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Evolution and behavioral adaptation of locomotor pattern generators in vertebrates

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Abstract

The fundamental evolutionary change in Vertebrate locomotion that allowed the transformation from aquatic swimming to overground stepping was related to a substantial reshaping of locomotor rhythm generating circuits located at the spinal cord level. The metameric organization of rhythm genesis in the lamprey, for example, evolved into neuronal pattern-generators re-centred at C7–T1 and L1–L2 spinal levels that are responsible for driving limb locomotor movements in quadrupeds such as the rat and rabbit. In Mammals, further locomotor adaptations such as the hopping hindlimb coordination in the rabbit, which is morphologically related to hindlimb lengthening, is achieved by a supraspinal reconfiguration of the basic alternate coupling of the bilateral lumbar locomotor generators found in all studied Mammals, including the rabbit. In this way therefore, evolution and behavioural adaptation of locomotion may involve utilisation of different neural mechanisms. ***To cite this article: D. Viala, C. R. Palevol 5 (2006)***.

Résumé

Évolution et adaptation comportementale des réseaux locomoteurs chez les Vertébrés. La locomotion des Vertébrés a fait l'objet d'une importante évolution et le passage de la nage à la marche s'accompagne d'un important remaniement dans l'organisation des générateurs de rythmes locomoteurs, situés au niveau spinal. D'une genèse métamérique du rythme locomoteur chez la lamproie, nous passons à une genèse destinée aux membres chez le rat, et limitée aux niveaux spinaux C7–T1 et L1–L2. Une forme d'adaptation locomotrice chez les Mammifères, comme le bond chez le lapin, liée à l'allongement des membres postérieurs, est réalisée dans le système nerveux par un contrôle supraspinal du couplage fondamental entre les deux générateurs lombaires. Au niveau spinal, le couplage fondamental est en alternance chez tous les Mammifères étudiés, y compris le lapin. Ainsi, dans les exemples étudiés, l'évolution et l'adaptation locomotrice utilisent des mécanismes nerveux différents. ***Pour citer cet article : D. Viala, C. R. Palevol 5 (2006)***.

Keywords: Evolution; Adaptation; Locomotor networks

Mots clés : Évolution ; Adaptation ; Réseaux locomoteurs

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1. Introduction

Locomotor patterns are diversified in Vertebrates and are necessarily adapted to an animal's environment and the way it feeds. The most drastic evolutionary alteration in locomotion has been associated with the change of habitat from aquatic to terrestrial living. This was in turn correlated with the appearance of limbs that became the main instruments of locomotion, replacing a fusiform body that propelled the aquatic animal by rostro-caudal undulatory waves. Even in limbed vertebrates, however, the locomotor pattern can be adapted to the development of morphological differences from the usual body plan. For instance, while most Mammals walk at low locomotor rates and gallop at high speed, a few species with substantially lengthened hindlimbs relative to their forelimbs (kangaroo, gerbil and rabbit) hop with bilaterally-synchronous hindlimb movements at all speeds. Locomotor adaptations of other limbed vertebrates can be considerably more varied than this example [12].

In this brief synthesis, interest is focused on the evolution and adaptation of neural pattern generators responsible for such different forms of locomotion. It was originally thought that mammalian locomotion is patterned at the spinal level via peripheral reflex processes [13]. However, it is now clear that the basic locomotor rhythmicity is generated within the spinal cord [2,14] by central pattern generators (CPGs). Here the evolution in distribution of such CPGs along the spinal cord from swimming in low vertebrates to walking in Mammals is considered, along with the mechanism by which CPG coordination is adapted to subsequent morphological changes such as hindlimb lengthening that occurred in the rabbit.

2. Evolution of the localization and organization of locomotor generators in lamprey and rat

2.1. Lamprey swimming

Swimming in the lamprey, as in other fish, is achieved via waves of body contraction that propagate from the head to the tail (Fig. 1A). As in Mammals, the rhythmic production of such propulsive rostro-caudal waves is generated at the spinal level [16]. The rhythmic neural activities responsible for the body muscle contractions in vivo can still be recorded from an isolated spinal cord maintained alive in vitro, after pharmacological activation of glutamate with NMDA [8]. Homolateral bursts with a rostro-caudal delay can be recorded on ventral motor roots along the cord and bilateral alternate bursts are present at a given metameric level (Fig. 1B). This pattern reflects the temporal organization observed with EMG recordings in intact preparations (Fig. 1A) and thus represents a valid model for further neurophysiological investigation at the cellular level [16]. In this way the in vitro preparation of the lamprey spinal cord has become an important system for establishing the metameric basis of rhythm genesis. Spinal transections and pharmacological activation (with NMDA) has allowed disclosure of the equivalent rhythmogenic capabilities of the different parts of the spinal cord [16]. Moreover, portions of the cord reduced to four segments were still able to generate rhythmic locomotor bursts, and considering that a zone of neural tissue is always damaged close to any transection, this result has been taken as evidence for the segmental genesis of locomotor activity involving a left and right interconnected CPG operating in alternation [4].

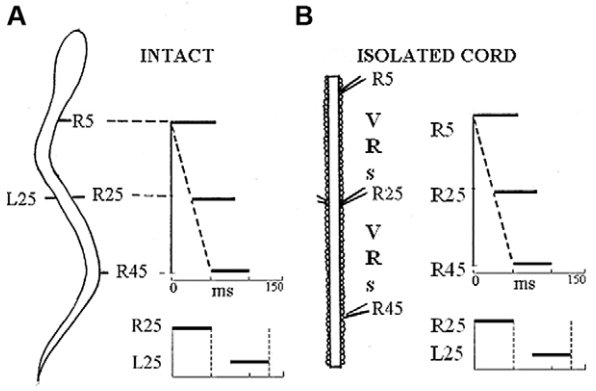

Fig. 1. Locomotor organization in the lamprey. (**A**) Schematized intact lamprey (INTACT) showing positions for electromyographic (EMG) recordings (right segments numbered R5, R25, R45 and left at L25). Horizontal bars at right indicate the duration of muscle activation at R5, R25 and R45 levels. Note the rostro-caudal delay and the bilateral alternation between R25 and L25. (**B**) Schematic of the spinal cord after isolation from the body and brain (ISOLATED CORD). When pharmacologically-activated with NMDA, this in vitro preparation can generate rhythmic bursts recorded with micropipettes on the ventral roots (VRs) at the same segmental levels as in (**A**). At right, the organization of the bursts recorded at the different levels is the same as in the intact preparation (compare with **A**).

Fig. 1. Organisation locomotrice chez la lamproie. (A) Lamproie intacte (INTACT) schématisée montrant l'emplacement des enregistrements électromyographiques (partie droite des segments R5, R25, R45 et gauche L25). À côté, les barres horizontales en gras schématisent la durée de chaque activation musculaire en R5, R25 et R45 et montrent le retard d'activation rostro-caudal ainsi que l'alternance bilatérale entre R25 et L25. (**B**) Schéma de la moelle isolée (ISOLATED CORD) et activée pharmacologiquement avec le NMDA, qui peut engendrer des décharges rythmiques enregistrées sur des racines ventrales (VRs) des mêmes segments qu'en (**A**). À côté, même présentation qu'en (**A**) : l'organisation des activités enregistrées aux différents niveaux est la même que sur la préparation intacte.

Thus in a low aquatic vertebrate such as the lamprey, and probably also in other swimming vertebrates, the general metameric organization of locomotion is supported by a neural drive from segmentally distributed CPGs within the spinal cord.

2.2. Locomotion in rat

Compared to the lamprey, mammalian evolution has mainly seen a re-centring of locomotor motions to the limb level, with more complex movement patterns now due to interplay between antagonistic flexor and extensor muscles to achieve normal stepping. With this evolutionary specialization of locomotion, how did the metameric CPGs of fish evolve to now drive localized appendicular parts of the body?

To a large extent, answers have been obtained from in vitro isolated brainstem-spinal cords of new born rats. (This perinatal model has been used because the high metabolic rate of Mammals does not allow survival of adult rat spinal cords in vitro.) Locomotor-like bursts were obtained from recordings on lumbar ventral roots (VRs) after a perfusion of a cocktail of NMDA and 5-HT that was at first limited to the L1 and L2 segments (Fig. 2A). Bursts were not limited to the perfused segments, but extended into the lumbar cord and were organized as expected into a coordinated pattern, i.e. with alternation of bursts on homolateral L2 and L5 VRs (which respectively drive flexor and extensor hindlimb muscles) and bilateral alternation at the same segmental level (Fig. 2A2). In contrast, if the pharmacological perfusion was restricted to lumbar segments more caudal to L2, locomotor-like activity was no longer induced at any spinal level (Fig. 2B2) [3]. Although some authors have claimed that an intrinsic rhythmic capability exists in segments more caudal to L2, they are in general agreement that the L1–L2 segments constitute the prevalent rhythmogenic locus [9]. Thus the main (if

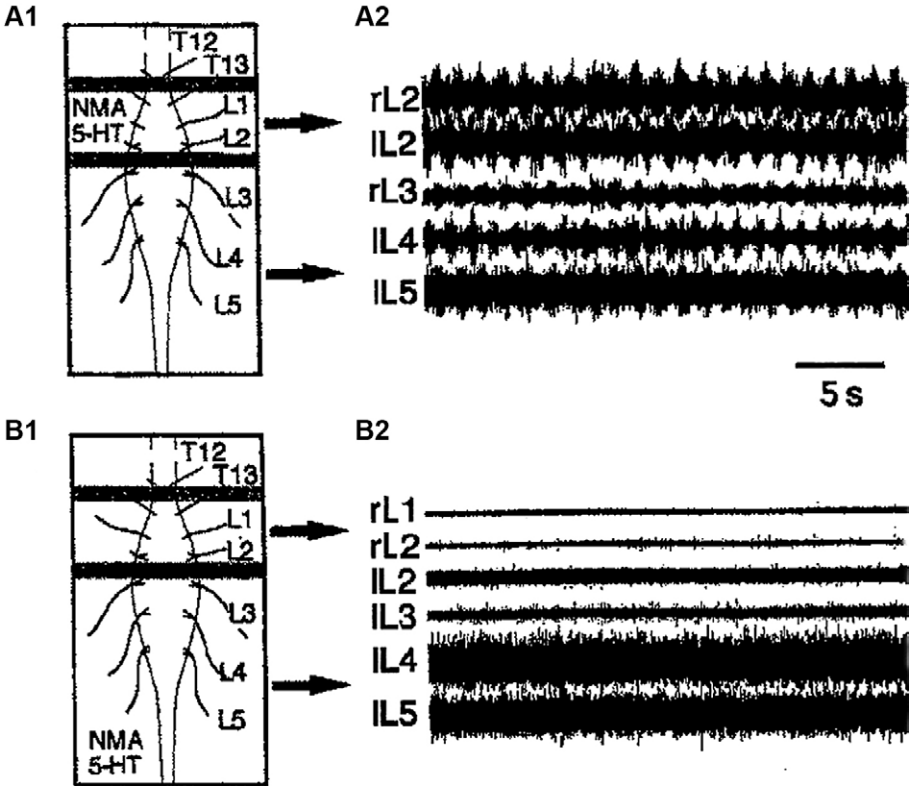

Fig. 2. Locomotor genesis in the lumbar cord of the neonatal rat. In vitro preparation and local activation with NMDA and 5-HT at L1–L2 in **A1** and at post-L2 in **B1**. Motor activities recorded on left (l) and right (r) lumbar (L) ventral roots: rL1, rL2, lL2⋯ Locomotor bursts are observed on all recorded VRs in **A2**. In contrast, bursting occurs in **B2**, indicating that the rhythmogenic capacity is limited to L1–L2 segments (from [3]).
Fig. 2. Genèse locomotrice dans la moelle lombaire du rat nouveau-né. Préparation in vitro activée localement par le NMDA et le 5-HT: en L1-L2 en **A1**, en arrière de L2 en **B1**. Activités motrices enregistrées sur des racines ventrales (VRs) lombaires (L) gauches (l) et droites (r) : rL1, rL2, ll2⋯ Des activités locomotrices sont observables sur toutes les VRs en A2. Aucune activité rythmique n'est présente en **B2** : la capacité rythmique est limitée aux segments L1–L2 (d'après [3]).

not the exclusive) left and right CPGs able to organize the homolateral and bilateral locomotor pattern are found in two segments, L1–L2, of the spinal cord and any rhythmic capacities of other lumbar segments may reflect vestigial remnants of a metameric organization.

Our investigations at the cervico-thoracic level of the same pharmacologically-activated preparations have also confirmed the restricted localization (limited to C7–C8–T1 levels) of the CPGs for forelimb locomotion [1]. Interestingly, moreover, when the whole spinal cord was activated with 5-HT and NMDA, rhythmic bursts occurred at all recorded levels (cervical, thoracic and lumbar) and at the same frequency (Fig. 3A1 and B1). If two spinal transections, one caudal to the cervico-thoracic generators and the second just rostral to the lumbar ones were made, the rostral (forelimb) and the caudal (hindlimb) generators remained active, but now produced locomotor-like rhythms at different frequen-

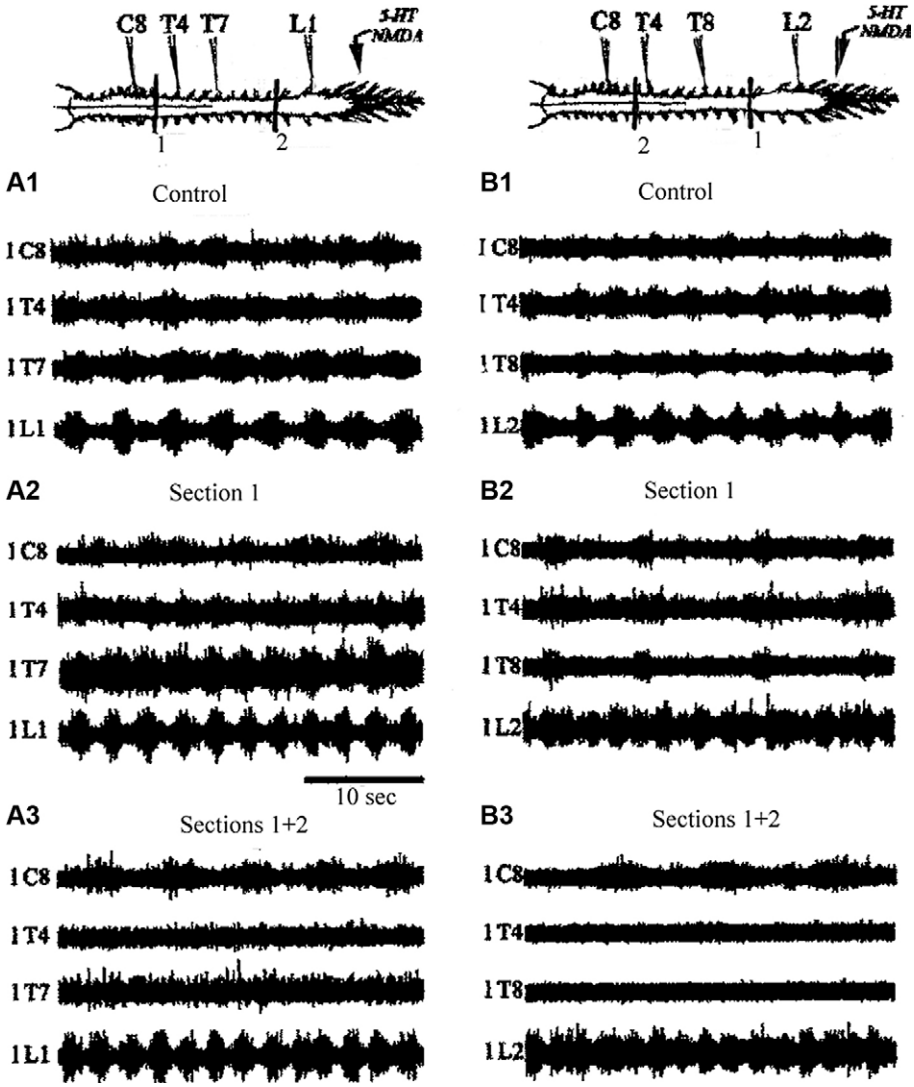

Fig. 3. Rhythmogenic capacity of the in vitro isolated cord of the new-born rat after activation with NMDA and 5-HT. Motor activity was recorded on homolateral ventral roots at cervical C8, thoracic T4, T7 or T8, and lumbar L1 or 2. **A** and **B** are from two different preparations. **A1, A2**: Locomotor bursts before any spinal transection. **A2**, **B2**: Changes in activity after a transection at point 1 (see schematic). **A3**, **B3**: After a second transection at point 2, rhythmicity is lacking at thoracic levels (from [1]).

Fig. 3. Capacités rythmogènes de la moelle de rat nouveau-né isolée, en préparation in vitro, et totalement activée pharmacologiquement (NMDA et 5-HTP). Activités motrices enregistrées sur les racines ventrales homolatérales C8, T4, T7 ou 8, L1 ou 2. **A** et **B** sont deux préparations différentes. **A1**, **B1** : Décharges locomotrices avant toute transection spinale. **A2**, **B2** : Modification de cette activité après la section n° 1 (voir schéma). **A3**, **B3** : Après la seconde transection n° 2, la rythmicité a disparu aux niveaux thoraciques (d'après [1]).

cies with the caudal generators having the highest intrinsic frequency (Fig. 3A3 and B3). Thus the rostral and caudal generators normally interact to coordinate the locomotor pattern to a common frequency, which is intermediate between their intrinsic rates of activity. Moreover, although both generators remained active after paired spinal transections, the isolated thoracic cord always fell silent. However, when the thoracic region was disconnected from only one group of generators, either rostral or caudal, the thoracic segments continued to express a rhythmic activity at the frequency of the generators to which they remained connected to (Fig. 3, A2, B2) [1].

To summarize therefore, in the newborn rat, locomotor-like rhythmogenesis is limited to a few spinal segments – L1–L2 and C7–T1 – that organize the limb locomotor pattern. Rhythmic activity at all other spinal levels essentially is passive and results from a drive from the rostral and caudal CPGs themselves. *Thus the evolution of locomotor movements from fish to mammals is correlated here with a relocalization and coupling of spinal neural circuitry involved in basic rhythmogenesis.*

3. Adaptation of locomotor generators for the hopping pattern in rabbit

Most Mammals, like the cat, the dog and the rat walk at relatively low speeds, moving their hindlimbs and forelimbs in alternation at each girdle. As described above, this alternating bilateral pattern is programmed centrally at the spinal level [2].

In the adult rabbit, in vivo recordings of both hindlimb locomotor activity in decerebrate, curarized preparations reflected the hopping pattern of the intact animal: bilateral flexor bursts occur synchronously and in anti-phase with bilateral extensor bursts (Fig. 4A). Surprisingly, however, after a low thoracic spinal transection and pharmacological activation with DOPA, 5-HTP or amphetamine, locomotor-like activity develops,

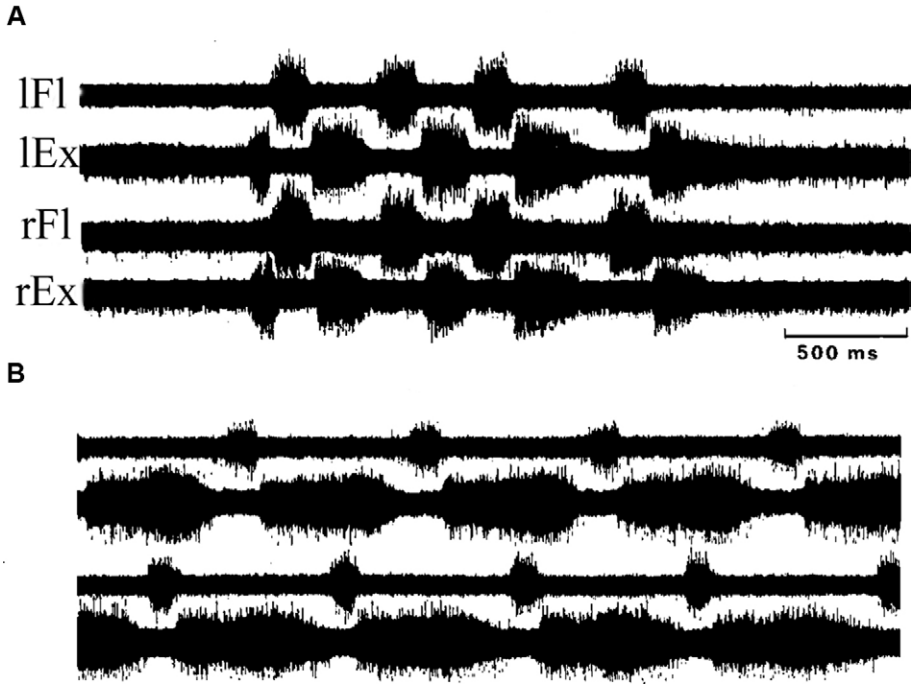

Fig. 4. Locomotor pattern of both hindlimbs in a decerebrate (**A**) then spinal (**B**) rabbit preparation. Locomotor bursts are recorded on muscle nerves (flexor, Fl, and extensor, Ex) of left (l) and right (r) hindlimbs: **A**, decerebrate curarized in vivo preparation; **B**, the same preparation after T12 spinal transection and pharmacological activation with amphetamine. The bilateral locomotor pattern, which was synchronous in **A**, is now alternating (from [15]).

Fig. 4. Organisation locomotrice bilatérale au niveau des membres postérieurs chez le lapin en préparation décérébrée, puis spinale. Les décharges rythmiques sont enregistrées sur des nerfs musculaires (fléchisseur, Fl et extenseur, Ex) des membres postérieurs gauche (l) et droit (r). En (**A**), préparation in vivo décérébrée et curarisée. En (**B**), la même préparation après transection spinale au niveau T12 et activation pharmacologique avec de l'amphétamine. Le couplage bilatéral, qui était en synchronisme en **A**, est maintenant en alternance (d'après [15]).

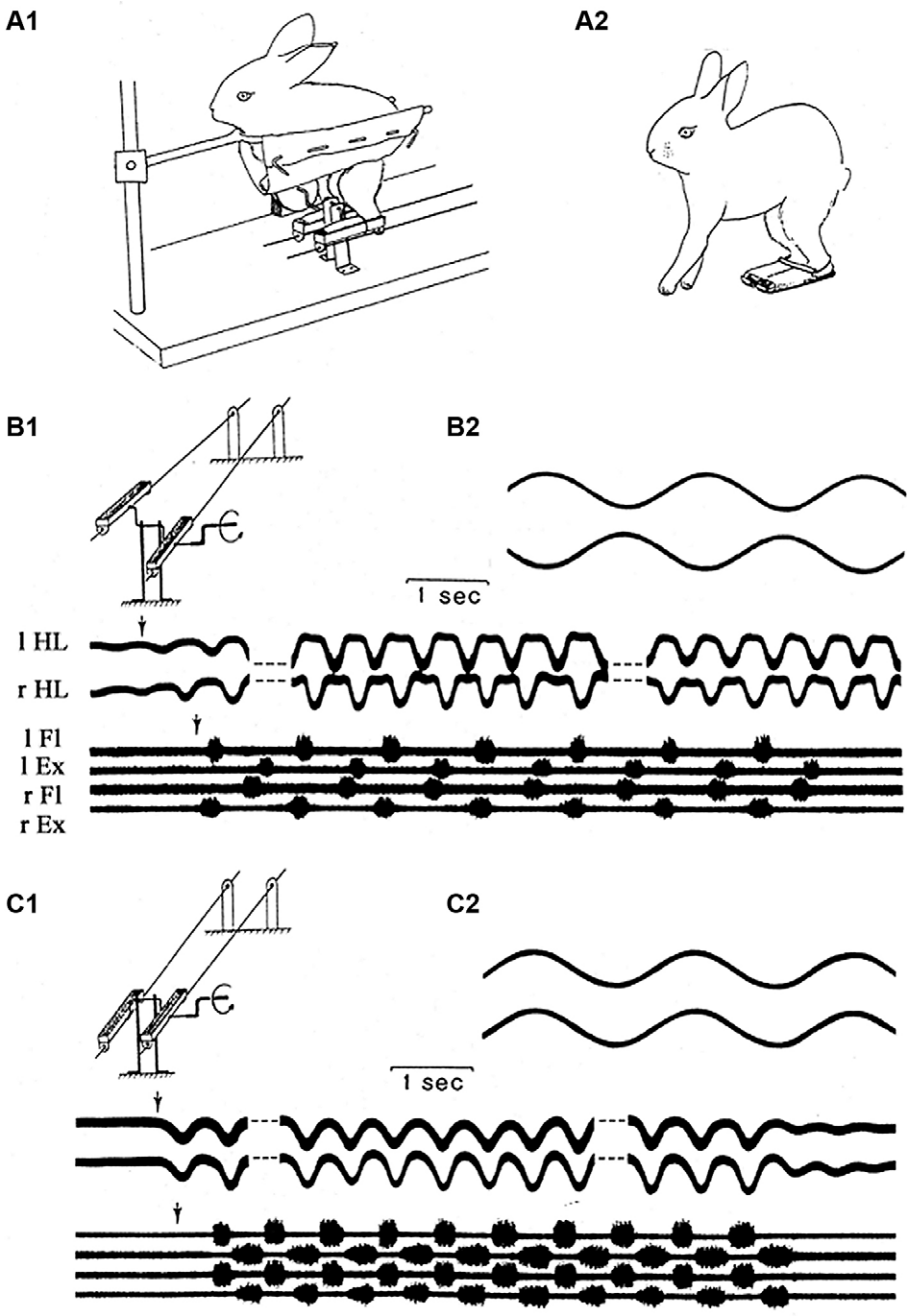

Fig. 5. Conditioning of the hindlimb bilateral locomotor pattern in infant rabbits spinalized shortly after birth. **A1**, **B1**, **C1**: schematic of the motor-driven 'bicycles' that allowed passive entrainment of hindlimbs either in alternation (**B1**) or in synchrony (**C1**). **A2**: Skis bound to the hindlimbs of animals trained in synchrony to maintain them in a symmetrical position between training sessions. **B2** and **C2** show three different recordings: the upper is a mecanogram (M) of pedal movements during training; the middle trace is a motion recording of the left hindlimb (lHL) and right hindlimb (rHL) during a locomotor sequence induced by a small pinch (at vertical arrow) to a 30-day-old animal. Hindlimb movements are alternate in **B2** and synchronous in **C2**, according to the training paradigm. The lower records were obtained soon after the middle sequence in the curarized preparation with hindlimb muscle nerve recordings (same presentation as in Fig. 4). They show clearly that the conditioned bilateral alternation (in **B2**) or synchrony (in **C2**) is programmed at a central, spinal level (from [15]).

Fig. 5. Conditionnement de l'organisation bilatérale au niveau des membres postérieurs chez le lapereau spinalisé rapidement après la naissance. **A1**, **A2**, **A3** : Schéma des « bicyclettes » motorisées permettant d'entraîner passivement les membres postérieurs du lapereau, soit en alternance (**B1**), soit en synchronisme (**C1**). **A2** : Skis de fixation des membres postérieurs des animaux entraînés en synchronisme et permettant de les maintenir en

but now with a typical bilateral alternating pattern, as in the cat or the rat (Fig. 4B) [14]. Does the capacity for synchronous coupling between left and right locomotor generators exist at the spinal level? Evidently the answer is no, since even with a reduction in pharmacological activation and with symmetrical bilateral stimulation of both hindlimbs, synchronous left–right motor output could not be maintained throughout the electrical stimulation and a return to alternate coupling occurred. Finally, it is noteworthy that in all studied Mammals, both alternate and synchronous interactions couple the lumbar locomotion generators, but the alternate one is totally dominant at the spinal level from birth [7,11,13]. From these observations, it can be concluded that the *hindlimb hopping pattern* of the intact adult rabbit has to be *imposed by a supraspinal control of spinal cross-cord couplings*, either by inhibition of the alternate coupling or by potentiation of the synchronous one, or both [15].

An interesting adaptation of the locomotor pattern to accommodate hindlimb lengthening occurs over a short period in the infant rabbit, from day 10 to 20 after birth. When the infant begins to leave the mother's nest at about 10 days, the fore- and hindlimbs are still roughly the same length and the young animal walks like a digitigrade cat. Within the ten following days, however, hindlimb hopping begins and is expressed more and more frequently in the young animal's walking pattern. At about 20 days after birth, the hindlimbs have grown much more rapidly than the forelimbs and the long hindfeet are now uniquely involved in a plantigrade hopping motion [15].

This period of 10 to 20 days of life is important in the rabbit's life since, as reported by Langworthy [10], the main descending pathways responsible for supraspinal motor control are myelinated at the lumbar level by about post-natal day 15. On this basis, we were interested in determining whether alternate stepping, like in-phase hopping could develop and be expressed if the lumbar locomotor generators were isolated from supraspinal levels before descending pathways had become functional. Following a low spinal transection two days after birth, animals were then trained daily from postnatal day 10 to 30. Training was performed on a motor-driven 'bicycle' with a gearing that could passively move the hindlimbs either synchronously, or in alternation like a human bicycle (see Fig. 5A1,B1, C1). At 30 days, hindlimb locomotor movements and the corresponding central motor pattern were entirely consistent with the training the animals had previously followed: expression of either a pure stepping or a pure hopping pattern (Fig. 5B2,C2). Nonetheless, the latter coordination mode was fully obtained only if both feet were bound to a pair of connected skis to maintain a synchronous hindlimb position between the conditioning sessions (Fig. 5A2). This training was no longer effective when the spinal transection was performed at 18 days of age [6,15].

These results demonstrate that shortly after birth, the bilateral spinal pattern of hindlimb locomotion in the rabbit is a *plastic phenomenon* capable of modification via either alternating or synchronous hindlimb sensory inflow occurring during behavioural training. In 1986, this provided not only the *first evidence of a plasticity arising from conditioning at the spinal level*, but also indicated that positive conditioning is possible as long as the rabbit's spinal CPGs have not been subjected to any critical supraspinal control approximately between 12 and 18 days of age.

4. Discussion

From a comparison of the evolutionary changes in Vertebrate locomotion and its adaptation in Mammals, it emerges that the neural mechanisms involved in the two processes are not the same. The basic neural phenomenon for locomotion found in all studied vertebrates is that rhythm genesis is achieved within the spinal cord via CPGs. The aim here was to show that with evolution, the organization of the CPGs throughout the cord, which is relatively uniform and metameric in swimming low vertebrates, has become re-centred in walking Mammals to segments controlling the limbs, with a loss of rhythmogenic capacity of most other metamers. In the example of behavioural adaptation described in the hopping rabbit, the basic spinal organization is maintained exactly as in most other walking Mammals. In the rabbit, however, the hopping adaptation is obtained via a supraspinal control from the brainstem onto the spinal couplings that link left and

position symétrique en dehors des séances d'entraînement. **B2** et **C2** montrent trois types d'enregistrements : le premier est un mécanogramme (M) des mouvements de chaque type de pédaliers (en alternance en **B2**, en synchronisme en **B3**) ; les enregistrements du milieu sont des enregistrements des mouvements locomoteurs des membres postérieurs gauche (lHL) et droit (rHL), induits par un léger pincement (à la flèche) de l'animal, alors âgé de 30 j. Ces mouvements sont en alternance en **B2** et en synchronisme en **C2**, en conformité avec l'entraînement. Les tracés du bas ont été obtenus rapidement après les précédents sur préparation curarisée et enregistrement sur des nerfs musculaires des membres postérieurs (même présentation que sur la Fig. 4). Ils montrent que l'alternance (en **B2**) comme le synchronisme (en **C2**), obtenus par conditionnement, sont programmés à un niveau central et spinal (d'après [15]).

right lumbar CPGs. While the bilateral pattern could be experimentally induced by behavioural conditioning of infant spinal rabbits, such an adaptation at the spinal level does not occur spontaneously. In this context it would be interesting to know whether the concept of employing different fundamental mechanisms in evolution and adaptation could be extended to other basic functions. To examine this possibility, Amphibians certainly represent a remarkable model of short-term evolutionary/adaptive processes that include drastic morphological and physiological changes that occur simultaneously to transform the same lamprey-like swimming tadpole into a walking (rat-like) or hopping (rabbit-like) adult [5].

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