Ontogeny of Rhythmic Motor Patterns Generated in the Embryonic Rat Spinal Cord

JUN REN AND JOHN J. GREER

Department of Physiology, Division of Neuroscience, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

Submitted 15 July 2002; accepted in final form 18 November 2002

Ren, Jun, and John J. Greer. Ontogeny of rhythmic motor patterns generated in the embryonic rat spinal cord. J Neurophysiol 89: 1187–1195, 2003; 10.1152/jn.00539.2002. Patterned spontaneous activity is generated in developing neuronal circuits throughout the CNS including the spinal cord. This activity is thought to be important for activity-dependent neuronal growth, synapse formation, and the establishment of neuronal networks. In this study, we examine the spatiotemporal distribution of motor patterns generated by rat spinal cord and medullary circuits from the time of initial axon outgrowth through to the inception of organized respiratory and locomotor rhythmogenesis during late gestation. This includes an analysis of the neuropharmacological control of spontaneous rhythms generated within the spinal cord at different developmental stages. In vitro spinal cord and medullary-spinal cord preparations isolated from rats at embryonic ages (E)13.5–E21.5 were studied. We found age-dependent changes in the spatiotemporal pattern, neurotransmitter control, and propensity for the generation of spontaneous rhythmic motor discharge during the prenatal period. The developmental profile of the neuropharmacological control of rhythmic bursting can be divided into three periods. At E13.5–E15.5, the spontaneous networks comprising cholinergic and glycineergic synaptic interconnections are capable of generating rhythmic activity, while GABAergic synapses play a role in supporting the spontaneous activity. At late stages (E16.5–E21.5), glutamate drive acting via non-N-methyl-D-aspartate (non-NMDA) receptors is primarily responsible for the rhythmic activity. During the middle stage (E16.5–E17.5), the spontaneous activity results from the combination of synaptic drive acting via non-NMDA glutamatergic, nicotinic acetylcholine, glycine, and GABA receptors. The modulatory actions of chloride-mediated conductances shifts from predominantly excitatory to inhibitory late in gestation.

M E T H O D S

In vitro prenatal rat preparations

Embryos (E13.5–E21.5; n = 117) were delivered from timed-pregnant rats anesthetized with halothane (1.2–1.5% delivered in 95% O2:5% CO2) and maintained at 37°C by radiant heat following procedures approved by the Animal Welfare Committee at the University of Alberta. The timing of pregnancies of dams was determined from the appearance of sperm plugs in the breeding cages. The ages of fetuses were confirmed by comparison of their crown-rump length measurements with those published by Angulo y González (1932). Immediately on delivery, the neuraxis was isolated from embryos as previously described (Greer et al. 1992). The spinal cord was dissected to include segments extending from the first cervical (C1) to the fourth sacral (S4) ventral roots. In other preparations, the medulla was left attached. The preparations were continuously perfused at 27 ± 1°C (perfusion rate 5 ml/min, volume of the chamber 1.5 ml) with mock cerebrospinal fluid (CSF) that contained (mM) 128 NaCl, 3.0 or 5.0 KCl, 1.5 CaCl2, 1.0 MgSO4, 24 NaHCO3, 0.5 NaH2PO4, and 30 D-glucose equilibrated with 95% O2:5% CO2 (pH = 7.4)

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests: J. J. Greer, Dept. of Physiology, 513 HMRC, University of Alberta, Edmonton, Alberta T6G 2S2 Canada (E-mail: john.greer@ualberta.ca).
Recording and analysis

Recordings of spinal motoneuron population activity in vitro were made with suction electrodes applied to the cut ends of spinal ventral roots and hypoglossal (XII) cranial roots. Signals were amplified, rectified, low-passed filtered, and recorded on computer via an analogue-digital converter (Digidata 1322A, Axon Instruments, Foster City, CA) and data-acquisition software (Axotape). Mean values relative to control for the period of motoneuron discharge were calculated pre- and postdrug delivery. Results are expressed as means ± SD and any differences tested using paired difference Student’s t-test; significance was accepted at P values < 0.05.

DRUGS. Stock solutions of drugs were prepared as concentrates. All drugs were added to the perfusate by switching to reservoirs containing the appropriate test solution. The following drugs were used: 2-amino-5-phosphonoveric acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), bicuculline, eserine, muscimol, strychnine, glycine, sarcosine, mecamylamine, dihydro-beta-erythroidine and t-tubocurarine. Drugs were purchased from Sigma (St. Louis, MO) or RBI (Oakville, ON, Canada).

FIG. 1. Developmental changes in the spontaneous rhythmic bursting recorded from cervical (C), thoracic (T), and lumbar (L) ventral roots of isolated prenatal rat spinal cord preparations. All recordings in this and subsequent figures are rectified and integrated suction electrode recordings from spinal ventral roots (C1–C7; T1–T13; L1–L4).

TABLE 1. Interval and duration of spontaneous bursts recorded before and after spinal cord transection at T1 and T13

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Cervical Cord</th>
<th>Thoracic Cord</th>
<th>Lumbar Cord</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact</td>
<td>Transected</td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td>Interval, min</td>
<td></td>
<td>6</td>
<td>1.9 ± 0.2</td>
<td>2.9 ± 0.6*</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.8 ± 0.5</td>
<td>5.9 ± 1.8*</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>E15.5</td>
<td>7</td>
<td>3.2 ± 0.9</td>
<td>20.5 ± 8.4*</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>E16.5</td>
<td>5</td>
<td>12.7 ± 4.6</td>
<td>&gt;60*</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>E17.5</td>
<td>5</td>
<td>2.7 ± 0.3</td>
<td>2.4 ± 0.5</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>Burst duration, s</td>
<td></td>
<td>6</td>
<td>6.1 ± 0.6</td>
<td>6.4 ± 0.8</td>
</tr>
<tr>
<td>E15.5</td>
<td>7</td>
<td>13.8 ± 3.2</td>
<td>19.5 ± 2.1*</td>
<td>13.5 ± 2.4</td>
</tr>
<tr>
<td>E16.5</td>
<td>5</td>
<td>21.6 ± 6.2</td>
<td>N/A</td>
<td>15.3 ± 5.8</td>
</tr>
<tr>
<td>E17.5</td>
<td>5</td>
<td>21.6 ± 6.2</td>
<td>N/A</td>
<td>15.3 ± 5.8</td>
</tr>
</tbody>
</table>

Values are means ± SD; * P < 0.05 compared with intact.

Ontogeny of rhythmic patterns

In the first series of experiments, we recorded from spinal ventral roots at different ages to determine the spatiotemporal patterns of spontaneous rhythmic motor discharge. Figure 1 illustrates representative traces of rhythmic motor patterns generated in cervical, thoracic and lumbar motor pools from E13.5 through E19.5. The spontaneous rhythmic bursts were generated in all E13.5 (n = 7), E14.5 (n = 23), E15.5 (n = 16), and E16.5 (n = 32) preparations studied. Rhythmic activity was observed in 10 of 13 E17.5 and 4 of 8 E18.5 preparations. The interburst intervals and burst durations increased with gestational age (Table 1). Robust, rhythmic motor patterns were not observed in spinal cord preparations bathed in normal Krebs solution containing 3 mM K+ beyond age E18.5 (n = 6). However, low-frequency, longer-duration rhythmic bursts were generated in E18.5–E21.5 preparations (n = 39) bathed in Krebs solution containing elevated extracellular potassium (5 mM) to raise the excitability by depolarizing spinal neurons.

There were age-dependent changes in the segmental location at which the rhythmic discharge started and the timing of activity on cervical, thoracic, and lumbar roots (Fig. 2). At E13.5, rhythmic activity was restricted to thoracic and cervical ventral roots. At E14.5, the rhythmic motor pattern appeared on thoracic ventral roots first and radiated in rostral and caudal directions to cervical and lumbar segments. By E16.5, the rhythmic activity appeared on lumbar ventral roots first approximately 90% of the time and radiated rostrally. The temporal relationships among bursts in the cervical, thoracic, and lumbar cord were less consistent at E15.5 with the loci of initiation varying between thoracic and lumbar segments. Figure 2B summarizes the age-dependent changes in the spatio-temporal relationships between cervical, lumbar, and thoracic root activity.

To examine the ability of cervical, thoracic, and lumbar cord segments to generate spontaneous activity independently, the spinal cord was transected with a scalpel at the first (T1) and last (T13) thoracic segments in E14.5–17.5 preparations (Fig. 3). The preparations were left for 30 min prior to recordings. Spontaneous activity was generated in each spinal segment at ages E14.5 to E16.5. At E17.5, only the lumbar segment generated a robust rhythm. The effects of the transection on
burst interval and burst duration are summarized in Table 1. The interval between spontaneous bursts was significantly ($P < 0.05$) prolonged in the cervical and lumbar segments by transection at E14.5, while the interval between bursts in the thoracic segment was not significantly affected and remained the most robust. At E15.5, transections prolonged the burst interval in each spinal cord segment. After transections at E16.5, the interval between bursts in cervical and thoracic segments was significantly increased ($P < 0.05$), while the interval between lumbar cord bursts was similar to control values and was most robust.

Age-dependent changes in the neurochemical control of patterned rhythmic activity

To determine the role of various neurotransmitters in the generation and modulation of patterned rhythmic discharge, we applied neurotransmitter receptor agonists, antagonists, and uptake inhibitors to the bathing medium at different developmental ages. Specifically, we assayed for the roles of acetylcholine, glycine, GABA, and glutamate by recording rectified integrated motor discharge. It should be noted that these data did not provide information on interneuronal or subthreshold rhythmic motor activity that may have persisted in the presence of the various pharmacological manipulations.

**ACETYLCHOLINE.** As shown in Fig. 4 A and B, rhythmic motor patterns were inhibited in the presence of the nicotinic acetylcholine receptor antagonist d-tubocurarine (dTC; 20 μM) at ages E13.5–E17.5. The rhythmic activity in E14.5–E15.5 preparations was also blocked by nicotinic receptor antagonists mecamylamine (15 μM; $n = 2$) and dihydro-beta-erythroidine (10 μM; $n = 2$). The activity was inhibited for the duration of the recording session (30–60 min). Beyond E17.5, dTC had little or no effect on rhythmic motor discharge. The rhythm was not affected by the muscarinic receptor antagonist atropine (1–10 μM) at any ages. To further examine the actions of acetylcholine, we administered the cholinesterase inhibitor eserine (1 μM) to the bathing medium (Fig. 4C). Increasing the endogenously released levels of acetylcholine with a 5-min application of eserine resulted in an increase in the duration of rhythmic bursts by 380 ± 87% ($n = 5$) and 160 ± 41% ($n = 6$) at ages E14.5 and E16.5, respectively. There was also a transient increase in the frequency of rhythmic bursting that was typically followed by a rebound depression.

**GLYCINE.** Figure 5 illustrates the age-dependent changes in the role of glycinergic transmission in the generation of spontaneous rhythmic motor discharge. Blocking glycine receptors with strychnine (STR; 25 μM) abolished the rhythmic discharge at ages E13.5–E17.5. The activity was inhibited for the duration of the recording session (30–60 min). In contrast, blocking glycinergic receptors post-E18.5 led to an increase in the frequency of rhythmic discharge. GABA. The age-dependent changes resulting from the blocking of GABA_A receptors with bicuculline (BIC; 50 μM) are illustrated in Fig. 6. At ages E13.5–E15.5, bicuculline caused a 53 ± 13% ($n = 13$) reduction in the frequency of rhythmic bursts. At E16.5, the same dose of bicuculline reduced the frequency to 12 ± 6% ($n = 5$) of control. The rhythmic activity was completely inhibited in four of five preparations at E17.5. Post-E17.5, blocking GABA_A receptors either had no effect or increased the frequency of rhythmic bursts (Fig. 6, C and D).

**AGE-DEPENDENT CHANGES IN CHLORIDE-MEDIATED CONDUCTANCES.** Pre-E17.5, activation of GABA_A receptors (Fig. 7, A and C) with muscimol (0.3 μM) resulted in an increase in the fre-

---

**FIG. 2.** Age-dependent changes in the spatiotemporal pattern of rhythmic bursting. A: fast sweeps of single spontaneous bursts recorded from cervical, thoracic, and lumbar ventral roots at E14.5 and E16.5. B: population data showing the delay onset (in seconds) of cervical (●) and lumbar (○) bursts in relation to those recorded from the thoracic ventral roots at different prenatal ages.

---

**INTACT**

---

**AFTER TRANSECTIONS**

---

**FIG. 3.** Comparison of rhythmic bursting activity generated in the intact vs. transected spinal cord. Recordings were made from cervical, thoracic, and lumbar ventral roots in the intact spinal cord at ages E14.5 and E16.5. The cord was subsequently transected at T1 and T13 to isolate cervical, thoracic, and lumbar segments. Population data for the effects of the transections on burst frequency and duration at various ages are provided in Table 1.
frequency of rhythmic discharge. In contrast, at late stages of embryonic development (more than E19), activation of chloride-mediated conductances decreased the frequency of rhythmic motor discharge (Fig. 7, B and C). Increasing the endogenous levels of glycine with sarcosine (1 mM; n/H1005 5) at E14.5 resulted in an increase of the frequency of rhythmic discharge by 181/52% (Fig. 7D).

GLUTAMATE. The spontaneous rhythmic discharge was not altered at ages E13.5–E15.5 in the presence of the non-NMDA receptor antagonist CNQX (6 μM; Fig. 8). At E16.5, CNQX transiently abolished the rhythm in cervical motor populations in all seven preparations studied. However, the rhythm reappeared within 10–20 min despite the continued exposure to CNQX. The amplitude of activity at E16.5 was decreased to 65 ± 13% (n = 7) of control on thoracic roots, but the frequency of rhythms was unaltered on thoracic and lumbar roots. At E17.5, CNQX abolished the rhythm on cervical, thoracic and lumbar roots. However, the spontaneous activity re-emerged on lumbar roots after approximately 15–20 min despite continued exposure to CNQX; activity remained absent on thoracic and cervical roots during the recording session (approximately 30 min). The rhythmic activity in E18.5–E21.5 preparations bathed in Krebs solution containing 5 mM potassium was blocked by CNQX but not by antagonists to acetylcholine, glycine, or GABA receptors. In fact, as reported in the preceding text, blocking of chloride-mediated conductances caused an increase in the frequency of rhythmic bursts. Blocking of NMDA receptors with the antagonist APV (30–100 μM; n = 8) and MK-801 (100 μM; n = 2) did not alter the rhythmic discharge at any age studied.

COMBINATORIAL ACTIONS OF NEUROTRANSMITTERS. It was clear from the data presented in the preceding text that multiple transmitters work together to regulate the generation of rhythmic bursting.
neurotransmitters were contributing to the generation of rhythms prior to E18.5. We investigated this further by performing a series of recordings from E16.5 preparations in the presence of multiple receptor antagonists in combination with agents that increased the endogenous levels of neurotransmitter (Fig. 9). In Fig. 9A, we demonstrate the interactions of glycine and non-NMDA receptor mediated effects. The addition of 10 μM strychnine (n = 6) to the perfusate caused a decrease (40 ± 11%; n = 6) of frequency of rhythmic motor discharge. Application of CNQX (6 μM) caused a marked decrease in the frequency (86 ± 6%; n = 6) of rhythmic activity on cervical motor pools with little effect on lumbar populations (decrease of 9 ± 10%). However, the combination of 10 μM strychnine and 6 μM CNQX abolished the rhythmic discharge in all spinal segments in all six preparations tested. The inhibition of rhythmic activity by higher concentrations of strychnine (25 μM) was countered by elevating the endogenous levels of acetylcholine with the cholinesterase inhibitor eserine (2 μM) in four of the six preparations tested (Fig. 9, B and D). The rhythm was reduced in frequency, but persisted, in the presence of strychnine (25 μM), CNQX (6 μM), and bicuculline (50 μM) in all four preparations tested. The rhythm was finally abolished with subsequent application of dTC (40 μM) in three of the four preparations tested. The combinatorial actions of acetylcholine and glycine are further emphasized in Fig. 9, C and E. The blocking of the rhythm by dTC (20 μM) was reversed by elevating endogenous glycine levels with sarcosine (2 mM). The rhythm was slowed by d-tubocurarine (20 μM), CNQX (6 μM), and bicuculline (50 μM) and finally abolished by strychnine (40 μM) in four of five preparations tested.

Interaction of rhythmic patterns generated by brain stem-spinal cord preparations during late gestation

In 19 of 25 brain stem-spinal cords isolated from E13.5 to E16.5 rats, the rhythmic motor pattern recorded from spinal ventral roots was also present on hypoglossal (XII) roots (Fig. 10). As described previously (Greer et al., 1992), brainstem...
spinal cord preparations start generating respiratory rhythms on XII and cervical roots at E17. We were interested in examining the interaction of the widespread spinally generated rhythms with the more spatially restricted and regulated respiratory rhythm. Figure 10 shows the spontaneous respiratory rhythm recorded from the hypoglossal (XII) rootlet and the longer-duration bursts on lumbar ventral roots in an E18.5 preparation. The respiratory rhythm was occluded in the presence of the spinal cord and into the medulla. The spatiotemporal organization of the pattern undergoes significant transformations during the period spanning E13.5–E18.5. The propensity for the generation of embryonic rhythms declines markedly during late gestation when the more spatially restricted and organized respiratory and locomotor patterns emerge. Multiple neurotransmitters act in concert to provide the excitatory drive necessary to generate rhythms prior to E18.5. Post-E18.5, the rhythms are generated primarily via glutamatergic transmission.

**Spatiotemporal development of spontaneous rhythmic bursts**

The data demonstrating that rhythmic patterns are generated as early as E13.5 in rostral spinal segments and at E14.5 in lumbar segments is consistent with results from previous work (Greer et al. 1992; Nakayama et al. 1999). However, past studies were rather incomplete and did not include recordings from motor pools along the full rostrocaudal extent of the brain stem and spinal cord. Thus the fact that the thoracic segments were a foci for the inception of spinal rhythmogenesis at early embryonic stages and that spontaneous rhythmic patterns spread into medullary motoneuron populations was not realized. The transection experiments demonstrated that cervical, thoracic, and lumbar segments were all capable of generating spontaneous rhythmic patterns. However, the thoracic and lumbar segment circuitry produced the most robust rhythms during early and late gestational ages, respectively.

At E13.5, when spontaneous rhythmic activity was first recorded, motor axons are migrating to and initiating contact with primordial muscle (Allan and Greer 1997). Spontaneous rhythmic bursting commences at a similar stage of chick spinal cord development (Milner and Landmesser 1999). The activity in the prenatal rat preparations persists until E18.5, which encompasses periods of intramuscular nerve branching, establishment of functional neuromuscular junctions, and myotube formation (Allan and Greer 1997; Bennett and Pettigrew 1974; Laskowski and Owens 1994; Noakes et al. 1983; Ross et al. 1987). Motoneurons also undergo some key developmental events during this period, including marked changes in the expression of voltage-dependent channels and neurotransmitter receptor subunits and a significant reduction in numbers via the process of naturally occurring cell death (Harris and McCaig 1984; Martin-Carballo and Greer 1999, 2000, 2001; Martin-Carballo et al. 2000; Ross et al. 1987; Xie and Ziskind-Conhaim 1995; Ziskind-Conhaim 1988). Beyond E17.5–E18.5, the spontaneous rhythms were seldom observed in preparations bathed in solutions containing physiological levels of extracellular potassium (3 mM). The timing of the decline of the widespread rhythms correlates with the inception of respiratory and alternating locomotor rhythmic discharge (Greer et al. 1992; Kobayashi et al. 2001; Ozaki et al. 1996). As illustrated in Fig. 10, the occurrence of the widespread motor discharge characteristic of early embryonic ages interferes with and occludes the respiratory pattern. This would be functionally inappropriate, resulting in the perturbation of fetal breathing movements that are critical for proper development of lung, motoneuron and muscle properties (Greer et al. 1999; Jansen and Chernick 1991; Kitterman 1996).

The underlying mechanisms responsible for the decline in the spontaneous rhythm pattern during late gestation are likely multifactorial. The spontaneous rhythm motor patterns arise from the combination of neurochemical synaptic events
Development of the neuropharmacological control of rhythmic bursts

The developmental profile of the neuropharmacological control of rhythmic bursting can be divided into three periods. At E13.5–E15.5, the spinal networks comprising cholinergic and glycnergic synaptic interconnections are capable of generating rhythmic activity, while GABAergic synapses play a role in supporting the spontaneous motor activity. It appears that each of the neurotransmitters contributes partial excitatory drive necessary for rhythm generation. Removal of anyone of the three can result in an inhibition or failure to achieve threshold for inducing rhythmic motor drive. These results are consistent with those of Milner and Landmesser, who found that cholinergic and GABAergic transmission contributed to the genesis of spontaneous rhythmic bursting during early stages of chick spinal cord development. The potential role of glycine receptors was not reported in that study.

At late stages (E18.5–E21.5), glutamatergic drive acting via non-NMDA receptors is primarily responsible for the rhythmic activity. This is consistent with what has been reported in past studies of chick (Barry and O’Donovan 1987) and rat spinal cord rhythmic activity (Nakayama et al. 1999). Further, a similar developmental transition from cholinergic to glutamatergic-mediated rhythm has been reported for the spontaneous bursting in the retina (Wong et al. 1998).

During the middle stage (E16.5–E17.5), the spontaneous activity involves synaptic drive acting via non-NMDA glutamatergic, nicotinic acetylcholine, glycine, and GABA_A receptors. All of these transmitter systems provide a component of excitation necessary to achieve rhythmicity. Blocking of any one of the receptor species can result in the loss of rhythm that can be subsequently re-established by increasing the endogenous levels of one of the other neurotransmitters. In the development of the neuropharmacological control of rhythmic bursts, there is a major transition in the neurochemical component that occurs by E18.5 that includes a restriction to a purely glutamatergic-mediated rhythmogenesis. There may also be a reorganization and/or pruning of synaptic connections along the rostrocaudal extent of the neuraxis. Electrical coupling persists during late gestation and into the newborn period (Chang et al. 1999; Fulton et al. 1980; Martin-Caraballo and Greer 1999). However, the coupling during the perinatal period is largely restricted to motoneurons innervating the same skeletal muscle (Walton and Navarrete 1991). Data from recent studies by Personius et al. (2002) demonstrate that at earlier embryonic ages, gap junctions among spinal neurons are much more widespread and thus likely to facilitate the spread of spontaneous activity.

FIG. 9. Combinatorial actions of neurotransmitters in the generation of spontaneous rhythmic activity at E16.5. A: interactions of glycine and excitatory amino acids. Strychnine (STR; 10 μM) caused an approximate 50% decrease in the frequency of rhythmic bursts on cervical and lumbar roots. CNQX (6 μM) caused a transient suppression of activity on cervical roots with little effect on lumbar activity. Concomitant application of strychnine (10 μM) and CNQX (6 μM) abolished the rhythm on all ventral roots. B: interaction of glycine and acetylcholine. Strychnine (STR; 25 μM) abolished the rhythm on lumbar ventral roots. The rhythm re-emerged after elevating the endogenous levels of acetylcholine via administration of eserine (2 μM). The rhythm persisted in the presence of strychnine. CNQX, and bicuculline (BIC; 50 μM) and was eventually suppressed by dTC (40 μM). C: interaction of acetylcholine and glycine. The suppression of rhythm by dTC was countered by elevating endogenous glycine levels with sarcosine (2 mM). The rhythm persisted in the presence of dTC, CNQX, and bicuculline (50 μM) and was eventually suppressed by strychnine (40 μM). Population data for paradigms examining the interaction of glycine and acetylcholine receptor activation are illustrated in D (n = 4) and E (n = 5).

FIG. 10. Rhythmic patterns recorded from brain stem-spinal cord preparations pre- and post-inception of respiratory rhythmic discharge. A: recordings from the hypoglossal (XII) and L1 ventral root from an E16.5 preparation showing that the spontaneous rhythm generated in the spinal cord spread to medullary motoneuron pools. B: recordings from an E18.5 preparation. Regular, rhythmic inspiratory activity was present on XII nerve roots except during times of spontaneously generated bursts from the lumbar cord which occluded the inspiratory discharge.
of initiation of these spontaneously generated rhythms under-robust rhythmic motor discharge patterns generated within the choline from motoneurons, or cholinergic release from parti-
motoneurons from arborizations of motoneuronal axons are expressed transiently within the spinal cord that could
rhythmogenesis at early stages is particularly puzzling. There
known. The source of cholinergic synaptic inputs involved in
of the speci

within the developing spinal cord (Ma et al. 1992; Schaffner et
Glycine, GABA, and glutamate are expressed in neurons
within the developing spinal cord (Ma et al. 1992; Schaffner et
Glycine receptors.

Neuroethological approaches to the study of motor development in
is a Senior Scholar of the Alberta Heritage Foundation for Medical Research

REFERENCES

Chub N and O’Donovan MJ. Blockade and recovery of spontaneous rhyth-
mic activity after application of neurotransmitter antagonists to spinal net-
Fühmann B, Miledi R, and Takahashi T. Electrical synapses between mo-
Laskowski MB and Owens JL. Embryonic expression of motoneuron topogra-
Ma W, Behar T, and Barker JL. Transient expression of GABA immuno-


