Neurobiological Bases of Rhythmic Motor Acts in Vertebrates

Sten Grillner

Summary. The general principles governing the nervous control of innate motor acts in vertebrates are discussed. Particular consideration is given to the control of locomotion in both mammals and lower vertebrates. One in vitro model of the lamprey central nervous system has been developed. It can be maintained in vitro for several days and the motor pattern underlying locomotion can be elicited in isolated sections of the spinal cord. These findings now allow a detailed analysis of the underlying neural mechanisms. The hypothesis that different parts of the network controlling locomotion can be used in a variety of other motor acts, including learned ones, is reviewed.

A characteristic of the human motor system is its high degree of versatility, which far surpasses that of other animals, even though particular types of movements can be carried out in individual species with a much higher degree of skill and perfection. A motor act initiated by will can be regarded as voluntary regardless of whether it results in a spoken word, an eye movement, or a step. Voluntary movements can thus be generated by both innate and learned motor programs. The same movement pattern used in a voluntary movement may in principle be used in a different context as part of an automatic movement sequence. Whether a motor act should be regarded as voluntary or automatic is not primarily a matter of which type of movement is involved, but rather the context in which it occurs (*1–3*).

In this article I consider genetically inherited, or innate, movement patterns. I use this expression to denote a motor act coordinated by neural circuits that develop as the nervous system matures before or after the birth of the animal. Such acts range from simple withdrawal reflexes to complex behavioral sequences used when an animal gives birth to and nurses its offspring. In this category fall the basic patterns of coordination humans use to express emotions. In fact, Darwin (*4*) noted that children born blind will smile as beautifully as children with normal eyesight. An innate movement pattern need not be very stereotyped, and it can be modified by sensory input for adaptation to the immediate environment as well as by experience.

Behavioral Repertoire of Decorticate and Decerebrate Vertebrates

In mammals as well as lower vertebrates complex patterns of behavior can be performed by animals lacking a cerebral cortex. Neonatally decorticated cats move around in a way that, to the casual observer, does not appear different from that of intact cats (*2, 5*). The locomotion appears goal-oriented, for such animals seek and eat food. They even perform exploratory forelimb movements. Decorticated female cats can copulate, give birth, and care for their newborn. A variety of apparently meaningful movements can thus be initiated and performed in cats lacking a cortex. From these results one may of course not conclude that, under normal conditions, the cortex is unimportant for initiation of such movement patterns. On the other hand, it is important that, with only the basal ganglia intact, the central nervous system (CNS) has the capacity to initiate and generate a complex movement repertoire adapted to the needs of the animal. This is in striking contrast to animals with a lesion at a lower diencephalic level that leaves only the mesencephalon and lower brainstem intact. Such animals can be made to walk, chew, or swallow, but the movements are stereotyped and machine-like and are no longer adapted to the needs of the animal or its environment (*2, 5*).

Let us now consider one type of movement, locomotion. In 1966 Shik, Orlovsky, and Severin demonstrated that decerebrate cats can be made to walk if specific brainstem areas are subjected to repetitive electrical stimulation at low strength (10 to 20 μA) (*6*). Moreover, if the stimulation strength is increased, the speed and form of locomotion can be modified from a slow walk to a trot to a gallop. A simple type of stimulus can thus elicit and control a complex pattern of behavior involving continuous coordination of the four legs and spine and thus a vast number of joints and muscles in different combinations.

One brainstem area, the mesencephalic locomotor region, which is very close to the caudal part of the nucleus cuneiformis, was recently studied in detail (*6–8*). Neurons with their cell bodies in this region appear to be responsible for, or at least to contribute to, the effects of stimulation on locomotion (*7, 8*). The output nuclei of the basal ganglia (Fig. 1), that is, the subthalamic and entopeduncular nuclei and part of the substantia nigra, project to the mesencephalic locomotor region (*7, 8*). Considering these findings and the fact that decorticated animals with the basal ganglia intact can initiate normal goal-oriented locomotor movements, it is not unreasonable to assume that part of these effects are exerted through the mesencephalic locomotor region and that they are further channeled to the spinal cord via pathways descending in the ventral funiculus (*7, 8*). Although the cat has been studied the most, the corresponding brainstem areas have also been found to elicit walking in primates as well as reptiles and swimming in bony, cartilaginous, and cyclostome fish (*8, 9*).

During the first part of the 19th century investigators asked whether basic movement synergies are generated by central networks or through some sort of reflex chain arrangement. This "either-or" way of posing the question was unfortunate in that it led even very competent investigators, such as Brown, von Holst, Wilson, and Gray to seek evidence (Fig. 2) in favor of only one view or the other (*2, 3, 10*). It was only during the 1970's that it became clear that the control strategy used to generate a variety of basic movement synergies utilizes both aspects, that is, a central network that generates essential features of the

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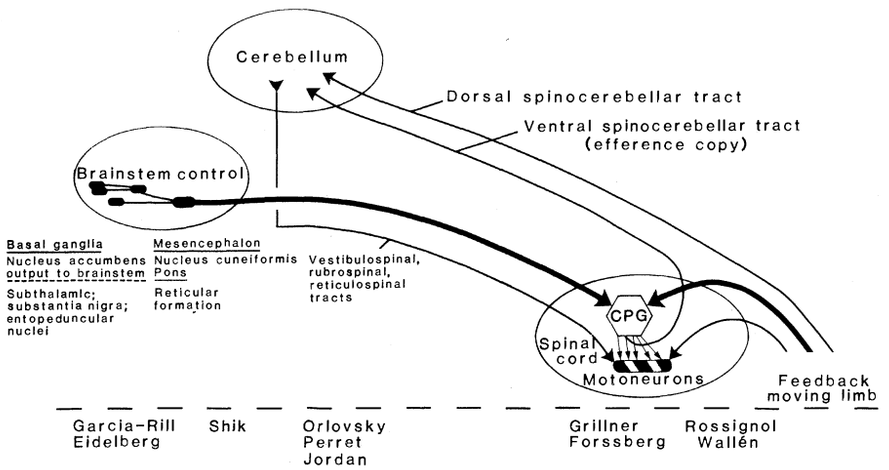


Fig. 1. Schematic diagram of the control system for locomotion in vertebrates, based mainly on data from mammals.

motor pattern and sensory feedback signals that form an integral and crucial part of the control system. In each particular motor system the specific role of sensory and central mechanisms will differ somewhat (*11, 12*).

Role of the Spinal Cord

The spinal cord itself contains neural circuits that, when activated, can be made to coordinate the different muscles to produce locomotor movements. Even when the spinal cord has been transected in the midthoracic region, a cat's hind limbs can be made to perform coordinated walking movements on a treadmill (Fig. 3), and as the speed increases it can suddenly switch to more or less simultaneous movements of the hind limbs as in a gallop (*13*). If a high spinal transection is performed, the fore- and hind limbs can each be made to generate alternating movements and the sets of limbs remain coordinated (*14*). Consequently, neural circuits in the spinal cord can generate different types of interlimb coordination. In mammals as well as lower vertebrates the spinal circuits generating the motor pattern underlying locomotion can be activated even when all movement-related afferent input from a limb or from the entire body has been abolished (*15*). Such findings indicate that the CNS and the spinal cord contain central pattern generators (CPG's) that can produce a complex motor output similar to that of locomotion. On the other hand, without sensory information the pattern can easily break down (*15*). Normally the feedback is crucial for adaptation of the movement synergy to what happens during each step (*12, 16*). The intraspinal locomotor circuitry is divided into smaller parts controlling, for instance, each limb; whenever one part of the circuitry is activated one limb will perform stepping movements, and the different limbs if active can be coordinated by different sets of coordinating neurons (*2, 17*).

The decision to walk is sufficient for the brain to initiate the activity. Presumably the basal ganglia are important in the decision process, and from these structures signals can be channeled from the output nuclei to the brainstem locomotor areas (*7–9*), which in turn activate the spinal cord CPG's, which contain all the necessary details of when and how motor neurons should be activated to produce a step at the appropriate speed.

Thus mammals, birds, and lower vertebrates with transections low in the spine can be made to perform walking movements with a pattern of coordination that closely resembles that of intact animals (*13*). Although this is important to the investigator, it is not of much help to the animal, which has a severe equilibrium deficit that as a rule causes the hindquarters to fall to one side or the other. If the animal walks on a treadmill it can be prevented from falling. It is then evident that the spinal animal can adapt the speed of locomotion to that of the treadmill. This evidently implies a sensory control of the step cycle duration and its components (*12, 16*).

On the spinal level, sensory signals activated by movements of the hip (muscle and joint receptors) are of major importance in the sensory control. As the limb approaches its most posterior position, sensory signals promote the switch from extensor to flexor activity (*18*). If the step has been slowed down, sensory signals arising during mid-step will prevent the limb from too early a flexion (Fig. 4). These effects are exerted on the spinal CPG's that generate detailed activation of individual muscles. Although the CPG's can without any feedback make the transition from flexion to extension and vice versa, when sensory information is available the movements become adapted to what is actually happening to the limb during each step cycle. Moreover, the sensory signals are of prime importance when the speed of locomotion changes rapidly or when walking uphill, downhill, or turning.

The direct reflex effects exerted on the CPG's are of fundamental importance, but other neuronal mechanisms are also at play which help to adapt the synergy to external events. If the trajectory of the limb movement is impeded during the forward swing of the limb, the spinal cord is programmed to counteract the impediment by gating the reflex effects to different muscle groups depending on the part of the cycle in which the perturbation occurs (*19*). Under these conditions the CPG itself can gate the reflex effects to the appropriate motoneurons. It is as yet too early to assess the relative contribution of muscle receptors to the synaptic effects in motoneurons during each step, but detailed description of muscle receptor activity of some different muscles and receptor types during locomotion is now at hand (*20*).

Refinement by the Cerebellum

The movement repertoire of spinal animals contains the essential features of the walking pattern, but lacks the refinement observed in intact and even decerebrate cats. The cerebellum appears crucial for the perfection of the movement pattern, as its removal (*21, 22*) leads to more coarse movements with an unsatisfactory coordination in which the limbs may touch each other and the feet be placed on the ground in an inexact manner. There are also equilibrium deficits. During each step cycle the cerebellum receives detailed information on the progress of different aspects of the movements of each limb. In addition, a blueprint (efference copy) of the output of the central program to the motoneurons in different phases of the step is sent to the cerebellum via ventral spinocerebellar and spinoreticulocerebellar pathways (*23*). After processing, the cerebellum influences motoneurons in each step cycle by indirect and direct projections to the motoneurons via vestibulo-, rubro-, and reticulospinal pathways. These signals contribute to perfecting the

movements by adapting each step cycle. It is not known to what extent the cerebellum contributes to the learning of new aspects of the locomotor pattern, such as the short- and long-term modifications necessary to walk on slippery ground or in shoes instead of bare feet.

Research on the Lamprey: A Simple Vertebrate Model

With the cat model we have substantially advanced our knowledge of how the CNS generates the complex sequence of movements that result in walking. In particular, we have learned much about how the control system works with different components for initiation, speed regulation, central pattern generation, and reflex interaction (*2, 12, 17, 22*). We know how these functions relate to different neural structures, and we have some grasp of the neuropharmacology of the systems and the patterns of activity during locomotion in different types of brainstem and spinal cord neurons. On the other hand, the vast complexity of the mammalian nervous system makes it unlikely that we will, at least with present methods, be able to explain locomotion on the basis of the interaction of individual neurons. To determine how a CPG operates, we need very detailed knowledge of the connectivity of the neurons that constitute the network, the properties of synaptic transmission between individual neurons, and the membrane properties of individual cells and how they vary with time during locomotion.

To answer such questions rather than pursue a gradually more refined description of the activity in certain presumed network components, we must study a simpler system. Since the general neural organization of the vertebrate locomotor system appears to be very conservative (compare type of brainstem control, spinal organization, feedback, and so forth), it would seem that lower vertebrates can serve as models of higher vertebrates. In our laboratory we have therefore redirected our research effort from the cat to the lamprey, a primitive jawless fish. The unmyelinated brainstem–spinal cord of this vertebrate can be maintained in vitro for several days since it is very thin. Furthermore, the neural activity that would normally lead to locomotor movements can be elicited even in vitro (Fig. 5). Since the motor pattern can occur without any movements, such neural activity is referred to as fictive locomotion (*2, 15*). Two crucial

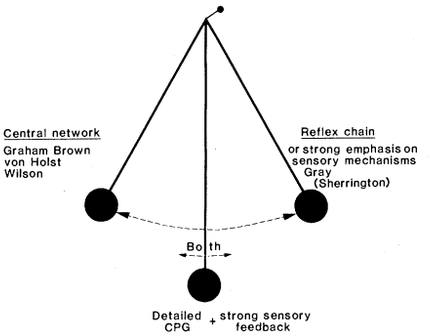


Fig. 2. Extreme positions held by investigators on the neural control of rhythmic motor acts.

experimental conditions are met here: (i) the experiments can be performed on an active nervous system "attempting" to perform the pattern of behavior and (ii) the in vitro condition allows practically all current neurobiological techniques to be used, unlike the situation in vivo.

Let us therefore summarize some findings on the lamprey model, obtained mainly in our own laboratory and that of Carl Rovainen.

Initiation. Fictive locomotion recorded in ventral roots or motoneurons can be initiated (i) by brainstem stimulation of specific areas (*9*), (ii) by sensory stimuli (*24*) if some skin areas are left innervated (such as the skin of the head or the tail fin), and (iii) by bath-applied excitatory amino acids activating *N*-methyl-D-aspartate (NMDA) or kainic acid receptors (*25*). Natural elicitation of locomotion depends on an activation of these two receptor types (*26*).

Distribution of the pattern generator networks. The ability to generate coordinated activity with appropriate phase lag along the spinal cord is distributed within the entire spinal cord, since any part of the cord can be subdivided into pieces of two to three segments that retain the ability to produce rhythmic alternating activity. The motor pattern of isolated spinal cord sections closely resembles that of the intact swimming lamprey (*27, 28*).

Output elements. The motoneurons are lined up in a long column along the spinal cord, with extensive dendritic trees projecting into the axonal bundles of the lateral and ventral funiculi. Motoneurons supplying different parts of the myotome are morphologically distinct (*29*). They appear to be pure output elements and not part of the pattern generator system (*30*).

Interneuronal pattern-generating network: Role of membrane properties. Motoneurons receive alternating excitation and inhibition during each fictive swim cycle (*31*). Crossed propriospinal inhibitory premotor interneurons contribute to the reciprocal inhibition of motoneurons across the midline (*32*). Small amacrine spiking interneurons are present in the

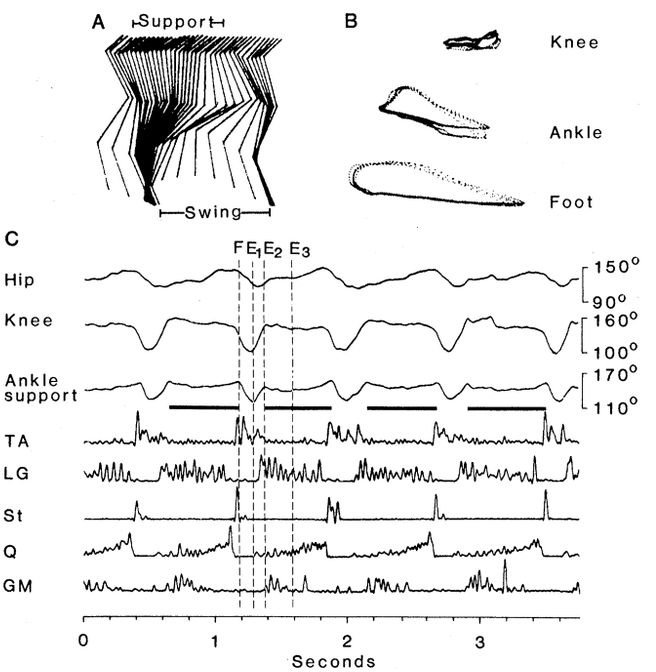


Fig. 3. Movements and muscular activity during slow walking (0.20 m/sec) in a spinal cat. (A) Forward movement of the right limb. The position of the limb throughout the step cycle is presented at 18-msec intervals. (B) Knee, ankle, and foot trajectories, displayed at 6-msec intervals during six consecutive step cycles. The positions are related to the position of the pelvis, which is fixed in the horizontal direction. (C) Angular movements of the hip, knee, and ankle (at the top), displayed together with the simultaneously recorded electromyograms of tibialis anterior (TA), gastrocnemius lateralis (LG), semitendinosus (St), quadriceps (Q), and gluteus medius (GM). The electromyograms are filtered and rectified. The swing and support phases are also indicated in the stick diagram. The onset of flexion (F) and the different extension phases (E_1, E_2, and E_3) are indicated in the angular movements of the ankle. The support phases are also indicated by schematic blocks. [Courtesy of *Acta Physiologica Scandinavica* (*13*)].

spinal cord and are active during fictive locomotion (*33*); their role is yet to be determined. The segmental interneuronal pattern–generating network contains presumed glycinergic interneurons (*34*), but probably lacks both γ-aminobutyric acid–containing interneurons and segmental serotonin-containing interneurons, which occur in a midline row below the central canal. They form a dense ventromedial plexus of terminals in which there are dendrites of motoneurons and premotor interneurons (*35*). The serotonin systems can probably influence the motor pattern.

Induction of rhythmic activity by NMDA in the spinal cord is presumably related to the fact that a negative slope conductance can be induced by the application of NMDA in some neurons (*36, 37*). This would tend to change a conventional neuron with a linear current–voltage curve to one that would tend to have a bistable or oscillatory membrane potential. If tetrodotoxin (TTX) is applied so that the sodium channels of the action potential are blocked and NMDA agonists are subsequently applied, some spinal cord neurons exhibit ongoing pacemaker-like membrane potential oscillations (*35, 37*). Presumably the same membrane properties contribute to the induction of fictive locomotion (Fig. 6).

Nonsynaptic mechanisms of possible importance in the CPG. The level of extracellular potassium influences the burst rate of the CPG. Within the gray matter of the spinal cord there are in addition phasic, albeit small, variations of extracellular potassium with each motor burst (*38*). Thus it will probably not be a major determinant of the motor pattern as has been discussed in other systems. In the hippocampus synchronous burst activity is accompanied by large extracellular fields, which play a major role in the generation of a burst (*38*). In the lamprey spinal cord the asynchronous activity of individual neurons appear to prevent such large fields from arising, although individual neurons may produce large extracellular fields.

Movement-related feedback caused by intraspinal mechanoreceptors. As the lamprey swims a mechanical wave travels down the body. The spinal CPG's are influenced by sensory feedback elicited by the movement, and artificially imposed movements can elicit an entrainment of the underlying motor pattern and thus exert direct effects on the CPG's as in higher animals (*39*). At least part of these effects are exerted by intraspinal mechanoreceptor neurons (Fig. 7) that sense the length of the spinal cord margin (*40*). As a lamprey or an eel swims, the spinal cord will always be bent to the same degree as the body. Consequently, in each swim cycle one side of the cord will first be stretched and then unloaded. The intraspinal mechanoreceptor neurons structurally resemble crayfish stretch receptor neurons and also have synaptic input to their dendrites.

The spinal cord apparently contains a

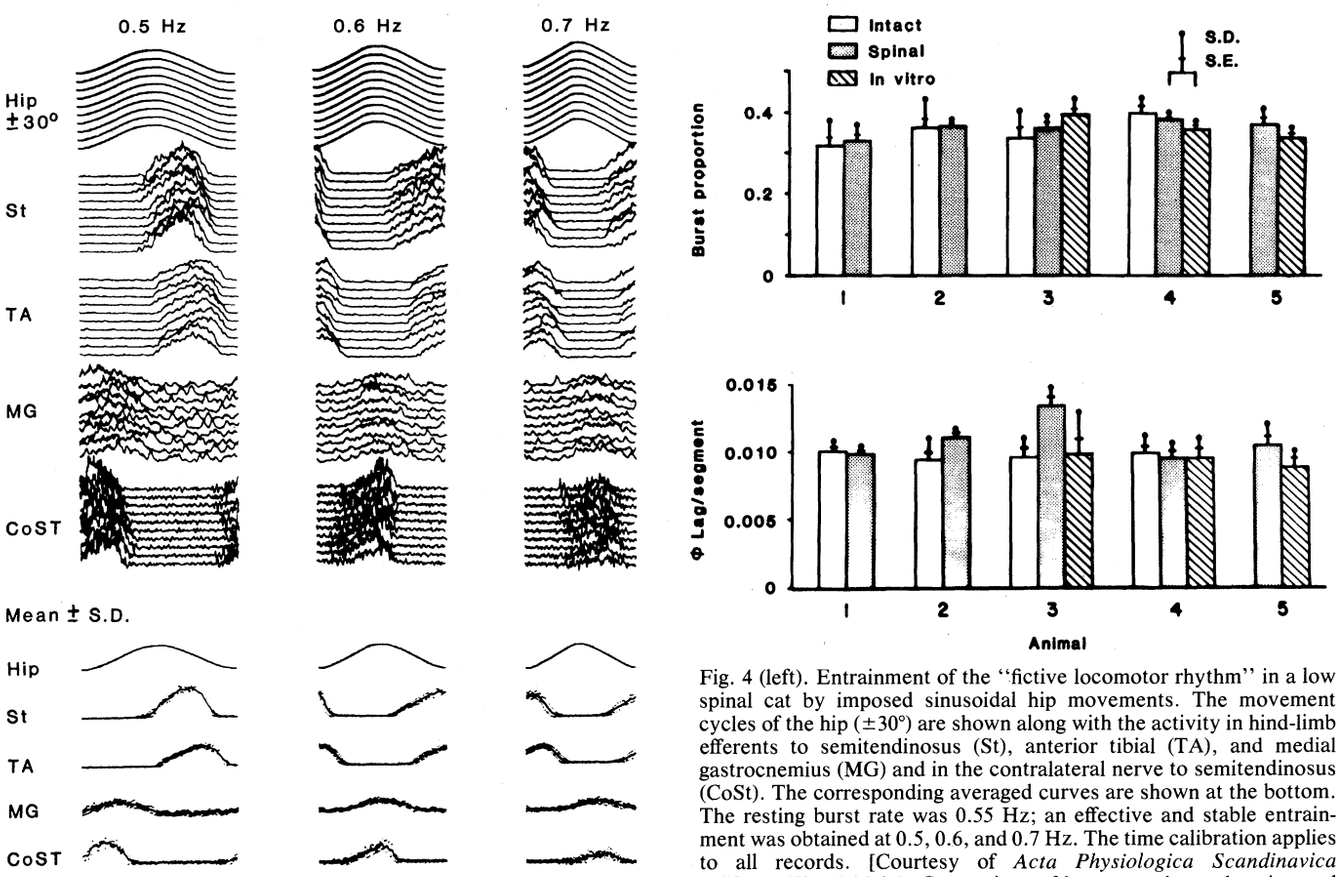


Fig. 4 (left). Entrainment of the "fictive locomotor rhythm" in a low spinal cat by imposed sinusoidal hip movements. The movement cycles of the hip (±30°) are shown along with the activity in hind-limb efferents to semitendinosus (St), anterior tibial (TA), and medial gastrocnemius (MG) and in the contralateral nerve to semitendinosus (CoSt). The corresponding averaged curves are shown at the bottom. The resting burst rate was 0.55 Hz; an effective and stable entrainment was obtained at 0.5, 0.6, and 0.7 Hz. The time calibration applies to all records. [Courtesy of *Acta Physiologica Scandinavica* (*18*)]. Fig. 5 (right). Comparison of locomotor burst duration and intersegmental phase lag between the intact lamprey and the spinal and in vitro preparations. The electromyographic activity of intact lampreys was recorded in different segments along the body while swimming freely (five preparations). The motor burst in each swim cycle constitutes a constant proportion of the cycle and the lag between the onset of the motor burst in adjacent segments constitutes a constant fraction (around 1 percent) of the swim cycle duration (that is Φ lag/segment) regardless of the actual cycle duration. The spinal cords of the same animals were subsequently transected and their swimming activity was recorded from the same electrodes. Later, the spinal cords were dissected from these animals, fictive swimming was induced by an activation of NMDA receptors, and the efferent activity was recorded from different ventral roots by suction electrodes. In these five animals the burst duration and phase lag remained very similar in the three different types of preparation. The swim frequency for the in vitro preparation ranged between 0.05 and 7 Hz and that for the intact animal from 0.5 to more than 7 Hz. [Modified from Wallén and Williams (*28*)]

network distributed throughout its length, which can generate a phase lag between the activation of each consecutive segment, resulting in an undulatory wave propagated along the body. Although much information has been gathered about both the neuronal components and the induction of altered membrane properties of importance in the pattern generation, the intrinsic operation of the spinal CPG's cannot yet be described in the same detailed manner that has been possible in some invertebrate systems such as *Tritonia* (*41*) and the crustacean stomatogastric system (*42*). The current concept of the organization is that in each half of the spinal cord there are a number of neuronal modules (possibly corresponding to segments), each of which can be made to produce a rhythmic output activating predominantly a subset of the motoneuronal pool. Each such module or unit CPG will be coupled with reciprocal inhibition to its neighbor on the contralateral side of the spinal cord, ensuring the required alternation in each segment. The unit CPG's along the spinal cord are presumably coordinated by coordinating neuronal elements providing mutual excitation and the necessary intersegmental phase lag.

Modifiability of Innate Motor Systems

A characteristic of most innate motor systems is that they must be modifiable. In fish locomotion the spinal cord cannot only coordinate the rostrocaudal phase lag resulting in forward swimming, the phase lag can also be reversed in the spinal state to make the animal move backward (*43*). Rather than having two separate interneuronal systems for forward and backward swimming, it appears likely that the same system of unit CPG's is used in both cases but that a different set of coordinating neurons predominates, making the caudal segments lead instead of the rostral ones. Similarly, burrowing, or swimming against a semisolid surface such as sand, is often regarded as a different pattern of behavior from swimming through water, since the movement patterns differ somewhat, with slow, large-amplitude movement occurring in burrowing. In burrowing the hydrodynamic situation changes radically, and the altered movement may be only a direct consequence of the altered mechanical situation and the sensory feedback acting on the central network coordinating the swimming movements (*33*).

In tetrapods the need to modify limb movements is perhaps even more apparent. Stein (*44*) recently described the characteristic changes the scratch reflex motor pattern in turtles undergoes as different parts of the body surface become the target of the scratch reflex. Not only does the relative degree of activation of the different muscles change, their phase relations do also. This points to a high degree of modifiability in the interneuronal network.

In tetrapod locomotion the interlimb

Fig. 6. Slowly oscillating membrane potential in spinal cord interneuron after administration of TTX to the bath ($2 \times 10^{-6} M$) containing 0.15 m*M* *N*-methylaspartate. [Modified from Grillner *et al.* (*36*)]

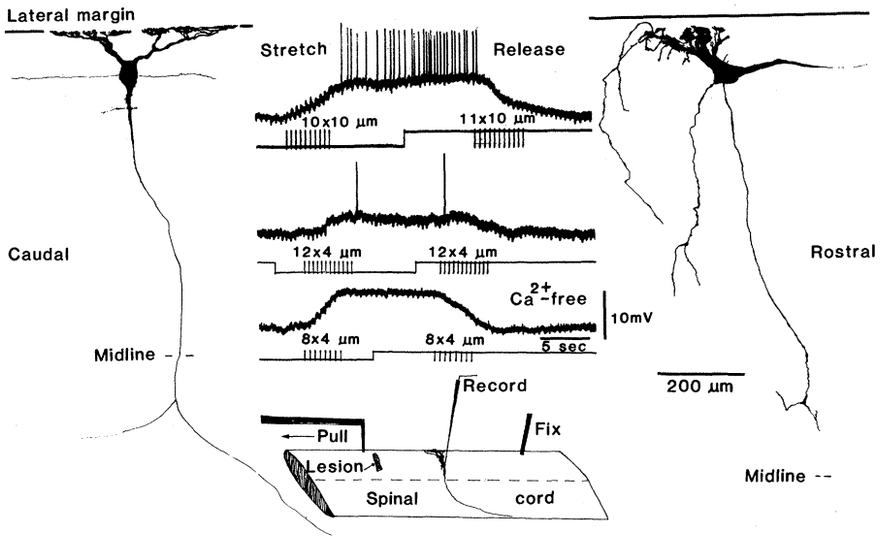
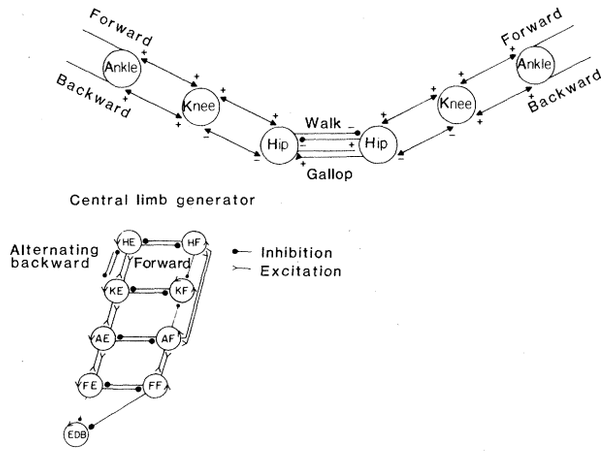


Fig. 7. Depolarizing responses to stretching of the spinal cord margin. The upper two sets of recordings are from the edge cell to the right (reconstruction, dorsal view, Lucifer yellow); the lower set is from the cell to the left. The upper traces are intracellular records (time and voltage calibration). The lower trace indicates the change in length of the lateral margin. Each vertical line indicates a brief 10- or 4-µm stretch. Over 4 to 5 seconds several 10- or 4-µm stretches were applied as indicated; the release is indicated in an analogous way. The inset shows the general experimental arrangement with the pull applied in the longitudinal direction along the lateral margin. The cell is depolarized on each stretch and returns to the resting level on release from stretch. Length of the cord at rest corresponds approximately to its length in situ [from (*40*)].

Fig. 8. Scheme of limb generator circuitry as a mosaic of unit CPG's. Interconnections between unit generators decide the relative phase of different muscle groups and how they are used during locomotion. Connections (inhibitory or excitatory) that could produce forward and backward locomotion have been indicated. Abbreviations: E, extensor; F, flexor; H, hip; K, knee; A, ankle; FE, foot extensor; FF, foot flexor; and EDB, extensor digitorum brevis (a short toe flexor). [Modified from Grillner (*2*) and Edgerton *et al.* (*45*)]



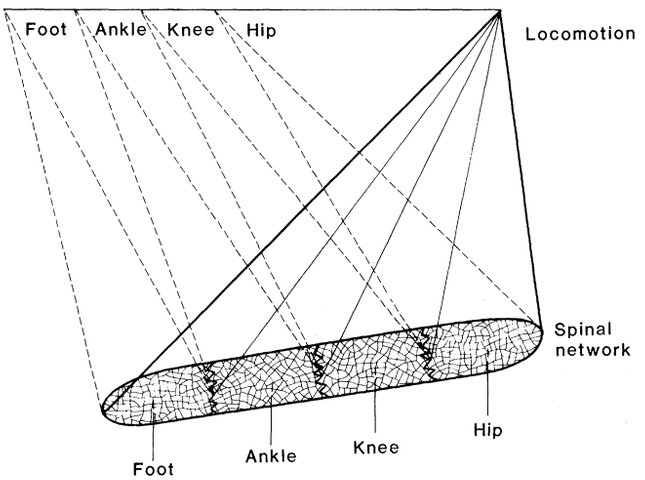


Fig. 9. Fractionation of the CPG network for locomotion. An activation of the entire network will give rise to locomotion, but an independent control of parts of this network could be utilized in (for instance) the volitional control of independent ankle, knee, or foot movements and even in the activation of individual muscle groups. [Modified from Grillner (*1*)]

coordination with walking, trotting, and galloping requires different sets of coordinating neurons to combine the limb CPG's in the different phase relations required for the particular type of gait (*2, 44*). On the level of the single limb the motor pattern must also be flexible. The angle in different joints may change and also the phase relations between joints, as when changing from forward to backward walking. In this case the movement of the hip changes in relation to the joints in the lower leg. A plausible explanation (*1, 2, 44, 45*) is that the CPG network of a limb is subdivided into unit CPG's, as discussed for the fish above, each controlling groups of closely synergistic muscles (Fig. 8). By changing from one set of interconnections to another between the hip unit CPG and those of the knee, the locomotion would automatically shift from a forward to a backward direction. This would be a very simple control strategy, with which higher centers could change the pattern of coordination.

Conclusion

A variety of pieces of indirect evidence are compatible with a unit CPG hypothesis (*1, 2*). If correct, the hypothesis suggests a very versatile motor organization in which the different components can be recombined in a variety of ways. This might permit not only modifications of gait types in a wide range but possibly also the utilization of unit CPG's in other patterns of behavior such as the scratch reflex and more complex limb movements. If descending pathways could activate individual unit CPG's, this would allow selective control of individual joints or muscle groups. That is, if one wants to wiggle the big toe, one may merely call on the unit CPG for the big toe, and so forth. Accordingly, the spinal cord might contain ready-made components for the control of different muscles and muscle groups. It seems plausible that part of the descending control eliciting limb movements could be exerted on such spinal modules rather than by utilizing private descending pathways transmitting detailed signals directly to the motoneurons, as is usually envisaged. Experimental lesions of pyramidal tracts in primates cause a loss of individual finger movements but no other motor deficits and an additional lesion of the lateral brainstem including the rubrospinal tract causes a further inability to generate independent hand movements (*46*). Whole-limb synergies are still intact, and the entire limb can reach out toward an object with full extension and subsequent flexion. The ability to fractionate whole-limb synergies into delicate movements of individual joints seems to have developed gradually during evolution, reaching an apex in man. In parallel, the descending control of the spinal cord networks might have become progressively more specific, so that particular descending fibers could call on small specific fractions of the spinal networks to generate specific movements. When we want to walk we may accordingly call on the entire ensemble of unit CPG's, but if more specific movements are desired we call on only particular parts thereof (Fig. 9). If a new type of movement is to be carried out, we have to learn which fraction of the network must be activated. Learning a new movement may thus require learning to combine and sequence specific fractions of the neuronal apparatus used to control the innate movement patterns in a novel way. If so, the clear-cut distinction often made between learned and innate movement patterns may be illusory rather than real.

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RESEARCH ARTICLE

Molecular Cloning of the Complementary DNA for Human Tumor Necrosis Factor

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A factor that became known as tumor necrosis factor (TNF) was first reported by Carswell and colleagues in the mid-1970's (*1*). Sera from endotoxin-treated mice, rabbits, or rats that had been previously sensitized with an immunopotentiator such as bacillus Calmette-Guérin (BCG) were found to contain a substance that, when injected into mice harboring transplanted tumors, caused extensive hemorrhaging of the tumors without undesirable side effects on the recipient. The sera were thus presumed to contain a substance that caused necrosis of tumor cells but had no effect on normal tissue; hence its designation TNF. The ability to cause selective tumor destruction when injected into whole animals became a standard assay for indicating the presence of TNF in vivo.

Several investigators (*2–4*) have attempted to isolate and purify native TNF from rabbit and mouse sera. The factor isolated from rabbit serum is a protein with a molecular weight of 39,000 (39K) to 55K on gel filtration and an isoelectric point of *p*H 5.1 to 5.2 (*2*). The factor from mouse serum had low (50K to 60K) (*4, 5*) and high (100K to 225K) (*4*) molecular weight forms and an isoelectric point of *p*H 4.8 (*3*). Purified preparations of murine TNF were tested against murine and human cell lines in vitro (*6*). In contrast to normal cells, tumor cell lines from both species were susceptible to the cytotoxic activity of the mouse TNF. Furthermore, the murine TNF was active against tumors transplanted from both humans and mice to nude mice (*7*).

Tumor necrosis factor is produced in the medium of mononuclear phagocytes from BCG-infected rabbits and macrophage-enriched peritoneal exudate cells from BCG-infected mice after induction with endotoxin (*5, 8, 9*). In addition, Williamson *et al.* (*10*) have reported the production of TNF by B-lymphoblastoid cells. This partially purified protein exhibited cytostatic and cytotoxic activity against human tumor cell lines in vitro and had tumor necrotic activity in animals.

We now describe the production and purification of human TNF from the human promyelocytic leukemia cell line HL-60 and the determination of its biologic characteristics and amino terminal amino acid sequence. We also describe the molecular cloning of the factor and its production in *Escherichia coli* and in mammalian cells.

Purification and biologic characterization. A number of human cell lines were compared as sources of TNF protein and messenger RNA (mRNA). These included several B-lymphoblastoid lines: Daudi and line 8866; monocyte line U937; a myelogenous leukemia cell line, ML2; and a promyelocytic leukemia line, HL-60. The HL-60 cell line was chosen because it can be induced to differentiate into monocytes upon treatment with phorbol myristate acetate (PMA) (*11*) and produces substantial amounts of TNF, which is thought to be generated by monocytes (*5, 8*) after treatment with endotoxin (lipopolysaccharide; LPS).

Previous attempts to purify TNF from cell culture supernatants have been unsuccessful because the protein is produced in minute amounts by human monocytes (*8*). Our success in purifying the protein was largely due to our ability to induce the HL-60 cell line to produce large amounts of TNF (*12*) and by our ability to determine amino acid sequences from minute quantities of protein samples.

About 4 to 8 liters of culture supernatant from induced HL-60 cells were concentrated by hollow-fiber ultrafiltration (1-square-foot cartridge with a 10K molecular weight cutoff; Amicon). The concentrated, conditioned medium was purified by DEAE ion-exchange chromatography, gel filtration on Sephadex G-75 (Superfine), preparative sodium dode-

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