Interaction Between Disinhibited Bursting and Fictive Locomotor Patterns in the Rat Isolated Spinal Cord

M. BEATO AND A. NISTRI
Biophysics Sector and Istituto Nazionale di Fisica della Materia Unit, International School for Advanced Studies (SISSA), 34014 Trieste, Italy

Beato, M. and A. Nistri. Interaction between disinhibited bursting and fictive locomotor patterns in the rat isolated spinal cord. *J. Neurophysiol.* 82: 2029–2038, 1999. Using a transverse barrier that allowed discrete application of neurochemicals to certain lumbar regions of the rat isolated spinal cord, we studied the intersegmental organization of rhythmic patterns recorded extracellularly from ventral roots and intracellularly from single motoneurons. Fictive locomotor patterns were elicited by serotonin (5-HT) and/or N-methyl-D-aspartate (NMDA) or high K⁺ solution applied to the rostral or caudal lumbar region of the cord. Neither 4-aminopyridine nor Mg²⁺-free solution shared this property. Coapplication of strychnine and bicuculline (blockers of fast synaptic inhibition) to the caudal part elicited slow bursting in the whole cord. These bursts could trigger episodes of fictive locomotion patterns in the rostral roots. When the rostral region was exposed to 5-HT and/or NMDA (during continuous application of strychnine and bicuculline caudally) a standard locomotor-like pattern was generated during each interburst interval and was surprisingly expressed with its typical pattern alternation even in the caudal area despite the local presence of strychnine and bicuculline. Midsagittal splitting of the caudal region did not change this alternating pattern, indicating that it was driven by rostral regions above the Midsagittal splitting. However, rhythmic behaviors were pharmacologically induced. For this purpose we simultaneously blocked synaptic inhibition in the caudal region and applied locomotor substances to the rostral portion.

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.

INTRODUCTION

In the neonatal rat spinal cord, in vitro locomotor-like rhythmic activity (usually termed as “fictive locomotion”) is generated by a ventrally located premotoneuronal network (Kjaerulff and Kiehn 1996) called central pattern generator (CPG) and typically activated by agents such as serotonin (5-HT) (Cazalets et al. 1992), N-methyl-D-aspartate (NMDA) (Kudo and Yamada 1987), or high K⁺ solutions (Bracci et al. 1998). The CPG is thought to be a modular assembly of functional units (unit burst generators) individually endowed with oscillatory activity transmitted to different muscles (Cheng et al. 1998; Edgerton et al. 1976; Grillner 1981; Grillner et al. 1991). In this neuronal network the coupling among unit burst generators would then change in accordance with preprogrammed patterns to be expressed as various stereotypic motor activities (Grillner 1985; Mortin and Stein 1989; Svoboda and Fetcho 1996). A major question is thus the nature of the CPG mechanisms that allow switching a certain motor pattern on. Different excitatory agents cannot apparently induce identical motor patterns as shown by Kiehn and Kjaerulff (1996), who reported that for instance the ventral root (VR) rhythmic activity induced by dopamine closely resembles the coordinated muscle contractions observed during locomotion, whereas the one elicited by 5-HT is closer to the muscle activity during swimming. In general, all these patterns are characterized by left-right and flexor-extensor alternating oscillations (at 1- to 5-s period, recorded intracellularly from single motoneurons or extracellularly from VR), although various muscle groups are differentially involved according to distinct motor behaviors.

Block of synaptic inhibition does not prevent rhythmicity because application of selective antagonists of GABA_A (Kremer and Lev-Tov 1997) or glycine receptors (Cowley and Schmidt 1995) can synchronize the alternating oscillations detected in antagonist motor pools. Indeed, full suppression of fast synaptic inhibition per se induces slow, synchronous bursting (~30-s period) (Bracci et al. 1996a,b), which can be accelerated to the range of standard locomotion periods by the same agents used to induce locomotion (Bracci et al. 1996a,b). Furthermore, disinhibited bursting shares with locomotor patterns the same sensitivity to block of either class of ionotropic glutamate receptors (Beato et al. 1997; Bracci et al. 1996a,b) and the same anatomic location (Bracci et al. 1996b; Kjaerulff and Kiehn 1996). These common features raise the possibility that disinhibited rhythmic bursting might be generated by the same oscillators that produce locomotor-like activity when their inhibitory connections are intact. One alternative possibility is that distinct networks are responsible for fictive locomotion and disinhibited rhythmicity.

The aim of the present experiments was to test whether a certain rhythmic pattern generated at a given segmental level may influence the operation of the CPG in adjacent spinal segments, if this interaction is bidirectional and depends on intact inhibitory circuits. As a tool to investigate these issues, the rat spinal cord in vitro was partitioned with a vertical barrier lowered onto the cord at lumbar level (Cazalets et al. 1995). Such a method allowed us to study the interaction between distinct regions of the spinal cord in which different rhythmic behaviors were pharmacologically induced. For this purpose we simultaneously blocked synaptic inhibition in the caudal spinal cord and applied locomotor substances to the rostral portion.
METHODS

Standard procedures were used to obtain spinal cord preparations of neonatal Wistar rats (0–4 days old) (Ballerni et al. 1997; Bracci et al. 1996a). The isolated spinal cord was fixed to the bottom of a recording chamber (~5 ml volume) and continuously superfused (~5 ml/min) with artificial cerebrospinal fluid (ACSF). The composition of the ACSF for both dissection and recording was (in mM) 113 NaCl, 4.5 KCl, 1 MgCl2·7H2O, 2 CaCl2, 1 NaH2PO4, 25 NaHCO3, and 11 glucose, gassed with 95% O2-5% CO2, pH 7.4. All agents were superfused at the concentrations mentioned in the text.

The recording chamber contained a transverse barrier that enabled us to superfuse adjacent spinal regions separately via inlets at the rostral and caudal end of the bath. Excess solution outflowed via two independent drains. The barrier was made up by two asymmetric components always placed at the same segmental position, namely the junction between L4 and L3 segments. The spinal cord was first pinned onto the cord in correspondence of the L3–L4 junction to complete the barrier with an inverse U-shaped recess was subsequently lowered up. A thin layer of petroleum jelly (Vaseline) was placed at the midline of the recording chamber across its whole width, and the plastic strip was then gently advanced through the Vaseline, which sealed it to the bottom of the recording chamber. A solid silicon barrier with an inverse U-shaped recess was subsequently lowered onto the cord in correspondence of the L1–L2 junction to complete the functional separation of the tissue in rostral and caudal portions. The thickness of the barrier was always less then one-third of the length of a segment. All leaks across the barrier were finally sealed up with Vaseline. The water tightness of the barrier was then tested by adding Phenol Red to one compartment and visually checking for any leakage of fluid from one compartment to the other. As a further precaution before starting the experiments, ACSF in one compartment was completely removed; the barrier was considered effective only if no fluid leak could be detected in this condition for at least 1 min. Leak tests were repeatedly performed during each experiment: whenever a leak was apparent, the experiment was interrupted. Caudal mid sagittal lesions were performed on a number of preparations after having carried out control tests with the split bath configuration. In this case, test substances were flushed out and replaced with control ACSF while the lower part of the spinal cord was bisected with a miniature blade. After checking that the barrier had remained leak-proof, test substances were applied again.

DC-coupled extracellular recordings were simultaneously performed from four VRs, namely pairs of L2 and L5 VRs, with tight fitting suction pipettes containing an Ag/Ag-Cl pellet. Intracellular recordings were obtained from L5 motoneurons functionally identified by stimulation of the corresponding VR. Sharp electrodes (40–60 MΩ) were filled with 3 M KCl, and recordings were performed in current-clamp configuration. During intracellular experiments VR activity was usually monitored from three VRs only. All electrodes were mounted on Narishige micromanipulators. Signals via extracellular electrodes were led to a custom made four channels AC/DC amplifier (1,000 gain), monitored on a Gould chart recorder, and digitized and recorded on a DAT tape for further off-line analysis. Period and relative phase measurements were performed over at least 25 randomly chosen cycles to minimize short-term sampling bias. Period was defined as the time between the onset of two cycles of locomotor activity, whereas phase between two roots was defined as the latency for the onset of a cycle in one root during the cycle of the other root, divided by the period and expressed in angular degrees whereby 180° represent complete phase alternation and 0 or 360° full phase coincidence (Kjaerulff and Kiehn 1996). All data were quantified as means ± SD; statistical significance was assessed with Student’s t-test (unless otherwise stated) or ANOVA. Forty-two spinal cord preparations were used for the present study. All drugs were purchased from Sigma.

RESULTS

Fictive locomotion induced by 5-HT, NMDA, or high potassium applied either at upper or lower lumbar level

As previously reported by Cazalets et al. (1995, 1996), selective application of 5-HT and NMDA to rostral segments elicited a locomotor pattern that could be detected from rostral as well as caudal VRs. Figure 1 (top left) shows simultaneous recordings from four VRs in the presence of 10 μM 5-HT and 2 μM NMDA when drugs were bath applied to the rostral compartment only (segments from T1 to L3). Within the first 2 min of application, we observed an upward baseline shift and a pronounced thickening (not shown) in the recordings of L2 VRs, which predominantly supply hindlimb flexor muscles (Kiehn and Kjaerulff 1996). Such an effect was undetectable in recordings from L5 VRs [mainly supplying hindlimb extensor muscles (Kiehn and Kjaerulff 1996)], thus further confirming that drugs could not diffuse across compartments. After 4 min superfusion with 5-HT and NMDA, a rhythmic alternating pattern appeared in L2 and L5 pairs, with typical phase-lock of IL5→rL5 and rL5→IL5 (Fig. 1, top left; period 3.5 ± 0.9 s). In 42/42 preparations in which the barrier was placed at the L3–L4 junction, oscillatory activity was detected in both rostral and caudal segments when the rostral cord only was exposed to various concentrations of NMDA (2–8 μM), 5-HT (5–30 μM), or a combination of these two agents. Because activation of the rostral segments can induce an alternating pattern in the more caudal portion of the spinal cord (Cazalets et al. 1995; Cowley and Schmidt 1997), it was necessary to investigate whether well coordinated locomotor patterns could equally be induced by bath application of excitatory agents to the caudal segments only. An example is shown in Fig. 1 (top right); caudal application of 10 μM 5-HT and 2 μM NMDA induced oscillations characterized by 2.8 ± 0.9 s period, alternating between left/right and flexor-related/extensor-related motor pools. In six of eight preparations, a combination of 5-HT and NMDA applied to caudal segments induced alternating oscillations in all four recorded VRs. It is known that application of high K+...
concentrations can induce locomotor-like patterns in the entire spinal cord (Bracci et al. 1998); it was thus tested whether a K⁺-elicited excitation restricted to caudal segments could also evoke alternating activity spreading to rostral segments. Figure 1 (bottom left) shows that well coordinated locomotor activity could be induced by application of 8.5 mM K⁺ (4.2 ± 1.3 s period). Similar results were observed in three of five preparations. It is worth noting that rostral drug applications induced locomotor patterns in all preparations tested and over a wide range of concentrations. On the contrary, caudal applications induced persistent patterns within a very narrow range of concentrations (2.5 μM 5-HT, 1 μM NMDA or 0.5 mM K⁺ for each preparation) and in some cases failed to elicit any rhythmic activity, thus confirming the greater sensitivity of rostral segments to locomotion-inducing agents (Kjaerulf and Kiehn 1996). Further investigations were undertaken to clarify whether any form of widespread excitation of the caudal network by treatments known to induce epileptiform activity could trigger episodes of locomotor activity in the rostral segments. To this end Mg²⁺-free Krebs solution was superfused via the caudal bath in six preparations. Mg²⁺-free solution induced paroxysmal bursts of activity lasting 1–10 s and with irregular frequency. At steady state (>20 min from removal of Mg²⁺) the onset of each burst was invariably synchronous among the four recorded roots (see arrow in Fig. 1, bottom right). For a small (15%) percentage of the analyzed events only, L₂ VR records were asynchronous. Such a phenomenon was observed occasionally during longer bursts (>5 s duration) and cannot be related to locomotor-like activity, because it lacked any rhythmicity. It is possible that the strong excitatory input received by the rostral segments might have transiently activated crossed inhibitory connections that prevented contralateral pools of motoneurons from firing simultaneously.

Further tests were performed by applying 50 μM 4-aminopyridine (4-AP) to the caudal bath: 4-AP induced an irregular bursting activity in VRs (not shown), characterized by frequent events of short-duration (between 1 and 2 s). In the three preparations tested, such bursts were always synchronous among the four recorded VRs and never triggered any alternating oscillations.

In summary, alternating oscillations could be detected in rostral segments following activation of caudal ones by agents that are commonly used to induce fictive locomotion, such as 5-HT, NMDA, or high K⁺ solutions.

**Bursting activity in the caudal spinal cord propagated to the rostral part and triggered well-coordinated locomotor episodes**

Because caudal application of excitatory agents (5-HT, NMDA, or high K⁺) could induce fictive locomotion, it was tested whether rostral locomotor episodes could also be triggered by spontaneous bursting resulting from pharmacological block of synaptic inhibition in caudal segments. The working hypothesis was that prolonged depolarization during each burst could spread rostrally to activate the portion of CPG in which inhibitory synapses were still operational.

In all these experiments, strychnine and bicuculline were applied at saturating concentrations [respectively, 1 μM and 20 μM (Bracci et al. 1996b)] via the caudal bath while recordings were obtained from L₅ and L₂ VRs. The early period (10–15 min) of strychnine and bicuculline application is characterized by random bursts that gradually turn into regular activity (Bracci et al. 1996a), which in the present study had an average period of 34 ± 12 s and an average burst duration of 16 ± 8 s. Figure 2A shows an early single burst recorded from L₅ VRs (top pair of traces) characterized by prolonged baseline deflection and tonic firing. This burst could be synchronously detected in the rostral L₂ segments also (bottom pair of traces in Fig. 2A) and was accompanied by alternating oscillations lasting ~25 s. The period (3.2 s on average) of such oscillations was within the range of typical locomotor frequencies and the phase shift between left and right L₂ VRs was 171 ± 22°, confirming their alternation. In 42/42 preparations during the early phase of application of strychnine and bicuculline, long-lasting (20–30 s) episodes of alternating activity were ob-

---

**FIG. 2.** Bursts induced in caudal L₅ segments evoke alternating oscillations in rostral L₂ ones. A: early burst (3 min after onset of drug application) induced by superfusion of the caudal compartment with strychnine (str) and bicuculline (bic). Burst onset is synchronous over the 4 recorded ventral roots (VRs): L₅ VRs fire tonically together during the burst, whereas L₂ VRs the plateau phase of the burst is accompanied by alternating oscillations. B: steady-state rhythmic activity 15 min from onset of drug application (different preparation from A) shows that regular rhythmic bursting in L₅ induces some alternating oscillation in the contralateral L₂ and L₁ VRs (extracellular traces are AC-coupled). Intracellular recording from a L₂ motoneuron (resting potential ~78 mV) shows that firing in the homolateral rL₁ VR is in phase with membrane potential oscillation in the motoneuron. Note also that during the interburst interval, spontaneous synaptic activity is detected. The underlined burst in B is represented on an expanded time scale in C (see arrows). Extracellular DC-coupled recordings allow to observe baseline oscillations in VRs recordings.

Downloaded from http://jn.physiology.org/ by IP 10.220.33.4 on September 27, 2016.
erved in the rostral segments in correspondence with the caudal bursts, thus demonstrating that disinhibition-activated caudal networks could invariably trigger the rostral locomotor CPG.

Figure 2B (different preparation from Fig. 2A) represents, in the presence of caudal strychnine and bicuculline, steady state bursting intracellularly recorded from a rostral L2 motoneuron (top trace), two rostral VRs (L1 and L2) and one caudal L5 VR. In this example all extracellular records were AC-coupled. Bursts in L5 VR consisted of sustained excitation whereas rL1 VR activity was characterized by alternating firing (usually no more than 2 or 3 cycles, with period ranging between 3 and 5 s). Firing in rL1 VR was always synchronous with voltage oscillations of the ipsilateral rL2 motoneuron (top record in Fig. 2B). The top trace of Fig. 2B depicts the intracellularly recorded bursts (22 ± 4 mV amplitude and ~150-ms rise time from baseline to peak). During the interburst interval, depolarizing events (that occasionally brought the neuron above threshold for firing) due to ongoing spontaneous synaptic activity frequently appeared. Motoneuron membrane potential (−78 mV) was not changed by application of strychnine and bicuculline (similar results were obtained in 23 cells from 17 different preparations). For each burst membrane potential oscillations (5- to 10-mV amplitude) were accompanied by an increase in firing during the rising and top phase of each oscillation. This phenomenon is depicted with expanded time scale in Fig. 2C. Extracellular DC coupled records show that motoneuronal oscillations (top record) were out of phase with the contralateral IL2 VR and in phase with the ipsilateral rL1 VR. In 27/42 preparations, such oscillatory episodes were consistently observed during the whole period of application, whereas in the remaining 15 preparations, alternating episodes were observed during early bursting, and only occasionally (but still time locked with the occurrence of a burst episode) after the disinhibited rhythmic activity reached steady state.

Simultaneous induction of disinhibited rhythm and fictive locomotion

A burst of activity in the caudal network could propagate to rostral segments and transiently activate the CPG for locomotion, suggesting that caudal commands could drive the main rostral CPG. Did this sort of entrainment prelude activation of the rostral CPG by the usual locomotor agents applied locally? This issue was tested in a series of experiments.

A locomotor pattern was induced by rostral application of 5 μM 5-HT and 4 μM NMDA and consisted of alternating activity clearly detected in rostral and caudal VRs (Fig. 3A). Phase relationships (open circles for 5-HT and NMDA treatment) were calculated for pairs of homolateral VRs (left or right IL2–L5; see bottom 2 polar plots in Fig. 4) and contralateral VRs (l, rL2 and l, rL5; see top polar plots in Fig. 4). Each dot represents the same preparation, the phase shift of 20–25 randomly chosen cycles recorded for the indicated pair of VRs (see METHODS). As data points were clustered around 180°, it is apparent that there was sustained alternation of activity between the indicated root pairs.

Application of strychnine and bicuculline to the caudal bath (Fig. 3B) induced bursting activity in L5 VRs as shown by the large and persistent depolarizations with superimposed firing (Fig. 3B, top 2 traces). Such a bursting activity could also be detected in the rostral L2 VRs (Fig. 3B, bottom 2 traces) as smaller amplitude sustained depolarizations synchronous with L2 bursts and intermingled with the ongoing fictive locomotor pattern due to the continuous presence of 5-HT and NMDA. During the plateau phase of each burst in all four roots, fictive locomotion was absent. When it resumed, it maintained its expected alternation between L2 VRs (due to intact inhibitory circuitry), but, rather unexpectedly, alternation also appeared between L5 VRs despite the presence of strychnine and bicuculline. This observation is clearly shown in Fig. 3C in which the four roots patterns are recorded on a faster time base. The fact that the L5 segments possessed an alternating pattern in the presence of pharmacological block of inhibition suggested that this activity was driven by the rostral segments in which inhibition was still functional. Further proof for this proposal was provided by the observation that alternation was also maintained along the rostrocaudal axis between the homolateral pairs of L2–L5.

Phase relationship data for the activity present during caudal strychnine and bicuculline application plus rostral 5-HT and NMDA application are shown as filled circles in Fig. 4. Note
that in this preparation, like in the other 41 tested, the phase values between contralateral L2 VRs completely overlapped those found in the absence of strychnine and bicuculline (compare filled and open circles, respectively). A similar overlap of phase values was observed for the other three pairs recorded in this experiment. Such oscillations were clearly detectable in L5 VRs in 25/42 preparations treated with 5-HT and/or NMDA. In 19/25 preparations, L5 oscillations were out of phase with homolateral L2 activity during fictive locomotion and so remained after caudal application of strychnine and bicuculline. In 6/25 spinal cords the phase between homolateral L2–L5 VRs was converted from alternating to synchronous after application of strychnine and bicuculline to the caudal bath. In the same six experiments rL2–IL2 and rL5–IL5 phases remained, however, in alternation (175 ± 21° and 169 ± 18° for l, rL2; 186 ± 26° and 195 ± 19° for l, rL5 for data before and after strychnine and bicuculline, respectively).

In summary then, in the majority of cases, alternation between VR activity during chemically induced locomotion was preserved between L2 and L5 VRs, despite pharmacological block of synaptic inhibition in caudal segments.

**Midsagittal lesion and partitioned spinal cord configuration**

The novel observation of clearly alternating oscillations in the caudal spinal cord, despite the presence of saturating concentrations of strychnine and bicuculline, raised the possibility that caudal alternation at L5 segmental level was due to inhibitory left-right synaptic transmission mediated by receptors insensitive to these convulsants. To test this hypothesis, we performed a midsagittal lesion (along the rostrocaudal axis) to bisect completely the spinal segments located in the caudal bath (see dashed line in Fig. 5C). Figure 5A shows the control locomotor pattern induced by rostral application of 5 μM 5-HT and 4 μM NMDA (3.2 ± 0.3 s period). Following wash out of 5-HT and NMDA, caudal application of strychnine and bicuculline elicited bursting activity that could be detected in the four recorded VRs (not shown). Steady-state bursting induced by strychnine and bicuculline was characterized by a period of 32 ± 8 s. Each burst comprised oscillations that were fast (200 ms period), synchronous, and occurred after a 20- to 40-min application of strychnine and bicuculline as previously described (Bracci et al. 1996a). In the early stage of strychnine

![FIG. 4. Diagram of the phase relationship among the recorded VRs before (○) and after (●) application of strychnine and bicuculline. Measurements refer to the preparation of Fig. 3. Each dot represents a single phase measurement between 2 cycles.](http://jn.physiology.org/)

![FIG. 5. Alternation in disinhibited segments persists following longitudinal lesion. A: locomotor pattern evoked by rostral application of 5 μM 5-HT and 4 μM NMDA. B: after application of strychnine and bicuculline to the caudal bath, the pattern between bursts is still alternating in the 4 recorded VRs. C: schematic representation of the midsagittal lesion that was performed from conus medullaris to L4 segment. D: the midsagittal lesion does not change alternation between VRs.](http://jn.physiology.org/)
Phase plot of VR activity

![Diagram](image)

**Fig. 6.** Diagram of phases between VRs during rostral fictive locomotion (○), after application of strychnine and bicuculline (●), and after midsagittal lesion (⊗) for the experiment described in Fig. 5. Each dot represents a single measure of the phase shift between oscillations in VRs for the preparation of Fig. 5.

Synergy between caudal bursts and chemical activation of the rostral segments

An interesting question was whether caudal bursts could trigger rostral locomotor activity when the concentration of chemical agents in the rostral bath was subthreshold for fictive locomotion. This type of facilitation could in fact allude to a common network mechanism responsible for disinhibited bursting and locomotor patterns. For this purpose we applied increasing concentrations of NMDA to the rostral compartment, while strychnine and bicuculline were applied to the caudal end. Single bursts in the presence of different concentrations of NMDA are shown in Fig. 7. With control solution in the rostral bath, and with strychnine and bicuculline in the caudal bath (Fig. 7, top left), alternating activity was virtually absent. When 1 µM NMDA was applied rostrally (Fig. 7, top right), alternating oscillatory activity was apparent during the entire double burst in the rostral VRs and then stopped. As the NMDA concentration was increased to 2 µM (Fig. 7, bottom left), alternating oscillations appeared more pronounced, started during the burst, and persisted after the burst for ~15 s. Application of 3 µM NMDA (Fig. 7, bottom right) induced a regular pattern of rostral alternating activity that was suppressed by the onset of a burst and recovered during its decay phase, persisting until a new burst episode occurred. Similar results were observed in six different preparations. From these records it appears that increasing concentrations of NMDA were also associated with a smaller amplitude of the rostral bursts elicited by strychnine and bicuculline. The origin of this reduction in burst amplitude when NMDA (with or without 5-HT) was applied was explored with intracellular recordings from L₂ motoneurons (n = 8). Burst peak amplitude, which in control conditions was 41 ± 6 mV, in the presence of NMDA and/or 5-HT was significantly reduced (28 ± 7 mV; P < 0.01 ANOVA test), whereas it was restored to a value not significantly different from control (38 ± 6 mV) by injection of negative DC current (n = 8 cells). In these tests the average depolarization induced by NMDA and/or 5-HT was 23 ± 7 mV. The observation that burst amplitude could return to...
DISCUSSION

The present study provides a novel description of the intersegmental interaction between rhythmogenic networks in the neonatal rat spinal cord. Data obtained during simultaneous, focal activation of caudal or rostral networks, which expressed disinhibition-induced rhythm or fictive locomotion respectively, enabled us to propose a wiring diagram to account for the operation of burst generators in distinct segments of the spinal cord.

Selective activation of caudal and rostral rhythmogenic networks

Activation of either rostral or caudal portions of the spinal cord by 5-HT and/or NMDA (or high K$^+$ solution) induced rhythmic alternating patterns that could be detected from both rostral (flexor-related) and caudal (extensor-related) VRs. The present findings thus support the conclusion that the CPG for locomotion is distributed along the rostrocaudal axis (Kjaerulff and Kiehn 1996).

An important finding of the present study is that rostral as well as caudal segments can be topically activated by 5-HT, NMDA, or high K$^+$ to produce locomotor patterns that spread to the other portion of the spinal cord not exposed to such substances. The present observations accord with data from Kjaerulff and Kiehn (1996), who used a mixture of NMDA and 5-HT but differ from those of Cazalets et al. (1995), who used a similar mixture of these agents. It is worth noting that Cazalets et al. (1995) used a substantially higher concentration of the locomotor pattern inducing drugs that were thus potentially liable to produce large and sustained depolarization of spinal neurons detected as tonic firing instead of rhythmic activity. Cowley and Schmidt (1997) have reported that, when applied caudally, 5-HT could not induce locomotor patterns recorded from peripheral nerves, whereas NMDA could elicit alternating patterns apparently unsuitable to locomotion. This result led Cowley and Schmidt to conclude that the locomotor CPG has a very rostral location at the thoracolumbar border. Our observations of a caudally originated motor pattern with 5-HT plus NMDA administration could not be explained simply on the basis of this latter agent because the NMDA concentration was usually below rhythm generating threshold (cf., Kjaerulff and Kiehn 1996). Our data therefore agree with those of Kjaerulff and Kiehn (1996) that the caudal areas are rhythmogenic, although less so than the rostral one. Furthermore, we have noted that very small changes in caudally applied drug concentrations were necessary to elicit a stable pattern without precipitating it into sustained tonic firing. Perhaps the experiments reported by Cowley and Schmidt (1997) did not rely on such a critical dosage of locomotor agents, which were often applied in much higher concentrations than the present ones.
The bidirectional propagation of locomotor patterns is compatible with the theory of unit burst generators distributed along the lumbar spinal cord (Grillner 1981). Hence the portions of the locomotor network that were not exposed to the excitatory agents might have received an excitatory drive (from the activated regions) that triggered the locomotor program. Phase alternation of VR outputs would be determined by the inhibitory/excitatory connections between burst generators supplying different muscles.

**Interaction between caudal and rostral networks**

Application of strychnine and bicuculline to the caudal bath invariably induced rhythmic bursting activity in all preparations tested. Onset and steady-state activity recorded from L5 VRs were similar to those observed during block of inhibition in the entire spinal cord (Bracci et al. 1996a). Individual bursts were usually characterized by a plateau phase, followed by several high-frequency (5–10 Hz) intraburst oscillations synchronously detected in L5 VRs. It is noteworthy that, during application of strychnine and bicuculline to the whole spinal cord, the interburst interval is characterized by a complete absence of synaptic activity (Ballerini et al. 1997; Bracci et al. 1996a, 1997). On the contrary, in the present conditions spontaneous synaptic potentials were frequently detected from L2 motoneurons during the interburst intervals, to indicate that the ongoing synaptic activity remained intact in the rostral networks, as strychnine and bicuculline were applied to the caudal end only.

The principal finding was, however, that bursts induced in caudal segments could trigger alternating, locomotor-like oscillations in L2 VRs. The excitation produced by each burst apparently propagated to the more rostral segments to activate the rostral unit burst generators. Although the input received by the rostral units was presumably synchronous for the left and right generators, the presence of intact inhibitory connections between antagonist units might have allowed the CPG to respond to this signal with left-right pattern alternation. For this phenomenon to occur, it is necessary that the strength of the inhibitory left-right connections prevailed over the synchronous excitatory input received from the caudal segments (Kremer and Lev-Tov 1997) so that antagonist motor pools never fired simultaneously. A rostral rhythmic alternating pattern could not be evoked by caudally applied Mg²⁺-free solution or 4-AP, suggesting that this response was not a nonspecific stereotype of the spinal network to any form of excitatory event.

The observation that disinhibition-induced bursting activity in caudal segments could trigger locomotor episodes in the rostral ones suggests that the network responsible for disinhibited rhythm impinges (directly or indirectly) on the CPG for locomotion. This possibility was confirmed by the experiments in which caudal application of strychnine and bicuculline was followed by rostral application of locomotion-inducing agents. In such conditions, persistent locomotor oscillations in L2 VRs were always changed by the occurrence of a burst through a repertoire that ranged from alteration in period to full suppression. In fact, discrete changes in the concentrations of the inducing agents in the rostral bath (i.e., of the amount of ongoing excitation induced in the rostral segments) elicited alternating oscillations exclusively during the burst, or in its decay phase, or in the interburst time, or even during and after bursts with related changes in periodicity. Suppression of locomotor-like oscillations during bursts (a commonly observed phenomenon) was likely due to excessive depolarization of L2 motoneurons because excitatory synaptic inputs coming from the caudal network summated with the direct depolarization induced by 5-HT and/or NMDA as demonstrated with intracellular recording. In addition, the input from the caudal network to the CPG itself might have been so strong to inactivate it [as observed when high doses of K⁺ or NMDA were applied to the whole spinal cord (Bracci et al. 1998)].

**Persistence of alternating oscillations despite application of strychnine and bicuculline**

Application of strychnine and bicuculline to the caudal bath to impair reciprocal inhibition between left and right motor pools (Bracci et al. 1996b) should have canceled any phase alternation of oscillations between the L5 VRs during fictive locomotion rostrally induced by 5-HT and NMDA. On the contrary, in the majority of preparations tested, locomotor-like oscillations characterized by left-right alternation were surprisingly found to persist in L5 VRs. Such oscillations were observed during the interburst intervals only, because the occurrence of a disinhibited burst invariably occluded them.

The presence of alternating oscillations in spinal cord segments, in which fast synaptic inhibition is demonstrably blocked (see Bracci et al. 1996b; Rozzo et al. 1999), might suggest that at the same segmental level inhibition was mediated by activation of receptors other than GABA_A and glycine ones. Possible candidates for this role might be GABA_B or GABA_C receptors (Rozzo et al. 1999), 5-HT_1 receptors (Beato and Nistri 1998; Elliott and Wallis 1992), or metabotropic glutamate receptors, that are known to depress synaptic transmission in many different systems (Scanziani et al. 1997; van den Pol et al. 1998). If this were the case, regardless of the particular transmitter system involved, alternation should have been disrupted following midsagittal lesion of the caudal segments as each side of the spinal cord would have become unable to produce reciprocal inhibition. Contrary to this possibility, lesion experiments showed that the alternating pattern remained unchanged despite full bisection of the spinal cord from _conus medullaris_ to L₅–L₃ segments. It seems thus likely that motor output alternation in the presence of strychnine and bicuculline in a sagittally split segment could only originate from segments unaffected by lesioning or pharmacological blockers of inhibition, namely those rostrally located beyond the transverse barrier.

**Wiring diagram for the locomotor CPG**

The present observations add further complexity to existing schemes of the spinal locomotor CPG (Cazalets et al. 1995; Cowley and Schmidt 1997; Kjaerulff and Kiehn 1997) and require a new minimal wiring diagram (Fig. 9). The building blocks of the spinal CPG remain as a series of unit burst generators (Grillner 1981) distributed along the rostrocaudal axis (these are shown as shaded squares in Fig. 9). The transverse barrier used in the present experiments is shown to divide the serially arranged burst generators into caudal and rostral ones. Motoneuronal pools are depicted as separate gray circles.
Because they are not an intrinsic part of the CPG as they merely provide the motor output (programmed by the CPG; note excitatory signals from unit generators to motoneurons) to muscles (Grillner 1981). The scheme of Fig. 9 is further simplified, for sake of clarity, by assuming just extensor motor pools in the caudal portion and flexor motor pools in the rostral one. Each burst generator supplies its contralateral equivalent and its homolateral antagonist with weak excitatory and strong inhibitory connections (see Kremer and Lev-Tov 1997). A scheme that relies on serially arranged connections alone remains, however, insufficient to explain why activation of certain unit generators can recruit distant ones with appropriate phase lock between them and between distant segments as indeed observed with the bidirectional ability of 5-HT or NMDA to evoke fictive locomotion regardless of the lumbar region of their application. One possibility is to assume that a strong excitatory signal from a given segment is conveyed through a major interlinking pathway (or via relay interneurons) to adjacent segments that would then be prompted to express their rhythmic output. Phase interlocking between segments might then be due to reciprocal inhibition. This hypothesis cannot, however, explain the typical locomotor-like rhythm with standard phase alternation in rostral and caudal segments despite the presence of strychnine and bicuculline caudally. This phenomenon persisted even after longitudinal splitting of the lower spinal cord. We are therefore inclined to believe that burst generators in distinct segments were connected by strong, crossed pathways as indicated in Fig. 9. Interssegmental cross linking would therefore provide the excitatory connections necessary to support rhythmic alternation in an area where inhibition was blocked. In addition, the crossover of this pathway should have taken place above the transverse barrier because lower segments surgically separated were still driven in a fully alternating fashion despite the splitting lesion and block of inhibition. The currently proposed circuit should be viewed as an operational scheme to be tested with computer modeling to reconstruct the CPG operation and predict certain pattern activities.

**Is a single CPG network sufficient to generate locomotor patterns and disinhibited rhythms?**

The still incomplete understanding of the identity of the interneurons that make up the locomotor CPG precludes a clear answer to this issue. Nevertheless, a number of observations concur to suggest that the single network hypothesis is the simplest to account for the experimental findings of the present (and other) studies. In particular, the intrinsically slow periodicity of disinhibited bursting is converted into a relatively fast (1–2 s) period (lacking alternation) by typical locomotor agents like 5-HT or NMDA (Bracci et al. 1996a,b); such an identical sensitivity of both patterns is best explained by a single network arrangement dynamically modulated and wired to produce different rhythms and patterns. Lesion studies show that the disinhibited rhythm and the locomotor pattern are similarly located in the anterior quadrant of the spinal cord (Bracci et al. 1996b; Kjaerulff and Kiehn 1996). In addition, both locomotor patterns and disinhibited rhythms can be expressed by the same rostral or caudal segments (Kjaerulff and Kiehn 1996; M. Beato, unpublished data and the present study). Furthermore, the strong synergy between disinhibited rhythm and locomotor pattern demonstrated by the present study (bursts could be the threshold crossing process to elicit a locomotor pattern or to modulate its periodicity) is also suggestive of a common CPG origin.

We thank Drs. Ole Kiehn and Ole Kjaerulff of the Panum Institute, Copenhagen, Denmark, for kindly demonstrating the transverse split bath arrangement used for the present study. This work was supported by grants from the Ministero dell’ Università e della Ricerca Scientifica e Tecnologica (co-finanziamento ricerca) and from the Istituto Nazionale Fisica della Materia. Address for reprint requests: A. Nistri, Biophysics Sector and Istituto Nazionale di Fisica della Materia Unit, International School for Advanced Studies (SISSA), Via Beirut 4, 34014 Trieste, Italy.

Received 20 April 1999; accepted in final form 18 June 1999.

**REFERENCES**


