

# Whole Cell Recordings of Lumbar Motoneurons During Locomotor-Like Activity in the In Vitro Neonatal Rat Spinal Cord

S. HOCHMAN AND B. J. SCHMIDT

*Department of Physiology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada*

**Hochman, S. and B. J. Schmidt.** Whole cell recordings of lumbar motoneurons during locomotor-like activity in the in vitro neonatal rat spinal cord. *J. Neurophysiol.* 79: 743–752, 1998. Whole cell current- and voltage-clamp recordings were obtained from lumbar motoneurons in the isolated neonatal rat spinal cord to characterize the behavior of motoneurons during neurochemically induced locomotor-like activity. Bath application of serotonin (10–100  $\mu\text{M}$ ) in combination with *N*-methyl-D-aspartate (1–12  $\mu\text{M}$ ) initially produced tonic membrane depolarization (mean = 26 mV), increased input resistance, decreased rheobase, and increased spike inactivation in response to depolarizing current pulse injections. After the initial tonic depolarization, rhythmic fluctuations of the motoneuron membrane potential (locomotor drive potentials; LDPs) developed that were modulated phasically in association with ventral root discharge. The peak and trough voltage levels of the LDP fluctuated above and below the membrane potential recorded immediately before the onset of rhythmic activity. Similarly, firing frequency was modulated above and below prelocomotion firing rates (in those motoneurons that displayed neurochemically induced tonic firing immediately before the onset of rhythmic activity). These observations are consistent with an alternation between phasic excitatory and inhibitory synaptic drives. The amplitude of LDPs and rhythmic excitatory drive current increased with membrane depolarization from –80 to –40 mV and then decreased with further depolarization, thus displaying nonlinear voltage-dependence. Faster frequency, small amplitude voltage fluctuations were observed superimposed on the depolarized phase of LDPs. In some motoneurons, the trajectory of these superimposed fluctuations was consistent with a synaptic origin, whereas in other cells, the regular sinusoidal appearance of the fluctuations and the occurrence of superimposed plateau potentials were more compatible with the activation of an intrinsic membrane property. One motoneuron displayed exclusively excitatory phasic drive, and another motoneuron was characterized by inhibitory phasic drive alone, during rhythmic activity. These findings are compatible with the concept of a central pattern generator that is capable of delivering both excitatory and inhibitory drive to motoneurons during locomotion. The data also suggest that the rhythmic excitatory and inhibitory outputs of the hypothetical half-center model can be dissociated and operate in isolation.

## INTRODUCTION

The isolated in vitro neonatal rat spinal cord preparation has greatly facilitated the pharmacological investigation of neural networks subserving mammalian locomotor rhythm generation (Atsuta et al. 1990, 1991; Cazalets et al. 1992, 1994; Cowley and Schmidt 1994b, 1995, 1997; Kiehn et al. 1992, 1996; Kudo and Yamada 1987; Smith and Feldman 1987; Smith et al. 1988; Sqalli-Houssaini et al. 1993). However, much remains to be learned about the functional organization and intrinsic properties of the neurons comprising the

central pattern generator (CPG) for locomotion (for review, see Gossard and Hultborn 1991). Progress in this area has been hampered, in part, by the inherent difficulties of trying to identify the essential interneuronal components of the CPG using electrophysiological criteria.

One approach to delineating CPG organization, as used in the present study, involves examining the effects of the rhythmogenic network on the final common output, the motoneuron. Intracellular recordings of motoneurons during fictive locomotion in cats (Edgerton et al. 1976) have demonstrated rhythmic oscillations of membrane potential known as locomotor drive potentials (LDPs) (Jordan 1983). In cat motoneurons, LDPs coincide with rhythmic alternation of excitatory and inhibitory synaptic inputs (Edgerton et al. 1976; Jordan 1983; Orsal et al. 1986; Pratt and Jordan 1987; Shefchyk and Jordan 1985), compatible with a symmetric half-center organization for the locomotor CPG (for review, see Jordan 1983). In contrast, recordings of motoneurons in the embryonic chick spinal cord suggest a different organization, wherein flexor and extensor motoneurons receive simultaneous depolarizing drive potentials during each step cycle, and flexor motoneurons demonstrate a pause in firing during peak depolarization as a result of a depolarizing GABAergic shunt conductance (O'Donovan 1989; Sernagor and O'Donovan 1991; Sernagor et al. 1995).

To date, only a few studies have employed intracellular recording techniques to examine the behavior of neurons during rhythmic motor activity in the in vitro neonatal rat model. It has been shown that LDPs occur in both motoneurons (Cazalets et al. 1996; Schmidt et al. 1989, 1994; Smith et al. 1988) and interneurons (Kiehn et al. 1996; MacLean et al. 1995), the amplitude of motoneuronal LDPs is sensitive to *N*-methyl-D-aspartate (NMDA) and non-NMDA excitatory amino acid antagonists (Cazalets et al. 1996; Schmidt et al. 1989), spike afterhyperpolarization is modulated (Schmidt 1994) in a manner similar to that observed in cat motoneurons during locomotion (Brownstone et al. 1992), and strychnine-sensitive inhibitory postsynaptic potential (IPSPs) with reversal potentials near –60 mV occur during the hyperpolarized phase of the LDP (Cazalets et al. 1996).

Voltage-dependent membrane properties add nonlinear response characteristics (Kiehn 1991; Schwindt and Crill 1977), which also may be important determinants of cell behavior during rhythmic behaviors. Indeed, excitatory components of LDPs in cat motoneurons have been shown to behave in a voltage-dependent manner (Brownstone et al. 1994). Similarly, NMDA receptor-mediated nonlinear membrane behavior and inherent oscillatory activity have been

recorded in neonatal rat interneurons (Hochman et al. 1994a; Kiehn et al. 1996) and motoneurons (Hochman et al. 1994b). In contrast, however, a recent study reported no evidence for a nonlinear change in motoneuronal LDP amplitude as a function of holding potential (Cazalets et al. 1996).

The present study uses whole cell recording methods to investigate motoneuron behavior during neurochemically induced rhythmic activity in the *in vitro* neonatal rat spinal cord. The results describe voltage-sensitive features of LDPs as well as the underlying phasic excitatory and inhibitory synaptic drive generated by the CPG. A preliminary report of some of this work has appeared in abstract form (Schmidt et al. 1994).

## METHODS

One- to 5-day-old neonatal Sprague-Dawley rats were decapitated and eviscerated. The spinal column with hindlimbs attached then was placed in a bath dissection chamber containing cooled (4°C), oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 128 NaCl, 3.0 KCl, 0.5 NaH<sub>2</sub>PO<sub>4</sub>, 1.5 CaCl<sub>2</sub>, 1.0 MgSO<sub>4</sub>, 21 NaHCO<sub>3</sub>, and 30 glucose, equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub>, pH 7.4. The spinal cord then was isolated, bilaterally intact, from rostral cervical to caudal sacral segments. The ACSF was gradually warmed to room temperature. The cord was stabilized with insect pins, ventral surface up, to the silicone elastomer (Sylgard) bottom of the chamber.

Glass suction electrodes were used to monitor ipsilateral ventral roots L<sub>3</sub>-L<sub>5</sub>. A switch box allowed selected suction electrodes to be used for either stimulation or recording. Whole cell patch-clamp recordings of motoneurons were obtained (Hamill et al. 1981) using a "blind" approach (Blanton et al. 1989). Glass capillary tubes (WPI; 1.5 mm OD) were pulled in a two-stage process (Narishige PP83) to create patch pipettes with resistances measured in ACSF ranging from 3 to 7 MΩ. Recordings were made with pipettes containing (in mM) 140 K-gluconate, 11 ethylene glycol-bis(β-amino ethyl ether) N,N,N',N'-tetraacetic acid, 35 KOH, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, and 1 CaCl<sub>2</sub>. In two motoneurons equimolar CsF replaced K-gluconate. The data shown in Figs. 1-6 were obtained using pipettes containing K-gluconate. In many motoneurons, 5 mM QX-314 also was included in the pipette to block Na<sup>+</sup> spikes.

Voltage- and current-clamp recordings were obtained using an Axopatch 1D amplifier (Axon Instruments) filtered at 5 kHz (4-pole low-pass Bessel). Voltage-clamp recordings were uncompensated with respect to series resistance and capacitance. Series resistance was compensated after switching to current-clamp mode by adjusting the series resistance potentiometer such that make-and-break points of voltage transients in response to current steps were balanced (bridge balance). The mean value for series resistance in the present sample was 31 ± 18 (SD) MΩ (n = 36). Series resistance was monitored frequently and readjusted if changes occurred. Liquid junction potentials ranged from 8 to 12 mV and were subtracted using the Axopatch 1D correction circuitry. Data were collected on computer with the pCLAMP acquisition software (v 6.0; Axon Instruments) typically at 4 kHz. Records also were digitized at 2 kHz and stored using a Vetter pulse code modulator videocassette adapter. A continuous paper copy of the data also was produced by an Astromed (MT9500) oscillographic recorder.

Figure 1 presents the experimental paradigm. Motoneurons were identified antidromically via stimulation of ventral roots. The combination of serotonin (5-HT) and NMDA is particularly effective in producing locomotor-like patterns of activity in this preparation (Cowley and Schmidt 1994b; Kiehn and Kjaerulff 1996; Sqallihoussaini et al. 1993). Therefore, rhythmogenesis usually was

induced by the bath application of both 5-HT (10-100 μM) and NMDA (1.0-12 μM). Rhythmic activity was induced in two motoneurons using 5-HT alone and in one motoneuron using NMDA alone. For the purpose of this study, the presence of rhythmically alternating activity between any two ipsilateral ventral roots (e.g., Fig. 1) was considered suggestive of a locomotor-like pattern. However, the inherent limitations of ventral root recordings for the recognition of specific motor patterns (Cowley and Schmidt 1994a) are acknowledged.

## RESULTS

Thirty-six antidromically identified motoneurons were examined during locomotor-like activity. The mean resting membrane potential was -74 ± 10 mV (n = 28; 8 cells were recorded in voltage-clamp mode only, and, therefore, no resting membrane potential is available). Input resistance was measured from a 10-mV hyperpolarizing voltage step and gave a mean value of 72 ± 41 MΩ (n = 24).

### *Effects of 5-HT and NMDA application on motoneuron recruitment and firing properties*

Addition of 5-HT alone, or in combination with NMDA, to the neonatal rat spinal cord consistently produced membrane depolarization. Quantification of depolarization was based on those recordings obtained using QX-314 in the patch electrode to block action potentials. The level of depolarization was measured before the onset of rhythmic oscillatory activity (e.g., see intracellular trace in Fig. 3A). Thus in six motoneurons initially exposed to 5-HT (10 μM) alone, the mean depolarization was 25 ± 5 mV (n = 6). When NMDA (1.5-4 μM) was coapplied with 5-HT, a similar level of mean depolarization was observed (26 ± 6 mV, n = 5). Administration of 5-HT and NMDA also was associated with an increase in membrane resistance from 57 ± 35 MΩ to 120 ± 60 MΩ (P < 0.01 paired t-test) in 7/7 cells, as reflected by an increase in voltage response to current pulse injection (e.g., Fig. 2A). Hence, in the presence of 5-HT and NMDA, less intense depolarizing current was capable of recruiting motoneuronal firing (reduced rheobase) compared with control conditions (Fig. 2B). In addition, spike inactivation during repetitive cell firing was more pronounced in the presence of bath applied 5-HT and NMDA, as observed in response to both current steps (Fig. 2B) and ramp depolarizations (Fig. 2C).

### *Development of rhythmic locomotor-like motor activity and LDPs*

After reaching a plateau level of depolarization, up to 30 s or more elapsed before the initiation of rhythmic activity (Fig. 3A). LDPs and cell firing were related to ventral root discharge (e.g., Figs. 1 and 5). Cells recorded with electrodes containing QX-314 also developed LDPs related to ventral root activity but without cell firing (Fig. 3A). The LDP trajectory fluctuated above and below the membrane potential recorded during the plateau phase in 8/10 motoneurons, as shown in Fig. 3Ai. Similar examples are shown in two other motoneurons (Fig. 3A, ii and iii). In two motoneurons, both the depolarized and hyperpolarized phases of the LDP were depolarized relative to the plateau level estab-

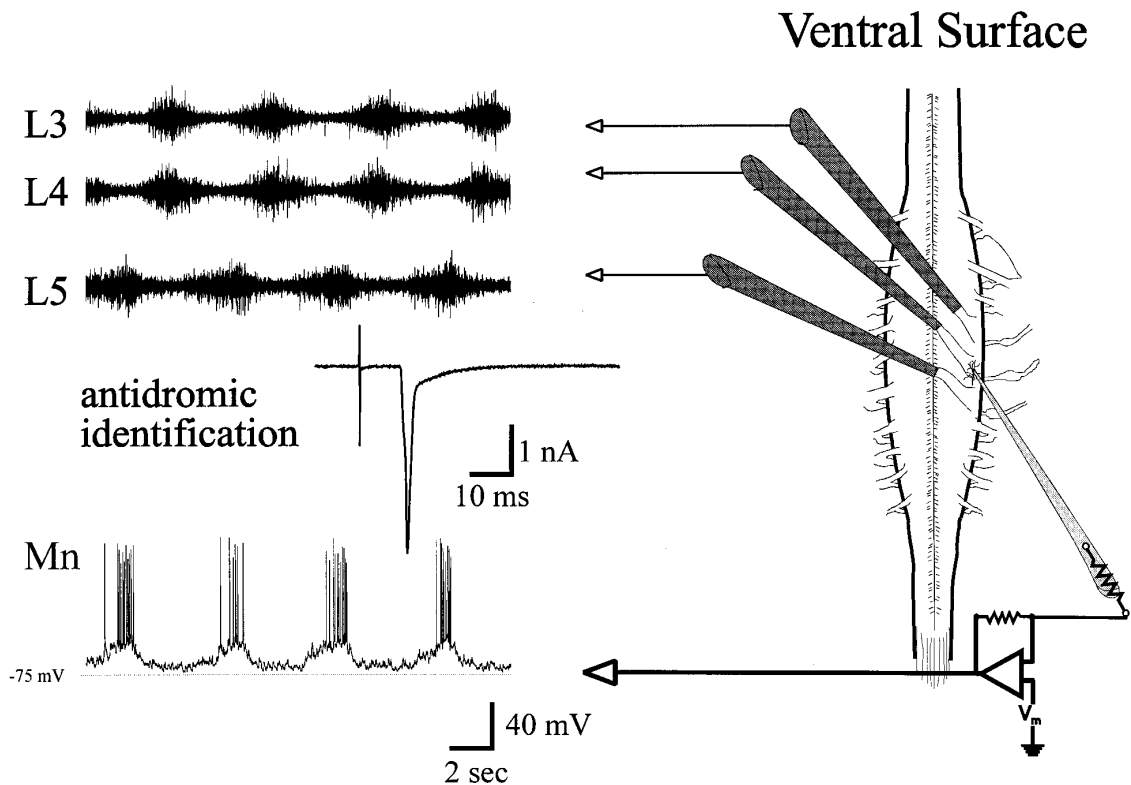


FIG. 1. Experimental arrangement. Spinal cord was isolated and stabilized ventral side facing upward. Suction electrodes were placed on lumbar ventral roots L<sub>3</sub>–L<sub>5</sub>. A customized box allowed switching between stimulation (for antidromic identification) and recording (for monitoring motor activity). Rhythmic activity was induced using serotonin (5-HT) 40  $\mu$ M and *N*-methyl-D-aspartate (NMDA) 2  $\mu$ M. Whole cell recordings were obtained from motoneurons (Mn) in voltage- (e.g., antidromic responses) or current-clamp mode (*bottom*). In this example, the resting potential of the cell before addition of 5-HT and NMDA, and in the absence of applied current, was  $-50$  mV. After establishing rhythmic activity, hyperpolarizing current (100 pA) was applied bringing the hyperpolarized phase of the LDP to approximately  $-73$  mV.

lished before the onset of rhythmic activity. The average LDP amplitude in the absence of injected current was  $10.3 \pm 4.0$  mV ( $n = 25$ ). The rectified and integrated ventral root recording shown in Fig. 3*Ai* demonstrates tonic firing of the motoneuron population before the onset of rhythmic activity. Similarly, in the absence of QX-314, bath application of 5-HT and NMDA, initially produced tonic cell firing as recorded from the patch electrode, which then was replaced by phasic modulation of the firing frequency related to the ventral root rhythm. The modulated frequency alternated between values that were greater and less than the tonic firing level recorded before the onset of rhythmic activity (Fig. 3*B*). These results suggest that the LDP in these motoneurons is characterized by alternating excitatory and inhibitory synaptic drive. However, the possibility that the LDPs may be related solely to modulation of excitatory drive cannot be absolutely excluded.

#### *Nonlinear voltage-sensitive properties of LDPs*

The amplitude of the LDP increased with depolarizing current injection (Fig. 4*Ai*). The drive potentials observed at two different membrane potentials are shown superimposed in Fig. 4*Aii*. During depolarizing current injection, there was also an increase in the amplitude of superimposed faster events (Fig. 4*Aii*), presumably synaptic in origin (see following text), in addition to the overall LDP amplitude

increase. The relationship of LDP size to membrane potential is shown for another motoneuron in Fig. 4*Bi*; in this example, averaged LDPs were normalized with respect to duration (Fig. 4*Bii*). The amplitude of the LDP was  $\sim 4$  mV at a holding potential of  $-90$  mV and increased to 8 and 12 mV at holding potentials of  $-70$  and  $-50$  mV, respectively (Fig. 4*Bii*). Again, membrane depolarization was associated with an increased amplitude of superimposed fast events (4*Biii*). Similar results were obtained in voltage-clamp mode, which demonstrated an increase in the amplitude of phasic drive currents (Fig. 4*Cii*) as well as superimposed fast events (Fig. 4*Ciii*, \*), at depolarized holding potentials.

Theoretically, the overall increase in LDP amplitude during tonic membrane depolarization, as noted in Fig. 4, *A* and *B*, could be related mainly to an increased driving force on inhibitory synaptic potentials during the hyperpolarized phase. However, analysis of the motoneuron shown in Fig. 5*A* suggests that the effects of holding potential on inhibitory drive are not likely the only explanation. This motoneuron displayed large amplitude LDPs (Fig. 5*Aii*). The presence of cell firing (no intrapipette QX-314) obscures LDP amplitude analysis in this cell. However, in voltage-clamp mode, phasic inward (excitatory) drive currents were noted to increase in amplitude at a holding potential of  $-40$  mV compared with  $-80$  mV and then decreased as the holding potential approached 0 mV (Fig. 5*Ai*). These observations suggest that at least part of the mechanism of LDP amplitude enhance-

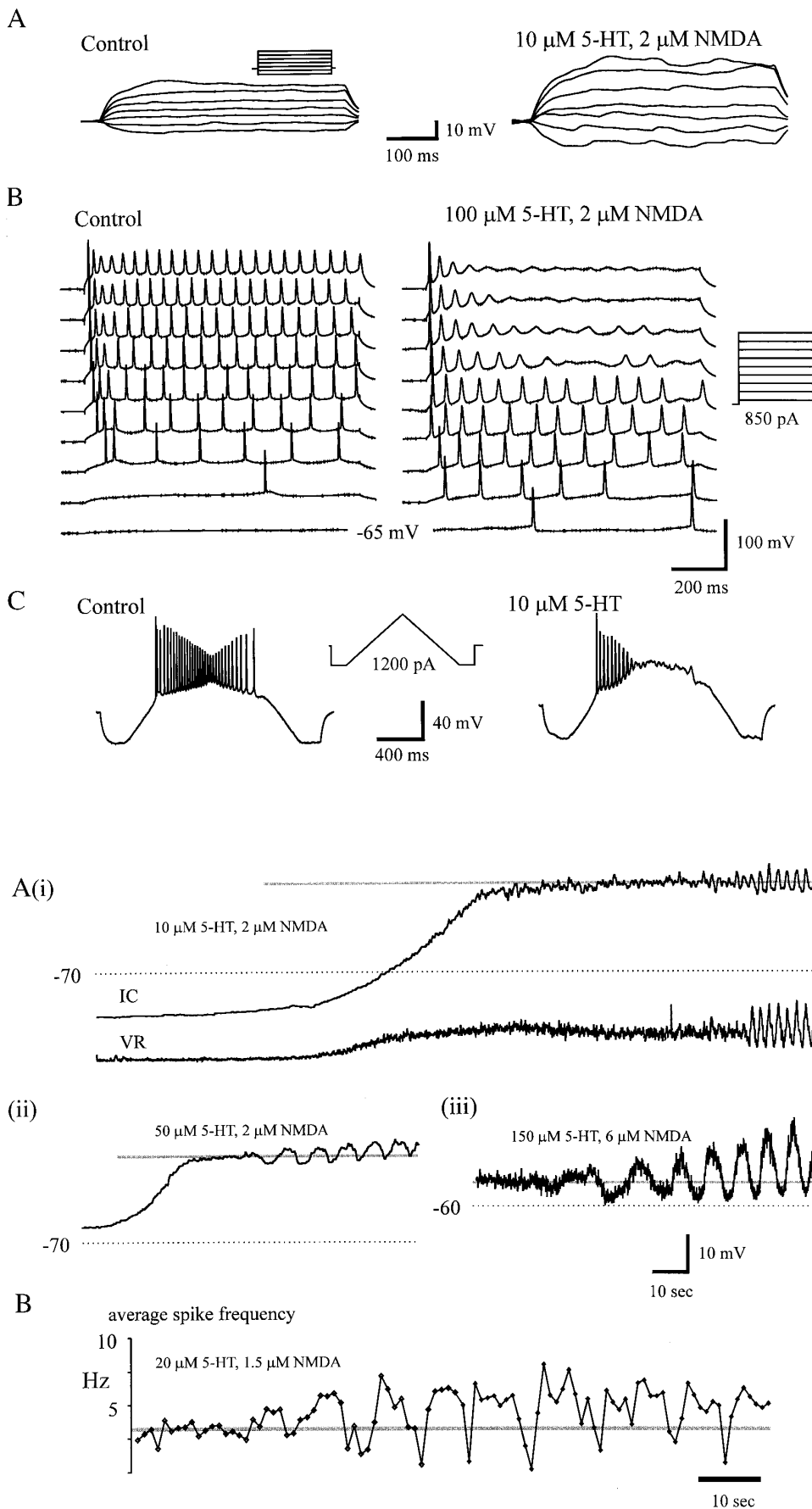


FIG. 2. Modification of motoneuron membrane properties by 5-HT and NMDA. *A*: voltage response to current steps in a motoneuron before and after the addition of 5-HT and NMDA, measured using a QX-314 filled electrode. *B*: repetitive firing of a motoneuron before and after bath application of 5-HT and NMDA. In the presence of applied neurochemicals, less depolarizing current injection was required to recruit action potentials and spike inactivation was more pronounced. *C*: repetitive firing induced by a current ramp also showed increased spike inactivation after bath application of 5-HT.

FIG. 3. Evidence of alternating excitatory and inhibitory synaptic drive in the production of LDPs. *A*: three nonspiking motoneurons (recorded with QX-314-filled electrodes): *i*: intracellular record (IC) shows tonic depolarization in response to NMDA and 5-HT followed by voltage fluctuation (0.35 Hz) above and below the depolarized baseline. *ii* and *iii*: voltage fluctuation above and below the depolarized baseline in 2 other motoneurons. *B*: in another motoneuron, firing frequency alternates above and below the frequency recorded immediately before the onset of rhythmic activity.

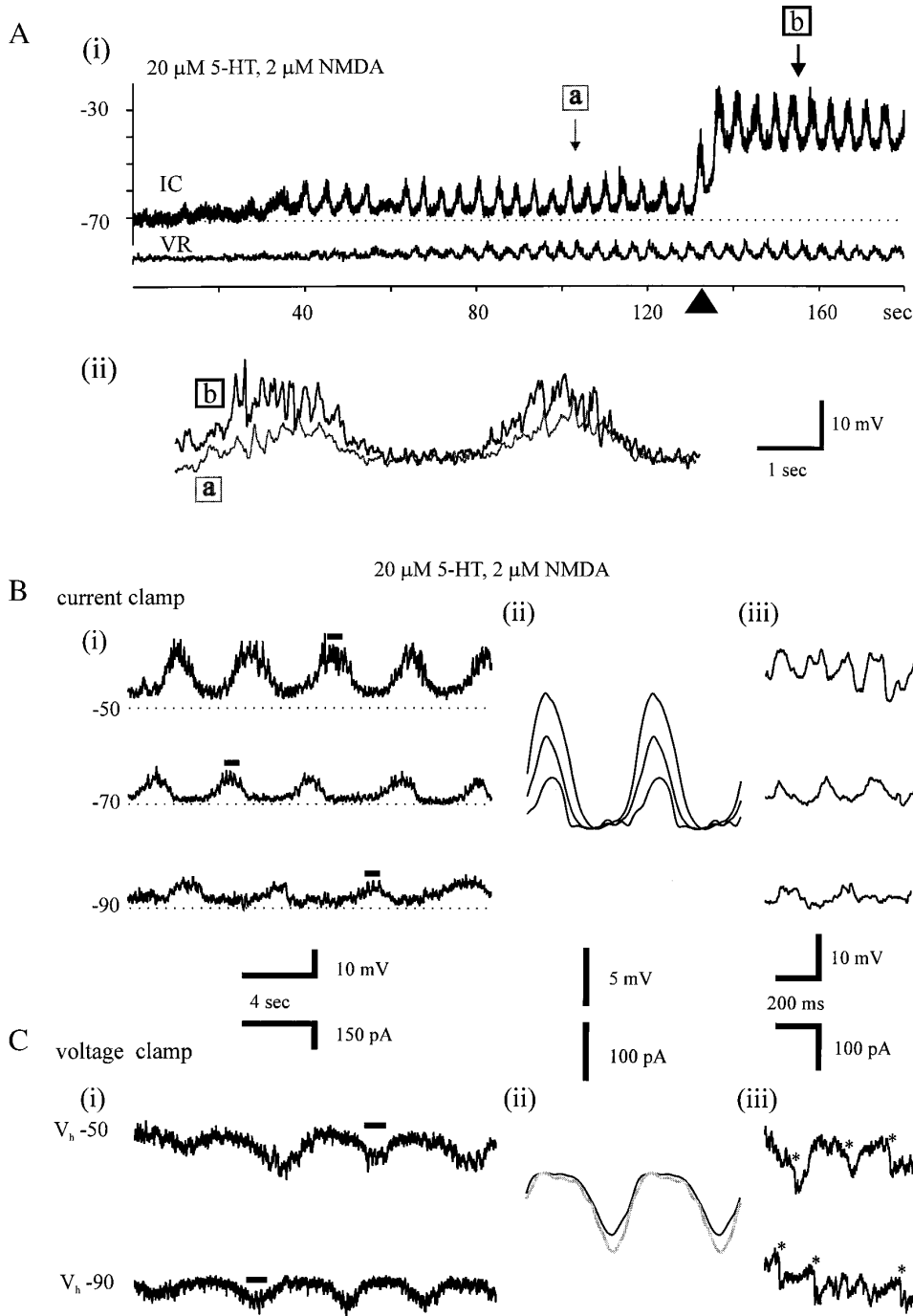


FIG. 4. Voltage dependence of drive potentials and currents. *Ai*: step current injection (at  $\blacktriangle$ ) depolarized the membrane from  $-60$  (*a*) to  $-30$  mV (*b*), as measured from the hyperpolarized phase of the locomotor drive potential (LDP). Depolarization was associated with an increase in LDP amplitude. *ii*: drive potentials from epoch "a" (gray trace) and "b" (black trace) were superimposed on a faster time base to facilitate their comparison. *Bi*: current-clamp records show the effect of different holding potentials on drive potential amplitude. *ii*: averaged drive potentials (normalized for duration) at the 3 different holding potentials. *iii*: superimposed, high-frequency events, sampled from the region under the bars in *Bi*, were also voltage dependent. *Ci*: voltage-clamp records show the effect of 2 different holding potentials on drive current amplitude. *ii*: averaged drive currents (normalized for duration) were larger at more depolarized ( $-50$  mV) holding potentials. *iii*: superimposed, high-frequency current fluctuations, sampled from under the bars in *Ci*, were also larger at more depolarized holding potentials. A synaptic origin of the high-frequency events is suggested by the rapid onset and slower repolarization of the potentials (e.g., see \*) as well as their persistence in voltage-clamp mode.

ment during membrane depolarization is related to the effects of voltage-sensitive excitatory inputs. In particular, these observations are compatible with a nonlinear NMDA receptor-mediated component.

Recordings of two motoneurons suggested that phasic excitatory and inhibitory drive components may occur in isolation. The motoneuron in Fig. 5*A* showed no evidence of an inhibitory component. Its resting membrane potential during locomotion was  $-40$  mV, which was above threshold for repetitive firing (Fig. 5*Aii*). Hyperpolarizing current injection brought the membrane potential to levels ( $-90$  mV) that presumably were hyperpolarized with respect to the reversal

potential for any inhibitory synaptic conductance (e.g., Cazals et al. 1996; Takahashi 1984; Wu et al. 1992). Yet, no depolarizing humps appeared between successive LDPs, as would be expected if there had been reversal of an inhibitory phase (Orsal et al. 1986; Perret 1983). It should be noted, however, that the Nernst equation predicts a reversal potential of  $-105$  mV based on the concentration of Cl in our recording pipettes and bath solution and assuming equilibration of the intrapipette and intracellular Cl concentrations. Therefore sufficient hyperpolarizing current may not have been applied to detect a reversed IPSP-mediated component of the LDP. However, against this argument is the observation from an-

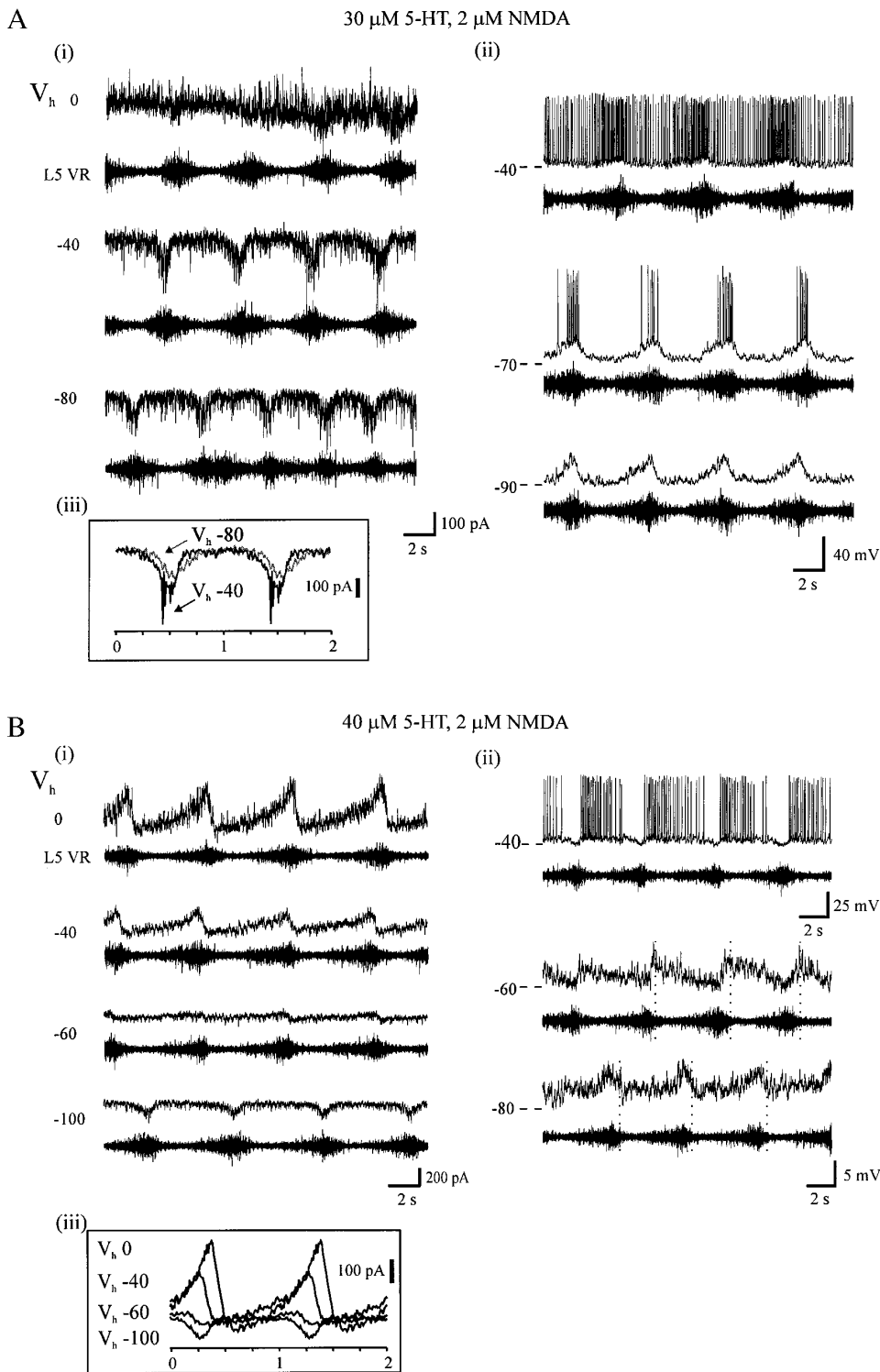


FIG. 5. Dissociated expression of rhythmic excitatory and inhibitory drive. *A*: in this motoneuron, excitatory drive alone generated rhythmic voltage fluctuations with superimposed firing in current-clamp mode (*ii*), and corresponding inward (excitatory) currents in voltage-clamp mode (*i*). Phasic inward currents were larger at a holding potential of  $-40$  mV compared with  $-80$  mV (*iii*) and equilibrated near  $0$  mV. *B*: in another motoneuron, inhibitory drive alone generated rhythmic voltage fluctuations in current-clamp mode (*ii*), which reverse between  $-80$  and  $-60$  mV and modulated cell firing at a holding potential of  $-40$  mV. In voltage-clamp mode (*i*), phasic outward currents were observed that reversed near  $-60$  mV (*iii*) consistent with an inhibitory conductance.

other cell (Fig. 5*B*) that the inhibitory component, when clearly present, does reverse at approximately  $-60$  mV using the same recording solutions. Thus Fig. 5*Bii* shows reversal of the phasic drive at holding potentials that were more negative than  $-60$  mV, confirming its inhibitory synaptic nature. The resting potential of this motoneuron was  $-66$  mV before the induction of rhythmic activity. A similar reversal of current drive was observed in voltage-clamp mode (Fig. 5*Bi*).

Therefore, inhibitory synaptic input shaped the phasic firing of this motoneuron. The lack of evidence of a phasic inward (excitatory) current suggest this motoneuron received exclusively inhibitory synaptic input from the CPG.

#### Fast events superimposed on LDPs

Fast events were superimposed on the depolarized phase of the LDP. The trajectory of the events in some motoneu-

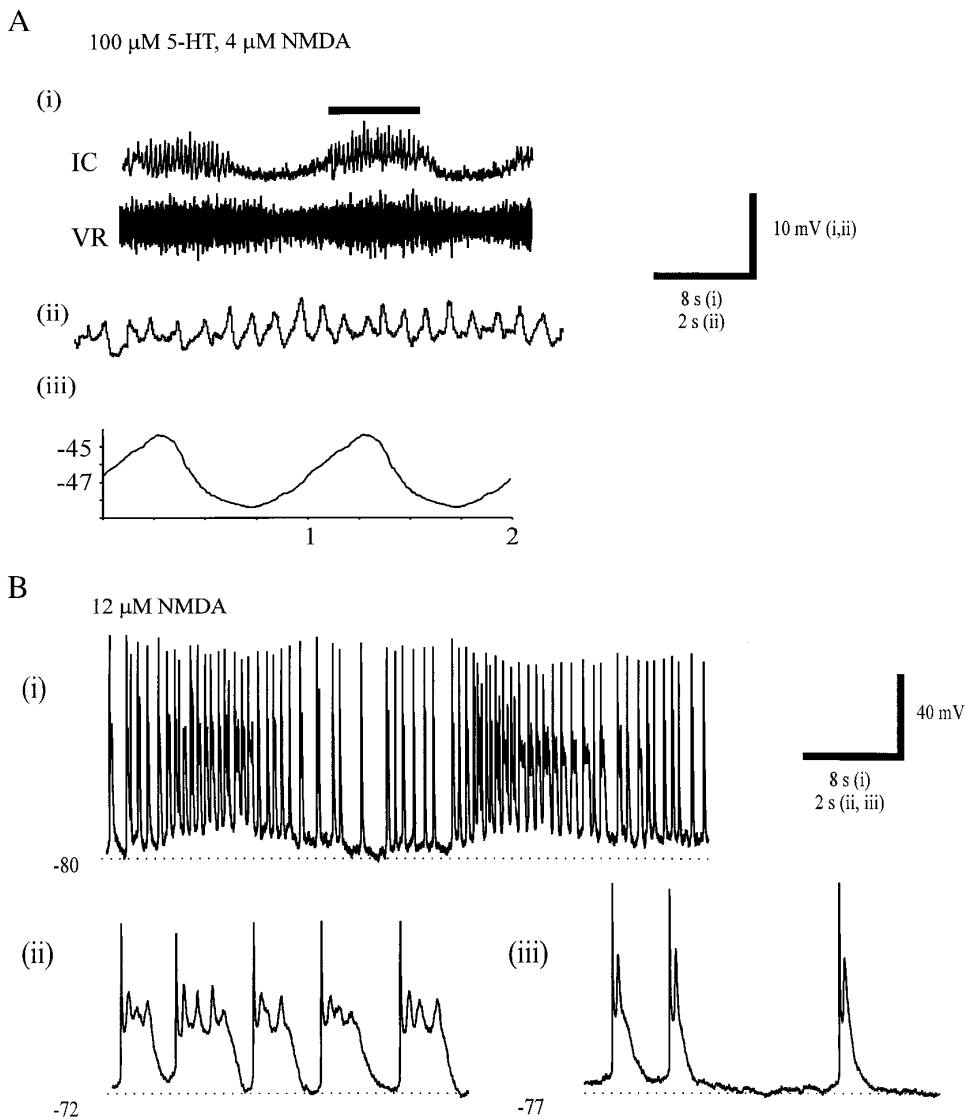


FIG. 6. Phasic recruitment of intrinsic membrane events during rhythmic activity. *Ai*: high-frequency oscillations ( $\sim 2.5$  Hz) recorded using a QX-314-filled pipette, were superimposed on the depolarized phase of the LDP (0.15–0.25 Hz). These oscillations are shown on an expanded time base in *ii*. Cycle normalized and averaged high-frequency events have a sinusoidal shape (*iii*). *Bi*: duration of plateau potentials increased during the depolarized phase of the LDP (*ii*) compared with the nondepolarized phase (*iii*).

rons was characterized by a rapid inward current and a relatively slow decay phase (see \* in Fig. 4*Cii*) compatible with an excitatory synaptic origin, as was the persistence of the events in voltage-clamp mode at  $-80$  mV (Fig. 4*Cii*). In other motoneurons, the superimposed voltage fluctuations were sinusoidal in appearance and more regular in occurrence (Fig. 6*A*), or depolarizing events of relatively long duration (plateau potentials) were recruited (Fig. 6*B*). The latter two observations suggest that inherent bistable properties also may produce fast events superimposed on the LDP (see following text).

#### DISCUSSION

These experiments demonstrate that rhythmic drive potentials in neonatal rat spinal motoneurons are generated by excitatory and inhibitory synaptic inputs and display voltage-sensitive properties. Because 5-HT typically induces a locomotor-like pattern (e.g., Cowley and Schmidt 1994a,b; Kiehn and Kjaerulff 1996; Kjaerulff et al. 1994; Sqalli-Housaini et al. 1993) and ipsilateral pairs of ventral roots dis-

played alternating activity, the present results are likely relevant to motoneuron behavior during locomotor network activation. However, reliance on ventral root recordings alone to detect specific motor patterns is not without limitations (Cowley and Schmidt 1994a). In the absence of a clearly defined sequence of hindlimb muscle activation, caution is required in assuming the drive potentials originate from a locomotor network source.

Compared with sharp electrode recordings, whole cell patch methods offer more reliable control of membrane voltage due to the lower resistance of the electrodes. The whole cell approach also enables a more accurate measure of membrane properties because the large shunt conductance due to cell impalement by sharp electrodes is eliminated (Spruston et al. 1994). Accordingly, the mean input resistance of neonatal motoneurons in the present study was fourfold higher than measurements obtained using sharp electrodes (Fulton and Walton 1986). In contrast, our measurements were four times smaller than the values obtained from whole cell recordings in newborn rat spinal cord slice experiments (Takahashi 1990). The latter difference may be due to loss of

dendritic arborizations in slice preparations, causing an artificially high-input resistance. Thus among the techniques available, patch-clamp recordings from fully intact cells, as obtained in the whole-cord preparation, may provide a more accurate estimate of membrane biophysical properties.

Activation of rhythmogenic circuits by application of neurochemicals to the entire cord may be associated with non-specific actions on motoneurons and interneurons projecting to motoneurons. This may obscure detection of those features of motoneuron behavior that specifically relate to the operation of rhythmogenic circuitry. For example, although tonic depolarization underlies the development of LDPs in cat motoneurons (Shefchyk and Jordan 1985), the tonic membrane depolarization observed in the present study may have been partly due to direct effects of 5-HT on the motoneuron. This may have involved activation of conductances such as the inward rectifier (Takahashi and Berger 1990) or enhancement of low-voltage-activated  $\text{Ca}^{2+}$  channels (Berger and Takahashi 1990). However, instead of a net conductance increase, we observed a decrease in input conductance suggesting other 5-HT actions such as a decrease in the  $\text{K}^+$  leak conductance (Larkman and Kelly 1992), inward rectifying conductance, "M"-type current, or  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductances (for review, see Anwyl 1990). An input conductance decrease during locomotor-like activity is consistent with the results of experiments using sharp electrodes (Schmidt 1994). However, increased input conductance during locomotion was reported by Cazalets et al. (1996). This discrepancy may be due to the exclusion of lumbar motoneurons from exposure to bath applied chemicals (via a bath partition) in the latter study.

The LDP trajectory fluctuated above and below the tonically depolarized membrane level recorded immediately before the onset of rhythmic activity. This observation is compatible with phasic alternation of CPG excitatory and inhibitory synaptic drive, as described in the cat (Jordan 1983; Perret 1983). However, if the tonic depolarization is mediated by continuous excitatory synaptic input, as suggested by Cazalets et al. (1996), then modulation of excitatory input alone, in theory, could yield the observed LDP trajectory and associated firing pattern. In this case, the hyperpolarized phase of the LDP and decreased firing frequency would be produced solely by a periodic decrease in the activity of the interneurons transmitting the excitatory drive. However, our data show that inhibitory and excitatory components sometimes occur in isolation, suggesting that the CPG can in fact deliver both types of drive to motoneurons.

Traditionally, the half-center model of the CPG implies that the two half-centers receive common tonic excitatory drive; it is the presence of mutual reciprocal inhibition that ensures the phasic quality of each half-center's output (Brown 1911; Gossard and Hultborn 1991; Jankowska et al. 1967; Lundberg 1981). Yet the present observations suggest that each half-center can generate rhythmic output in the absence of phasic reciprocal inhibitory input from the antagonist half-center (Fig. 7), consistent with the results of experiments wherein inhibitory neurotransmission was blocked (Cowley and Schmidt 1995). It is unclear, however, whether the production of purely excitatory or inhibitory rhythmic drive to some motoneurons reflects anomalous CPG behavior, a fundamentally different organization of the rhythmogenic circuitry in this preparation (i.e., compared with the cat) or the method of network activation.

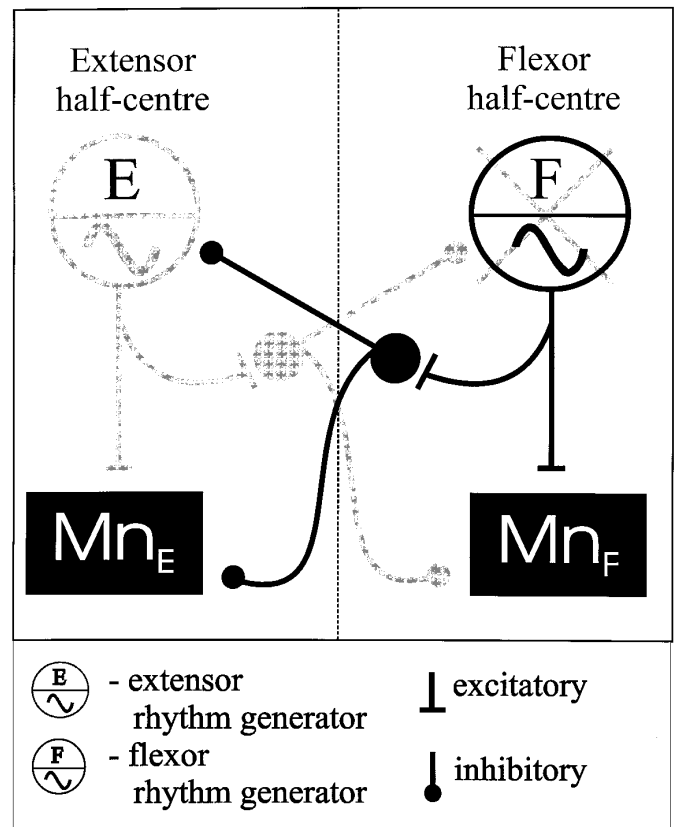


FIG. 7. A hypothetical half-center organization consistent with alternation between inhibitory and excitatory synaptic actions on motoneurons during locomotion. Loss of activity in one half-center (e.g., flexor) would result in flexor motoneurons ( $\text{Mn}_F$ ) receiving phasic inhibitory input only, whereas extensor motoneurons ( $\text{Mn}_E$ ) would receive exclusively excitatory input, possibly accounting for the observations shown in Fig. 5.

genic circuitry in this preparation (i.e., compared with the cat) or the method of network activation.

Brownstone et al. (1992) showed that repetitive firing of motoneurons during an LDP is not simply related to the level of net synaptic current they receive, in contrast to what one might predict from classical studies (e.g., Baldissera and Gustafsson 1971; Granit et al. 1966; Kernell 1965; Schwindt and Calvin 1973). The present demonstration of discrete, presumably synaptic, events superimposed on LDPs (Fig. 4) suggests that the timing of spike activation may be determined, at least in part, by these extrinsic inputs. In addition, the occurrence in some recordings of LDPs with superimposed sinusoidal oscillations (Fig. 6A) or plateau potentials (Fig. 6B), which resemble NMDA-mediated oscillations and plateau potentials produced in tetrodotoxin-isolated neurons (Hochman et al. 1994a,b; Kim and Chandler 1995), suggests that inherent membrane bistable properties also may play a role in controlling spike activation during locomotion.

In contrast to the present results, Cazalets et al. (1996) reported a linear relationship of LDP amplitude with respect to membrane potential. However, this relationship was examined only in the range of  $-40$  and  $-80$  mV (Cazalets et al. 1996). In the present study, LDP amplitude displayed a nonlinear voltage-sensitive response to membrane potential, in agreement with observations of mesencephalic locomotor

region (MLR)-induced locomotion in the cat (Brownstone et al. 1994). In particular, our voltage-clamp recordings showed increased phasic inward (excitatory) currents at holding potentials of  $-40$  mV compared with  $-80$  mV, which then decreased as the holding potential was further depolarized toward 0 mV. These findings are consistent with an NMDA receptor-mediated effect.

In conclusion, the results are consistent with a complex interplay between synaptic and intrinsic membrane properties, which in combination give rise to LDPs and nonlinear regulation of motoneuron firing during rhythmic behaviors. Phasic synaptic drive from the CPG includes both excitation and inhibition, either component of which, in some instances, may be transmitted to motoneurons independently. Future studies aimed at understanding mammalian locomotor circuitry will need to explore the role of similar properties in premotoneuronal elements of the CPG.

This work was supported by the Health Sciences Centre Foundation.

Address for reprint requests: B. J. Schmidt, Dept. of Physiology, 770 Bannatyne Ave., University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada.

Received 22 August 1996; accepted in final form 29 September 1997.

## REFERENCES

- ANWYL, R. Neurophysiological actions of 5-hydroxytryptamine in the vertebrate nervous system. *Prog. Neurobiol.* 35: 451–468, 1990.
- ATSUTA, Y., ABRAHAM, P., IWAHARA, T., GARCIA-RILL, E., AND SKINNER, R. D. Control of locomotion in vitro: II. chemical stimulation. *Somatosens. Mot. Res.* 8: 55–63, 1991.
- ATSUTA, Y., GARCIA-RILL, E., AND SKINNER, R. D. Characteristics of electrically induced locomotion in rat in vitro brain stem-spinal cord preparation. *J. Neurophysiol.* 64: 727–735, 1990.
- BALDISSERA, F. AND GUSTAFSSON, B. Regulation of repetitive firing in motoneurons by the afterhyperpolarization conductance. *Brain Res.* 30: 431–434, 1971.
- BERGER, A. J. AND TAKAHASHI, T. Serotonin enhances a low-voltage-activated calcium current in rat spinal motoneurons. *J. Neurosci.* 10: 1922–1928, 1990.
- BLANTON, M. G., LO TORCO, J. J., AND KRIEGSTEIN, A. R. Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J. Neurosci. Methods* 30: 203–210, 1989.
- BROWN, T. G. The intrinsic factors in the act of progression in the mammal. *Proc. R. Soc. Lond. B Biol. Sci.* 84: 308–319, 1911.
- BROWNSTONE, R. M., GOSSARD, J.-P., AND HULTBORN, H. Voltage-dependent excitation of motoneurons from spinal locomotor centres in the cat. *Exp. Brain Res.* 102: 34–44, 1994.
- BROWNSTONE, R. M., JORDAN, L. M., KRIELLAARS, D. J., NOGA, B. R., AND SHEFCHYK, S. J. On the regulation of repetitive firing in lumbar motoneurons during fictive locomotion in the cat. *Exp. Brain Res.* 90: 441–455, 1992.
- CAZALET, J.-R., BORDE, M., AND CLARAC, F. The synaptic drive from the locomotor network to motoneurons in the newborn rat. *J. Neurosci.* 16: 298–306, 1996.
- CAZALET, J. R., SQALLI-HOUSSAINI, Y., AND CLARAC, F. Activation of the central pattern generators for locomotion by serotonin and excitatory amino acids in neonatal rat. *J. Physiol. (Lond.)* 455: 187–204, 1992.
- CAZALET, J. R., SQALLI-HOUSSAINI, Y., AND CLARAC, F. GABAergic inactivation of the central pattern generators for locomotion in isolated neonatal rat spinal cord. *J. Physiol. (Lond.)* 474: 173–181, 1994.
- COWLEY, K. C. AND SCHMIDT, B. J. Some limitations of ventral root recordings for monitoring locomotion in the in vitro neonatal rat spinal cord preparation. *Neurosci. Lett.* 171: 142–146, 1994a.
- COWLEY, K. C. AND SCHMIDT, B. J. A comparison of motor patterns induced by *N*-methyl-D-aspartate, acetylcholine and serotonin in the in vitro neonatal rat spinal cord. *Neurosci. Lett.* 171: 147–150, 1994b.
- COWLEY, K. C. AND SCHMIDT, B. J. Effects of inhibitory amino acid antagonists on reciprocal inhibitory interactions during rhythmic motor activity in the in vitro neonatal rat spinal cord. *J. Neurophysiol.* 74: 1109–1117, 1995.
- COWLEY, K. C. AND SCHMIDT, B. J. Regional distribution of the locomotor pattern generating network in the neonatal rat spinal cord. *J. Neurophysiol.* 77: 247–259, 1997.
- EDGERTON, V. R., GRILLNER, S., SJOSTROM, A., AND ZANGGER, P. Central generation of locomotion in vertebrates. In: *Neural Control of Locomotion*. New York: Plenum, 1976, p. 439–464.
- FULTON, B. P. AND WALTON, K. Electrophysiological properties of neonatal rat motoneurons studied in vitro. *J. Physiol. (Lond.)* 370: 651–678, 1986.
- GOSSARD, J. P. AND HULTBORN, H. The organization of the spinal rhythm generation in locomotion. In: *Plasticity of Motoneuronal Connections*, edited by A. Wernig. Amsterdam: Elsevier Science Publishers, 1991, p. 385–404.
- GRANIT, R., KERNELL, D., AND LAMARRE, Y. Algebraical summation in synaptic activation of motoneurons firing within the “primary range” to injected currents. *J. Physiol. (Lond.)* 187: 379–399, 1966.
- HAMILL, O. P., MARTY, A., NEHER, E., SAKMANN, B., AND SIGWORTH, F. J. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* 391: 85–100, 1981.
- HOCHMAN, S., JORDAN, L. M., AND MACDONALD, J. F. *N*-methyl-D-aspartate receptor-mediated voltage oscillations in neurons surrounding the central canal in slices of rat spinal cord. *J. Neurophysiol.* 72: 565–577, 1994a.
- HOCHMAN, S., JORDAN, L. M., AND SCHMIDT, B. J. TTX-resistant NMDA receptor-mediated voltage oscillations in mammalian lumbar motoneurons. *J. Neurophysiol.* 72: 2559–2562, 1994b.
- JANKOWSKA, E., JUKES, M.G.M., LUND, S., AND LUNDBERG, A. The effect of DOPA on the spinal cord. VI. Half-centre organization of interneurons transmitting effects from the flexor reflex afferents. *Acta Physiol. Scand.* 68: 389–403, 1967.
- JORDAN, L. M. Factors determining motoneuron rhythmicity during fictive locomotion. *Soc. Exp. Biol. Symp.* 37: 423–444, 1983.
- KERNELL, D. Synaptic influences on the repetitive activity elicited in cat lumbosacral motoneurons by longlasting injected currents. *Acta Physiol. Scand.* 63: 409–410, 1965.
- KIEHN, O. Plateau potentials and active integration in the “final common pathway” for motor behaviour. *Trends Neurosci.* 14: 68–73, 1991.
- KIEHN, O., IZUKA, M., AND KUDO, N. Resetting from low threshold afferents of *N*-methyl-D-aspartate-induced locomotor rhythm in the isolated spinal cord-hindlimb preparation from newborn rats. *Neurosci. Lett.* 148: 43–46, 1992.
- KIEHN, O., JOHNSON, B. R., AND RAASTAD, M. Plateau properties in mammalian spinal interneurons during transmitter-induced locomotor activity. *Neuroscience* 75: 263–273, 1996.
- KIEHN, O. AND KJAERULFF, O. Spatiotemporal characteristics of 5-HT and dopamine-induced rhythmic hindlimb activity in the in vitro neonatal rat. *J. Neurophysiol.* 75: 1472–1482, 1996.
- KIM, Y. I. AND CHANDLER, S. H. NMDA-induced burst discharge in guinea pig trigeminal motoneurons in vitro. *J. Neurophysiol.* 74: 334–346, 1995.
- KJAERULFF, O., ISABELLA, B., AND KIEHN, O. Sulphorhodamine-labelled cells in the neonatal rat spinal cord following chemically induced locomotor activity in vitro. *J. Physiol. (Lond.)* 478: 265–273, 1994.
- KUDO, N. AND YAMADA, T. *N*-methyl-D,L-aspartate-induced locomotor activity in a spinal cord-hindlimb muscles preparation of the newborn rat studied in vitro. *Neurosci. Lett.* 75: 43–48, 1987.
- LARKMAN, P. M. AND KELLY, J. S. Ionic mechanisms mediating 5-hydroxytryptamine- and noradrenaline-evoked depolarizations of adult rat facial motoneurons. *J. Physiol. (Lond.)* 456: 473–490, 1992.
- LUNDBERG, A. Half-centres revisited. *J. Adv. Physiol. Sci.* 1: 155–167, 1981.
- MACLEAN, J. N., HOCHMAN, S., AND MAGNUSON, D.S.K. Lamina VII neurons are rhythmically active during locomotor-like activity in the neonatal rat spinal cord. *Neurosci. Lett.* 197: 9–12, 1995.
- O'DONOVAN, M. J. Motor activity in the isolated spinal cord of the chick embryo: synaptic drive and firing pattern of single motoneurons. *J. Neurosci.* 9: 943–958, 1989.
- ORSAL, D., PERRET, C., AND CABELGUEN, J.-M. Evidence of rhythmic inhibitory synaptic influences in hindlimb motoneurons during fictive locomotion in the thalamic cat. *Exp. Brain Res.* 64: 217–224, 1986.
- PERRET, C. Centrally generated pattern of motoneuron activity during locomotion in the cat. *Soc. Exp. Biol. Symp.* 37: 405–422, 1983.
- PRATT, C. A. AND JORDAN, L. M. Ia inhibitory interneurons and Renshaw

- cells as contributors to the spinal mechanisms of fictive locomotion. *J. Neurophysiol.* 57: 56–71, 1987.
- SCHMIDT, B. J. Afterhyperpolarization modulation in lumbar motoneurons during locomotor-like rhythmic activity in the neonatal rat spinal cord in vitro. *Exp. Brain Res.* 99: 214–222, 1994.
- SCHMIDT, B. J., HOCHMAN, S., AND JORDAN, L. M. Voltage-dependent oscillations of motoneuron membrane potential during rhythmic motor activity in the in vitro neonatal rat spinal cord. *Soc. Neurosci. Abstr.* 20: 717.9, 1994.
- SCHMIDT, B. J., SMITH, J. C., AND FELDMAN, J. L. Involvement of excitatory amino acids in neurotransmission of rhythmic excitatory drive to motoneurons during locomotion in the rat spinal cord in vitro. *Soc. Neurosci. Abstr.* 15: 207.9, 1989.
- SCHWINDT, P. C. AND CALVIN, W. H. Equivalence of synaptic and injected current in determining the membrane potential trajectory during motoneuron rhythmic firing. *Brain Res.* 59: 389–394, 1973.
- SCHWINDT, P. C. AND CRILL, W. E. A persistent negative resistance in cat lumbar motoneurons. *Brain Res.* 120: 173–178, 1977.
- SERNAGOR, E., CHUB, N., RITTER, A., AND O'DONOVAN, M. J. Pharmacological characteristics of the rhythmic synaptic drive onto lumbosacral motoneurons in the chick embryo spinal cord. *J. Neurosci.* 15: 7452–7464, 1995.
- SERNAGOR, E. AND O'DONOVAN, M. J. Whole-cell patch clamp recordings from rhythmically active motoneurons in the isolated spinal cord of the chick embryo. *Neurosci. Lett.* 128: 211–216, 1991.
- SHEFCHYK, S. J. AND JORDAN, L. M. Motoneuron input-resistance changes during fictive locomotion produced by stimulation of the mesencephalic locomotor region. *J. Neurophysiol.* 54: 1101–1108, 1985.
- SMITH, J. C. AND FELDMAN, J. L. In vitro brainstem-spinal cord preparations for study of motor systems for mammalian respiration and locomotion. *J. Neurosci. Methods* 21: 321–333, 1987.
- SMITH, J. C., FELDMAN, J. L., AND SCHMIDT, B. J. Neural mechanisms generating locomotion studied in mammalian brain stem-spinal cord in vitro. *FASEB J.* 2: 2283–2288, 1988.
- SPRUSTON, N., JAFFE, D. B., AND JOHNSTON, D. Dendritic attenuation of synaptic potentials and currents: the role of passive membrane properties. *Trends Neurosci.* 17: 161–166, 1994.
- SQALLI-HOUSSAINI, Y., CAZALETS, J.-R., AND CLARAC, F. Oscillatory properties of the central pattern generator for locomotion in neonatal rats. *J. Neurophysiol.* 70: 803–813, 1993.
- TAKAHASHI, T. Inhibitory miniature synaptic potentials in rat motoneurons. *Proc. R. Soc. Lond. B Biol. Sci.* 221: 103–109, 1984.
- TAKAHASHI, T. Membrane currents in visually identified motoneurons of neonatal rat spinal cord. *J. Physiol. (Lond.)* 423: 27–46, 1990.
- TAKAHASHI, T. AND BERGER, A. J. Direct excitation of rat spinal motoneurons by serotonin. *J. Physiol. (Lond.)* 423: 63–76, 1990.
- WU, W.-L., ZISKIND-CONHAIM, L., AND SWEET, M. A. Early development of glycine- and GABA-mediated synapses in rat spinal cord. *J. Neurosci.* 12: 3935–3945, 1992.