We were surprised to find that some runs of the extraction program generated a parameter set that produced an excellent fit to the input data (Fig. 12B, Table 4; Table 5).

![FIG. 10. Full model fit to averaged depression curves for the 32°C data as in Fig. 8, C and D, with $f$, $\Delta P$, and $\Delta M$ fixed to the values found at 24°C (see also Table 3).](image)
4), although a majority of runs led to several other local minima that produced parameter sets with larger RMS errors. Tests with artificial data sets suggested that irregular sequences of EPSPs contain less information than the R2, R3, and tail data but nevertheless have enough content to constrain the parameter extraction process (see APPENDIX).

DISCUSSION

A great deal of recent work on these mechanisms in CNS synapses has been done using in vitro slice preparations that offer particular structural advantages, such as the calyx of Held (e.g., Bellingham et al. 1998; Borst and Sakmann 1998; Forsythe et al. 1998; Wu and Borst 1999), the parallel fiber synapses on cerebellar Purkinje cells (e.g., Atluri and Regehr 1996; Dittman and Regehr 1998), and the climbing fiber synapses on cerebellar Purkinje cells (Silver et al. 1998). We chose to study the less accessible spinal cord system for several reasons. Monosynaptic contacts from group Ia muscle spindle afferents onto spinal motoneurons represent a functionally well-defined synaptic system that has been intensively studied in adult mammals (Burke and Rudomin 1977; Eccles 1964; Redman 1990). This system exhibits functional immaturity at birth in comparison to propriospinal excitatory systems that are formed earlier in embryonic life (Pinco and Lev-Tov 1994) but changes rapidly during the first 2 wk of postnatal life (Seebach and Mendell 1996), making it a potentially useful model of synaptic maturation. We hoped to be able to use information that has been developed in more accessible synaptic systems to understand this process. Finally, the mouse is rapidly becoming an important model system for neuroscience research because of the availability of neurological mutant animals. Baseline information about normal development of synapses and circuits in the mouse is essential to elucidate functional abnormalities in such animal models.

Initial DR EPSPs are monosynaptic

We assume that DR EPSPs are produced by group Ia muscle spindle afferents that project directly to motoneurons as in the adult cat (Burke and Glenn 1996; Burke and Rudomin 1977; Eccles 1964). DR afferents reach the ventral horn by day E17 in embryonic rats and mice, produce functional excitation of

### TABLE 4. Parameters of fit to data obtained at 24°C and $[Ca^{2+}]_o = 2.0$ mM during pseudo-random stimulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Irregular Firing</th>
<th>Baseline</th>
</tr>
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<tbody>
<tr>
<td>$\tau_N$, s</td>
<td>6.1</td>
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</tr>
<tr>
<td>$\tau_S$, s</td>
<td>3.3</td>
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</tr>
<tr>
<td>$f$</td>
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</tr>
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<td>$\Delta P$</td>
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<tr>
<td>$\tau_{PS}$, s</td>
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</tr>
<tr>
<td>$\Delta M$</td>
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</tr>
<tr>
<td>$\tau_{PS}$, s</td>
<td>2.0</td>
<td>2.3</td>
</tr>
<tr>
<td>RMS err, %</td>
<td>1.0</td>
<td>2.2</td>
</tr>
</tbody>
</table>
motoneurons shortly thereafter, and are well established by postnatal day two (Kudo and Yamada 1985, 1987; Mears and Frank 1997; Ziskind-Conhaim 1990). Much of the relatively long latency exhibited by the EPSPs produced by these afferents at room temperature appears to be due to slow conduction along intraspinal collaterals (Fig. 3C) (see also Lev-Tov and Pinco 1992). As measured from VH terminal potentials in the neonatal mouse, local synaptic delay is ≤1.0 ms (Fig. 3, A and B) (see also Lev-Tov and Pinco 1992). Moreover, increasing the bath temperature to 32°C produced marked decreases in these latencies, without changes in the initial EPSP rising phases (Fig. 4). We conclude that the initial DR EPSP in the mouse spinal cord are monosynaptic.

As expected from earlier studies (Jahr and Yoshioka 1986; Konnerth et al. 1990; Pinco and Lev-Tov 1993b; Ziskind-Conhaim 1990), the DR EPSPs are markedly sensitive to blockade by the AMPA-receptor blocker CNQX but also exhibit a relatively minor component that is reduced by the NMDA receptor blocker, AP5 (Fig. 2). Pinco and Lev-Tov (1993b) have presented evidence that both types of glutamate receptor are co-activated by release from the same monosynaptic synapses (see also Konnerth et al. 1990; von Gersdorff et al. 1997). Therefore we assume that interpretations of the present observations are unlikely to be distorted by the use of AP5 to reduce background activity.

**EPSP depression is not due to failure of afferent activation or presynaptic inhibition**

The constancy of afferent volleys entering the spinal cord (e.g., Fig. 3) confirmed the findings of others that the range of stimulation frequencies do not change the number of DR afferents activated (Kudo and Yamada 1985; Seebach and Mendell 1996). The extracellular VH terminal potentials recorded in the ventral horn at different stimulation frequencies are constant (Fig. 3B), suggesting that conduction in intraspinal collaterals is also secure at the stimulus frequencies used in this work (Lev-Tov and Pinco 1992). The relative facilitation of R2 and R3 responses at higher stimulus frequencies fits this conclusion because propagation failures would be expected to increase as frequency rises (Streit et al. 1992).

It is possible that DR stimulation can activate pathways that produce presynaptic inhibition, which could complicate interpretation of depression curves. Indeed, low-frequency depression of monosynaptic transmission in the adult rat has been attributed mainly to presynaptic inhibition (e.g., Seburn and Cope 1997). In the present work, addition of GABA_A receptor blocker bicuculline produced no changes in observed depression curves when mephenesin plus AP5 was used to suppress paroxysmal and stimulus-evoked polysynaptic activity. Similarly, addition of 2-hydroxysaclofen to depress GABAB receptors also produced depression curves that were essentially identical to control curves in the same motoneurons. We conclude that presynaptic inhibition, if it exists in the neonatal mouse cord, does not contribute to the present observations. It is unknown whether the recently described non-GABA-related PAD, attributed to increases in extracellular $[K^+]_o$ (Kremmer and Lev-Tov 1998), can modulate synaptic transmission.

Presynaptic metabotropic glutamate receptors can produce powerful modulation of synaptic transmission (reviewed in MacDermott et al. 1999). Preliminary experiments with group II/III metabotropic GluR (mGluR) agonists exhibited strong reductions of DR EPSPs and concomitant reduction in synaptic depression in the mouse cord (unpublished data). Although activation of presynaptic mGluRs appear to play only a minor role in short-term synaptic depression at the calyx of Held (von Gersdorff et al. 1999), further experiments are underway to examine this possibility in the neonatal mouse cord.

We were unable to evaluate the possibility that GluR desensitization plays a role in the depression observed in the mouse cord because CTZ, which is customarily used to block this effect, produced marked paroxysmal discharges in the mouse cord when doses larger than 15 μM were applied. Interpretation of CTZ effects were also complicated by recent evidence that CTZ can also affect presynaptic transmitter release mechanisms (Bellingham and Walmsley 1999). However the inter-stimulus intervals employed in this study were all considerably longer than the reported duration of GluR desensitization (Raman and Trussell 1995; Turecek and Trussell 2000), so it seems unlikely that this factor distorted the present results.

On the basis of the preceding observations, we have attributed the observed modulation of monosynaptic DR EPSP amplitudes during repetitive stimulation to presynaptic mechanisms involved in transmitter mobilization and release. We developed quantitative phenomenological models of the system because the complex effects of stimulation frequency, and changes in $[Ca^{2+}]_i$, and temperature, on DR EPSP amplitudes were difficult to understand intuitively.

**Nature of model elements**

In the adult cat, composite monosynaptic group Ia EPSPs are produced by multiple afferents, each having multiple synaptic boutons on an individual motoneuron (Burke and Glenn 1996; Redman and Walmsley 1983). The synaptic potentials produced by an individual group Ia afferent exhibit all-or-none quantal increments (Burke 1967; Kuno 1964; Redman 1990; Redman and Walmsley 1983), which may or may not represent saturating actions at individual boutons. For simplicity, the present empirical model treated the anatomically and functionally complex system as if it were a single synaptic entity. We assume that the $N$ compartment represents the net behavior of readily releasable transmitter vesicles while the $S$ compartment presumably reflects the net behavior of release sites in the synaptic boutons. This two-compartment system provided better fits to the experimental data than single-compartment models (Table 1) (see Varela et al. 1997).

There is no doubt that the entry of $Ca^{2+}$ into synaptic terminals is the major factor that initiates evoked transmitter release (Magleby 1987; Zucker 1989, 1999). The rapidly decaying $P$ process that produces overt facilitation of R2 and R3 only at higher frequencies is compatible with the notion of “residual calcium,” in which the effect of voltage-sensitive $Ca^{2+}$ entry decays over a finite time, producing paired-pulse facilitation (Dittman and Regehr 1998; Pinco and Lev-Tov 1993a; reviewed in Magleby 1987 and Zucker 1999). In the present results, the covert facilitation of R2 and R3 at higher stimulus frequencies was only evident in relation to the amplitudes of those responses at lower frequencies (Figs. 5–7). The estimated time constant for the short-term facilitatory $P$ process at 24°C ($\tau_P = 140$ ms; Fig. 8) is compatible with the rapid decay of paired-pulse facilitation in synapses from cer-
ebellar granule cells to Purkinje cells (100–200 ms) (Atluri and Regehr 1996; Dittman and Regehr 1998). Directly visualized free intra-terminal Ca\(^{2+}\) decays more rapidly than this paired-pulse facilitation, implying the operation of an indirect Ca\(^{2+}\)-activated mechanism rather than simply amplifying low-affinity triggered release (Atluri and Regehr 1996; Zucker 1999). Whatever Ca\(^{2+}\)-related molecular events result in residual Ca\(^{2+}\) facilitation, it seems reasonable to attribute the present P process to this mechanism.

We interpret the parameter \(f\) as the proportion of release-capable sites that do in fact liberate readily releasable transmitter rather than a probability associated with individual release sites. The distinction is important here because, with \(\Delta P = 2.1\) (Fig. 8), \(f \cdot (1 + P)\) more than triples as \(\Delta t\) approaches zero. Although \(f\) during synaptic activity need not be constant (Silver et al. 1998), a value \(>1\) would be meaningless for a probability. However, if we interpret \(S_i \cdot f \cdot (1 + P)\) as the proportion of release-ready sites at a given time, values \(>1\) could signify an increase in the total number of release-ready sites (i.e., \(S_{\text{max}}\), used to normalize \(S_i\)) above its original value (for example, see Wang and Zucker 1998). In other words, if \(f\) is assumed to be constant, a value of \(P_i > 1\) could indicate that the number of release-ready sites is larger than the original absolute \(S_{\text{max}}\) in the baseline state. Of course, the present model cannot differentiate between changes in \(f\) versus \(S_{\text{max}}\) and/or \(N_{\text{max}}\), any or all of which may be altered by repetitive activation (e.g., Schneggenburger et al. 1999; Wu and Borst 1999). One possible interpretation of a change in \(S_{\text{max}}\) is that repetitive activation may reveal the existence of synapses that do not release transmitter in the baseline state (i.e., “silent” synapses).

The relatively subtle effects added by the \(M\) process fit with the classical distinction between a rapidly decaying “facilitation” and a more slowly decaying “augmentation” during repetitive synaptic activation (Magleby 1987; Zucker 1989). There is evidence that repetitive activation increases the rate of renewal of readily-releasable transmitter in some synaptic systems (Stevens and Wesseling 1998; Wang and Kaczmarek 1998; Wang and Zucker 1998). This effect was implemented by modifying \(\tau_S\), which governs the rate of transmitter renewal by a saturating process that grows during stimulus trains and decays exponentially after each stimulus (Eqs. A4a, A8, and A9). It should be noted that the time constant for renewal of release sites \(\tau_S\) was assumed to be constant, which enabled the parameter extraction program to differentiate between \(\tau_N\) and \(\tau_S\), despite their analogous effects on the calculated responses (Eq. A6).

Effect of altered \([Ca^{2+}]_o\) on synaptic transmission

The relief of synaptic depression that occurs when \([Ca^{2+}]_o\) is lowered is usually attributed to reduction in transmitter depletion (Betz 1970; Lev-Tov and Pinco 1992). As found by Pinco and Lev-Tov (1993a), lowering \([Ca^{2+}]_o\) from 2.0 to 0.8 mM, without changing \([Mg^{2+}]_o\), reduced the average amplitude of R1 EPSPs by \(\sim 50\%\) and significantly reduced the paired-pulse depression of R2 (Fig. 7A). On the other hand, the depression of tail responses was little changed by either low or high \([Ca^{2+}]_o\) (Fig. 7B). This differs from the observations of Lev-Tov and Pinco (Lev-Tov and Pinco 1992; Pinco and Lev-Tov 1993a), who used older rat pups (P6–P10) and low \([Ca^{2+}]_o\) ACSF (0.75 mM) with high \(Mg^{2+}\) (6–7 mM) and mephenesin. Under these conditions, they found markedly decreased steady-state (i.e., tail) depression, accompanied by a much larger (about fourfold) decrease in absolute R1 amplitudes than was observed in our experiments. The composition of our normal bathing solution was virtually identical to theirs, except that we did not change the \(Mg^{2+}\) concentration when \([Ca^{2+}]_o\) was altered nor did we use mephenesin in these experiments. Such differences presumably account for the divergent observations.

The empirical model provided clues to the mechanisms that may underlie these complex changes. The R2 depression curves in the three \([Ca^{2+}]_o\) conditions (Fig. 7A) were offset in response amplitude but were similar in shape. This fits with model analysis (Fig. 11A), which suggested that changing \([Ca^{2+}]_o\) entry alters the release fraction \(f\) and the increments of the \(P\) and \(M\) processes (\(\Delta P\) and \(\Delta M\)), all of which affect EPSP amplitudes, rather than the system time constants (Table 2). Interactions between these factors in the initial EPSPs in each train produced fractional depletions of the \(N\) and \(S\) compartments that were roughly similar in all three conditions.

Effect of increased temperature

It has long been known that temperature markedly affects synaptic transmission as well as the intrinsic properties of neurons (Katz and Miledi 1965; Pierau et al. 1976). In the present work, increasing bath temperature from 24 to 32°C produced considerable decreases in central latencies of monosynaptic DR EPSPs (Fig. 4), presumably because of increased conduction velocities in afferent collaterals and perhaps also by shortening synaptic delay (Katz and Miledi 1965). Although the average amplitudes of DR-evoked composite EPSPs were about the same at 24 and 32°C, the R2 and tail depression curves showed less relative depression at the higher temperature except for R2 responses at frequencies \(>4\) Hz (Fig. 7, C and D).

In contrast to the results with altered \([Ca^{2+}]_o\), temperature increase changed the shape of the depression curves (Fig. 7, C and D), notably introducing a convergence in the R2 depression curves for higher frequencies at different temperatures (Fig. 7C). The model suggests that the major factor producing this convergence is the marked shortening of \(\tau_T\) from 140 to 80 ms (Table 3), which limits facilitation to interpulse intervals \(<0.250\) ms (4 Hz) at 32°C. This would be difficult to explain by changes in the postsynaptic motoneurons (e.g., see Hardingham and Larkman 1998). The reduction in \(\tau_T\) with increased temperature is compatible with the faster decay of paired-pulse facilitation and free \([Ca^{2+}]_o\) found by Atluri and Regehr (1996, their Fig. 3) in cerebellar granule cell synapses with a similar temperature increase. The more rapid restoration of the \(N\) and \(S\) pools, coupled with the apparent lack of change in \(f\) (Table 3), produced the smaller relative synaptic depression of all EPSPs.

Concluding comment

Although the participation of other factors cannot be ruled out, the relative success of the present model formulation suggests that short-term synaptic depression of monosynaptic (presumably group Ia) EPSPs in the P2–P4 mouse spinal cord results primarily from mechanisms operating within presynap-
tic terminals. The present results are consistent with the view that short-term synaptic depression results mainly from depletion of readily releasable transmitter and competent release sites, plus a release fraction that is relatively high (Zucker 1989, 1999; but cf. Markram et al. 1998). The results also suggest the existence of rapidly decaying facilitation and more slowly decaying augmentation processes with stimulus-evoked increments that depend on Ca\textsuperscript{2+} entry (Zucker 1999). Because of the complex interactions between these processes, these conclusions are not intuitively obvious from inspection of the data, but they are all compatible with findings in more accessible systems. The present results provide a framework for examination of the postnatal maturation of group Ia synaptic transmission in the neonatal mouse.

**APPENDIX**

**Model assumptions and components**

Although the models used in this work progressed from simple to more complex, it is more convenient to describe the full model used for parameter searches and then show how it can be reduced to test all six formulations tested.

1) The full model includes two independent pools of N_max and S_max release-ready elements with unknown absolute values, which are both required for transmitter liberation. The release-ready populations are expressed as fractional populations, so that N_i = N/N_max = 1 and S_i = S/S_max = 1 in the baseline state. The product N_i · S_i represents the fractional population that is capable of releasing transmitter at the ith activation.

2) A constant proportion, f, of release-ready N_i · S_i elements actually release transmitter at each ith activation. When the N_i and S_i elements release transmitter to give an EPSP, R_i, they are depleted, or become refractory (N^*_i and S^*_i). Thus the first response, R_1, in a train is simply

\[ R_1 = N_1 \cdot S_1 \cdot f \quad (A1) \]

3) Immediately after activation, the N_i and S_i fractional populations that actually released transmitter are temporarily inactivated, leaving N^*_i and S^*_i fractions still able to release transmitter

\[ N^*_i = N_i - R_i \quad (A2) \]
\[ S^*_i = S_i - R_i \quad (A3) \]

4) The N^*_i and S^*_i populations are restored toward N_max and S_max during the inter-pulse intervals, Delta_t, by independent, saturating, exponential processes

\[ N_{i+1} = N^*_i \cdot [(1 - N^*_i) \cdot [1 - \exp(-\Delta_t/\tau_{N,0})]] \quad (A4) \]

\[ S_{i+1} = S^*_i \cdot [(1 - S^*_i) \cdot [1 - \exp(-\Delta_t/\tau_{S,0})]] \quad (A5) \]

One-compartment models are simulated by setting S_i = 1 at all times.

5) The relative facilitation of R2 and R3 at higher frequencies (Fig. 6) is produced by a saturating process \( P \) in which each activation adds an increment \( \Delta P \) that changes transmitter release from competent N_i or N_i · S_i elements, so that the ith EPSP amplitude Eq. A1 becomes

\[ R_i = N_i \cdot S_i \cdot f \cdot (1 + P_i) \quad (A6) \]

where \( P_i = 0 \) and \( P_i \) decays exponentially from its value at the ith activation as:

\[ P_{i+1} = (P_i + \Delta P) \cdot \exp(-\Delta_t/\tau_P) \quad (A7) \]

This effect is removed from some models by setting \( \Delta P = 0 \).

6) Based on evidence that repetitive synaptic activation can increase the availability of readily-releasable transmitter during repetitive stimulation (see DISCUSSION). This effect is simulation by a saturating process, M, with increment \( \Delta M \) and exponential decay time constant \( \tau_{M,0} \) by which synaptic activation can change the initial time constant \( \tau_{N,0} \) in Eq. A4

\[ M_{i+1} = (M_i + \Delta M) \cdot \exp(-\Delta_t/\tau_M) \quad (A8) \]

which alters \( \tau_N \) as

\[ \tau_{N,i+1} = \tau_{N,0}/(1 + M_i) \quad (A9) \]

With positive values of \( \Delta M \), \( \tau_{N,i+1} \) decreases during repetitive activation, effectively increasing the renewal rate of \( N_i \), so that Eq. A4 becomes

\[ N_{i+1} = N^*_i \cdot [(1 - N^*_i) \cdot [1 - \exp(-\Delta_t/\tau_{N,i+1})]] \quad (A4a) \]

This effect is removed from some models by setting \( \Delta M = 0 \).

7) The amplitudes of all simulated responses in a given train were normalized by that of the first EPSP, R_1, as done with the observed data (Fig. 6).

**Model assessment**

The behavior of this system of equations was explored using a spreadsheet with graphics (Microsoft Excel). One-compartment simulations were obtained by setting \( S_i = 1.0 \) in Eq. A6. Setting \( \Delta P \) to zero in Eq. A7 removes the facilitating \( P \) process, while setting \( \Delta M \) to zero in Eq. A8 removes the augmenting \( M \) process. The parameters exhibited complex interactions in their effects of the shapes of the R2, R3, and tail depression curves. To extract the parameter set that best fit each elaboration of the model, Eqs. A1–A9 were embodied in a computer program written in PASCAL (CodeWarrior, Metrowerks; Macintosh G3 computer). The program used an iterative search algorithm to find the set of parameters that minimized the RMS error between simulated and experimental R2, R3, and tail responses at all frequencies tested (e.g., Fig. 8). The program began with randomly chosen values for the free parameters in the selected model type and used these to generate 10 responses, R1–R10, with \( \Delta \) values of 0.125, 0.25, 0.5, 1, 2, 4, and 8 s. The summed differences between all observed (\( V_{obs,i} \)) and simulated (\( V_{calc,i} \)) responses gave the root mean square (RMS) error for each test parameter set

\[ \text{RMS error} = \sqrt{\frac{\sum_{i=1}^{n} (V_{obs,i} - V_{calc,i})^2}{n}} \]

After each iteration, all of the parameters were randomly perturbed by a factor weighted by RMS error and the process was repeated until the new parameter set produced an RMS error less than the original. The new parameter set and its error value then became the standards for comparison, the weighting factor was adjusted for the new (decreased) error, and the process was iterated until the total error was <0.1% or there had been 10^5 iterations.

Despite the large number of iterations, a complete parameter search with 10^5 iterations took <10 s on a G3 Macintosh computer. The source code is available on request.

**Sensitivity tests**

Tests of the full model’s sensitivity to changes in each of the seven parameters were done in two ways. First, we generated R2, R3, and tail responses using the baseline parameter set (Fig. 8), but with the individual parameters perturbed up to ±20% from the baseline values, one at a time. The resulting RMS errors were slightly asymmetrical with positive versus negative perturbations but were almost linearly related to perturbation magnitude. Changing the release fraction, \( f \),
produced the largest total errors while equivalent changes in $\Delta M$ produced the smallest. For 20% perturbation, the relative total RMS errors varied in the following descending sequence: $f$, $\tau_{s}$, $\Delta P$, $\tau_{r}$, $\tau_{D}$, and $\Delta M$, with ratios of 13.2:4.5:2.4:2.0:1.8:1.0:1.0, respectively.

It was also important to determine the error in parameter that are extracted from test data created with a known parameter set. This was done by using the program repeatedly to extract parameters from the test data with different levels of added random noise (0, 5, 10, 20, or 30% random noise, with 10 trials on each set). Test data with $\pm 20$% added random noise converged to similar parameter sets. Convergence to more than one parameter set was sometimes encountered with 30% noise, although the majority of runs still converged to a set that exhibited similar minimum RMS error. Percent parameter error was defined as 1 – (test/target) · 100. Without added noise, the average error was $<1.0$% for $f$, $\tau_{s}$, $\Delta P$, and $\tau_{D}$, $\tau_{r}$, and 5 and 6.6% for $\tau_{s}$ and $\Delta M$, respectively. As expected, adding $\pm 20$% random noise increased the average error introduced with were larger for all extracted parameters, but still largest for $\tau_{s}$ and $\Delta M$.

The variability in each parameter during repeated extractions was assessed by the percentage coefficients of variation (CV = SD · 100/mean) of the extracted parameter errors during repeated trials with noisy input. The error and CV values were all dimensionless ratios. To encapsulate both average error and its variability in repeated trials, we took the average of the dimensionless error and CV ratios. To encapsulate both average error and its variability in repeated trials, we took the average of the dimensionless error and CV ratios.

Responses at the limits

The final model formulation includes one equation (Eq. A7) that introduced an unbounded term ($P$) into the calculation of the normalized responses (Eq. A4a), which produced the upward curvature of R2 depression at higher frequencies. Equations 2–7 can be compressed into a single expression for normalized R2

\[
R2 = \left[ 1 - f \exp \left( -\frac{\Delta t}{\tau_{s}} \right) \right] \cdot \left[ 1 - f \exp \left( -\frac{\Delta t}{\tau_{r}} \right) \right] \cdot \left[ 1 + \Delta P \exp \left( -\frac{\Delta t}{\tau_{D}} \right) \right]
\]

in which the first term represents changes in $S$, the second changes in $N$, and the third the influence of $P$ on both. As $\Delta t \rightarrow 0$, this equation reduces to

\[
R2 = (1 + \Delta P) \cdot (1 - f)^2
\]

For the parameters that fit the observed baseline data (Fig. 8), normalized R2 approaches a maximum of about 0.63 as $\Delta t$ approaches zero.

We also used the final model equations to develop a single closed-form equation to calculate the steady-state normalized response, $R_{c}$. The calculated $R_{c}$ values were within 1% of those of the model responses to the tenth pulse (i.e., R10) at frequencies $\leq 1$ Hz but, as expected, deviated at the higher frequencies because the system requires 10 pulses to reach equilibrium at those frequencies.

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