Université de Montréal

PHYSIOLOGICAL, PHARMACOLOGICAL AND ANATOMICAL STUDIES OF THE LOCOMOTION IN CATS SUBJECTED TO VENTRAL AND VENTROLATERAL SPINAL LESION

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PHYSIOLOGICAL, PHARMACOLOGICAL AND ANATOMICAL STUDIES OF THE LOCOMOTION IN CATS SUBJECTED TO VENTRAL AND VENTROLATERAL SPINAL LESION

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SUMMARY

The aim of this study was to better understand the contribution of descending pathways in the ventral and ventrolateral spinal quadrants, such as the reticulo- and the vestibulospinal pathways, to the control of locomotion in the adult cat. Former studies (Eidelberg 1981, Afelt 1974) have suggested that these pathways are "essential and necessary" for locomotion. However, this conclusion was mainly based on spinal lesions designed to spare only small patches of tissue in the ventral and the ventrolateral quadrants and the ability to walk in the absence of these pathways was not directly addressed. Therefore, in the first part of this study the ability to walk in the absence of the ventral and ventrolateral pathways was addressed by lesioning the ventral and ventrolateral pathways (at the thoracic level) while leaving intact dorsolateral pathways such as the cortico- and rubrospinal pathways. This lesion paradigm provides insight on the direct implication of the ventral and ventrolateral pathways in locomotor control and also allows one to evaluate whether remaining descending pathways in the dorsolateral quadrants are sufficient to sustain locomotion in the absence of these important ventral and ventrolateral pathways.

The locomotor capacities after such lesions were evaluated by following the recovery of treadmill locomotion of 8 adult cats which were

documented daily using electromyographic (EMG) recordings and kinematic methods. To evaluate the extent of the spinal lesion, WGA-HRP was injected caudal to the site of the lesion and labelled cells were counted in brain stem nuclei and the motor cortex.

The data show that all cats eventually recovered quadrupedal voluntary locomotion despite the extensive damage to the reticulospinal and the vestibulospinal pathways. Immediately post-lesion the animals could not support or walk with their hindlimbs. Subsequently, progressive locomotor improvement occurred over a period of 1 to 4 weeks, until a more stable locomotor performance was reached (plateau-period). The time for recovery was longer for cats with more extensive lesions and their locomotor deficits, during the plateau-period, were more pronounced. These deficits included poor lateral stability, irregular stepping of the hindlimbs and inconsistent homolateral fore- and hindlimb coupling. Overall, the results from this study showed that some recovery of quadrupedal locomotion is possible even after a massive lesion to the ventral and ventrolateral quadrants (severing the vestibulospinal pathway and causing extensive, although incomplete, damage to the reticulospinal tract) as long as the dorsolateral pathways, such as the corticospinal tract, are present. The results also emphasize the importance of ventral and ventrolatrolateral pathways for regulating the walking in a step by step manner and providing the appropriate postural adjustments.

It was found clinically important to try to improve the locomotor capacities of the partially lesioned cats as a useful animal model for patients with partial spinal lesions. Therefore, in the second part of the thesis, the locomotion of two cats that were subjected to the partial spinal lesions, was compared before and after application of Serotoninergic and Noradrenergic drugs through a chronically implanted intrathecal cannula. EMG and kinematic analyses showed that Noradrenaline (NE) injected in either the immediate post-lesion stage or during the plateau-period, improved weight support of the hindquarters, stabilized the hindlimb stepping and the interlimb coupling, permitting the cats to maintain regular quadrupedal locomotion for longer periods of time and at a higher speed. Methoxamine, an α_1- agonist (tested only at the plateau-period) had similar effects. In contrast, the α_2- agonist, Clonidine, tended to decrease weight support, perhaps through a decrease of reflex excitability of the hindlimbs, and therefore caused a deterioration of the walking. Serotoninergic drugs, such as 5HT, its precursor 5HTP, or the agonist Quipazine also improved the locomotion by increasing regularity and the duration of the hindlimb step cycle. In contrast, the $5HT_{1A}$ agonist, DPAT, affected only one of the cats, in which it caused foot drag and frequent stumbling. Injection of a combination of Methoxamine and Quipazine resulted in maintained, regular stepping with smooth movements and good weight support. Our results show that some drugs, such as Methoxamine or/and Quipazine can improve the residual voluntary

locomotor capacities of the partially lesioned cat and improve some of its postural aspects. However, the effects of other drugs (such as Clonidine) may depend on whether or not the spinal lesion is complete as well on the given doses. In a clinical context, this may suggest that different classes of drugs should be used in patients with different types of spinal cord injuries.

The studies in this thesis taken in perspective with former studies (Eidelberg 1981, Afelt 1974, Bem et al 1995, Gorska 1993a, Jiang and Drew 1996, Nathan 1994) show that patches of intact tissue of various tracts, whether located in the ventral or the dorsal quadrants of the spinal cord are probably sufficient to sustain locomotor function. The long period of recovery observed for the most extensively lesioned cats suggests that plastic changes underlie the recovery process. The plastic changes can occur at many control levels of locomotion. Among the proposed changes are: amplification of the function in the remaining descending pathways which normally participate in the control of locomotion and also "take-over" by other intact descending pathways. The results of the assays with clonidine indicate that the partial lesioned cat may also depend on the excitability of segmental reflexes to maintain weight support. From the clinical point of view, these encouraging findings suggest that the locomotor system can adapt to extensive lesions given a minimal contribution from remaining pathways. Therefore, in the clinical context, it is of major importance to maintain survival of remaining axons after injury

and also apply proper training programs to accelerate or improve the functional "take over" process by other pathways. Adequate pharmacotherapy can be applied as well to improve these residual locomotor functions.

RÉSUMÉ

Cette étude a pour but de mieux comprendre la contribution des faisceaux descendants des quadrants ventraux et ventrolatéraux de la moelle épinière, telles que les voies réticulo et vestibulospinales, dans le contrôle de la locomotion chez le chat adulte. Des études antérieures (Eidelberg 1981 et Afelt 1974) suggéraient que ces faisceaux étaient "essentiels et nécessaires" à la locomotion. Leur conclusion était cependant basée principalement sur des lésions spinales ne laissant que de petites parties de tissus intacts dans les portions ventrales et ventrolatérales. Ce protocole ne permettait donc pas de déterminer l'habilité et la capacité de marcher en l'absence de ces voies descendantes.

La première phase de cette étude a pour but de clarifier l'apport de ces voies descendantes en utilisant une nouvelle approche qui consiste à pratiquer des lésions des quadrants ventraux et ventrolatéraux au niveau thoracique en laissant intactes les voies dorsolatérales, telles que les voies rubrospinales et corticospinales. Ce type de lésion a permis de cerner l'apport des voies ventrales et ventrolatérales dans le contrôle de la locomotion. De plus, il a été possible d'évaluer si les voies descendantes encore présentes dans les quadrants dorsolatéraux étaient suffisantes pour maintenir un rythme locomoteur en l'absence de ces

voies ventrales et ventrolatérales si importantes dans le contrôle de la locomotion.

L'évaluation de la récupération locomotrice a été faite chez huit chats adultes qui ont subi des lésions spinales partielles au niveau thoracique bas. L'implantation chronique d'électrodes électromyographiques a permis de documenter les principales caractéristiques de la locomotion dans les mêmes conditions d'enregistrement pré et post-lésion. L'activité électromyographique (EMG) a été enregistrée quotidiennement et chaque expérience a été documentée à l'aide de vidéos synchronisés avec l'activité musculaire afin de suivre l'évolution de la récupération par une analyse cinématique détaillée. Lorsque l'animal eut récupéré et après avoir complété toutes les expériences pertinentes, nous avons injecté de la peroxidase raifort (WGA-HRP) caudalement au site de la lésion, ce qui nous a permis de documenter exactement les lésions que nous avions faites en marquant les cellules dans les noyaux du tronc cérébral et dans le cortex moteur, lieu d'origine des voies corticospinales. La comparaison de ces résultats avec des mesures effectuées chez le chat intact a permis de savoir quelles étaient les voies encore présentes.

Nos résultats ont démontré que tous les chats peuvent récupérer la capacité de marcher à quatre pattes, volontairement, et ce malgré les dommages importants de faisceaux descendants (voies réticulospinales et vestibulospinales) qui ont été confirmés par le compte des cellules

marquées dans le tronc cérébral. Au stade aigu suivant la lésion spinale, tous les chats présentaient des déficits posturaux et locomoteurs et ne pouvaient soulever leur arrière-train ou marcher volontairement avec leurs membres postérieurs. Les chats ont graduellement récupéré la marche à quatre pattes à plus ou moins long terme, dépendamment des lésions subies et malgré des problèmes de débalancement, de synchronisation et de stabilité latérale. L'analyse électromyographique et cinématique a aussi démontré une augmentation de la variabilité de la durée du cycle de marche, sans toutefois révéler de changement majeur dans la structure du cycle ou dans la coordination entre les articulations des membres. Le couplage homolatéral (entre les membres antérieurs et postérieurs) a été sérieusement perturbé chez les chats avec les lésions les plus importantes. Même si le patron alterné des extenseurs et fléchisseurs a été maintenu, de grandes variations ont été notées dans l'amplitude et la durée des bouffées d'EMG ainsi qu'un manque de modulation d'amplitude durant la marche sur tapis roulant en pentes ascendantes ou descendantes.

Chez les chats avec de plus grandes lésions, les membres antérieurs semblent jouer un rôle de propulsion plus important, tel que le démontre l'augmentation d'activité des triceps. Chez les chats ayant subi de petites lésions, ces déficits étaient transitoires. Cependant, la majorité des chats avec de grandes lésions présentaient des déficits prononcés qui persistaient longtemps après la lésion, même après avoir atteint un

certain plateau de récupération locomotrice. Ceci nous a permis de conclure que la récupération de la locomotion quadrupède était possible même après des lésions massives des quadrants ventraux et ventrolatéraux (endommageant les voies vestibulospinales et causant des dommages sévères mais incomplets aux voies réticulospinales) en autant que les voies dorsolatérales telles que corticospinales et autres soient présentes. Ces résultats démontrent l'importance des voies ventrales et ventrolatérales qui régularisent la marche et produisent les ajustements posturaux nécessaires à celle-ci.

Par la suite, nos recherches se sont tournées vers l'amélioration de la capacité locomotrice résiduelle chez le chat spinal partiel puisqu'il s'agit d'un bon modèle d'étude pour les patients ayant subi des lésions partielles de la moelle épinière. Dans la seconde partie de cette thèse, la locomotion chez deux chats présentant des lésions extensives de la moelle épinière a été comparée avant et après l'injection intrathécale de drogues noradrénergiques et sérotoninergiques par une canule implantée chroniquement. Ces drogues ont été sélectionnées car leurs effets sur la locomotion des chats spinaux complets sont bien documentés et ils sont de plus utilisés dans les essais cliniques chez les patients. Les analyses électromyographique et cinématique montrent que la Norépinéphrine injectée au stade aigu et durant la période de plateau améliore la locomotion quadrupède. Suivant l'injection de Norépinéphrine apparaîssent une plus grande régularité de la marche des membres

postérieurs et un meilleur couplage entre les membres, ce qui permet conséquemment aux chats de maintenir une locomotion stable pour de longues périodes. La Methoxamine, un agoniste alpha-1 (injecté seulement durant la période de plateau) a eu des effets similaires. Par opposition, l'agoniste alpha-2 Clonidine entraîne une diminution du support de poids des membres postérieurs résultant en une détérioration de la marche. Les drogues sérotoninergiques, telles que le neurotransmetteur 5-HT, son précurseur 5-HTP et l'agoniste Quipazine améliorent la locomotion en agissant sur la régularité des mouvements des membres postérieurs et en amplifiant la durée du cycle de marche. Par contre, l'agoniste 5HT1A, DPAT, a produit un traînement de pied chez un chat, entraînant des chutes fréquentes. L'injection d'une combinaison de Methoxamine et de Quipazine a permis d'obtenir une marche régulière avec des mouvements souples et une bonne stabilité latérale.

Nos résultats démontrent que l'effet de la drogue peut être intégré à la capacité locomotrice volontaire résiduelle et produit une amélioration de certains aspects posturaux. De plus, ce travail montre clairement que les effets de drogues (telle que la Clonidine) sont directement réliés à la localisation et à l'étendue de la lésion spinale, ainsi qu'aux concentrations de doses injectées. Dans un contexte clinique, ceci peut suggérer que différentes classes de drogues peuvent être utilisées chez des patients ayant subi différents types de lésions spinales.

Ces résultats, lorsque combinés à ceux déjà obtenus (Eidelberg 1981, Afelt 1974, Bem et al 1995 Gorska 1993a, Jiang et Drew 1996, Nathan 1994), démontrent que des résidus de tissu intact de différents faisceaux , s'ils sont localisés dans les quadrants ventraux ou dorsaux de la moelle épinière, sont probablement suffisants pour maintenir des fonctions locomotrices.

Les longues périodes de récupération observées chez les chats ayant de grandes lésions suggèrent que des changements plastiques jouent un rôle dans le processus de récupération. Ces changements de plasticité peuvent intervenir à différents niveaux du système locomoteur spinal. Parmi les changements proposés, citons l'amplification des fonctions des faisceaux descendants encore présents qui participent normalement au contrôle de la locomotion, ainsi que la compensation par les autres voies descendantes qui sont demeurées intactes. Les résultats des expériences avec la Clonidine indiquent que les chats avec des lésions partielles de la moelle épinière dépendent de la transmission périphérique et des réflexes segmentaires pour maintenir le support de poids.

D'un point de vue clinique, ces résultats encourageants suggèrent que le système locomoteur peut s'adapter à des lésions extensives de la moelle épinière grâce à la contribution des faisceaux encore présents. Il est donc de première importance de maintenir la survie des axones encore intacts après une lésion spinale et d'adopter un programme

d'entraînement adéquat pour accélérer le processus de récupération de la locomotion ou améliorer la compensation par les autres faisceaux. Une pharmacothérapie adéquate peut aussi être utilisée pour améliorer les fonctions locomotrices résiduelles.

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LIST OF ABBREVIATIONS

(General introduction and discussion)

CNS:	Central nervous system
CPG:	Central pattern generator
DLF:	Dorsolateral funiculus
DOPA:	Dihydroxyphenyalanine
DPAT:	8-Hydroxy-dipropylaminotetralin
DTF:	Dorsal tegmental field
E_1:	First extension phase of the step cycle
E_2:	Second extension phase of the step cycle
E_3:	Third extension phase of the step cycle
EMG:	Electromyographic
EN:	Entopeduncular nucleus
F:	Flexion phase
FRA:	Flexor reflex afferent
GABA:	γ-aminobutyric acid
5-HT:	Serotonine
5-HTP:	5-hydroxytryptophan
HRP (WGA-HRP):	Horseradish Peroxidase
i.p:	Intraperitoneal
i.v:	Intravenous
IC:	Inferior colliculus
LVN:	Lateral vestibular nucleus
MLR:	Mesencephalic locomotor region
MN:	Motoneuron
MRF:	Medullary reticular formation

5N:	Trigeminal nucleus complex
NE:	Noradrenaline
NRGc:	Nucleus reticularis gigantocellularis
NRPo:	Nucleus reticularis pontis oralis
NRM:	Nucleus reticularis magnocellularis
6-OHDA:	6-hydroxydopamine
PLR (PLS, PMLS):	Pontomedullary locomotor strip
PPN:	Pedunculopontine nucleus
RN:	Red nucleus
SLR:	Subthalamic locomotor region
SN:	Substantia nigra
St:	Semitendinosus
Str:	Striatum
T:	Thoracic
VLF:	Ventrolateral funiculus
VTF:	Ventral tegmental field

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to Fanny

my scientific mother

GENERAL INTRODUCTION

The ability to walk from one place to another requires the generation of basic stereotypic stepping movements, maintenance of adequate posture and equilibrium during walking and adaptation of the basic movement pattern to on-going changes in the environment (Grillner, 1981, Armstrong, 1986, Rossignol, 1996). The importance of descending information from the brain stem and the motor cortex to the control of these locomotor aspects is usually demonstrated by interrupting the descending pathways by a complete spinal cord transection. Although adult cats spinalized at a low thoracic level can regain the ability to step with the hindlimbs on a moving treadmill belt (Grillner, 1973, Forssberg et al. 1980, Barbeau and Rossignol, 1987, Belanger et al. 1996, Chau et al. 1998a), they cannot voluntarily initiate locomotion or maintain weight support and lateral stability of the hindquarters. Furthermore, when complete spinal cats are placed on the floor, they cannot execute a coordinated locomotion with all four limbs; instead, they propel themselves using their forelimbs only, while the hindlimbs drag behind. These findings indicate that a well-integrated voluntary quadrupedal locomotion depends to a large extent on descending information, exerted on the spinal system, by brain stem nuclei and the motor cortex.

For the general introduction to this thesis, a special emphasis will be placed on the contribution of descending pathways to the different locomotor aspects, (initiation, postural adjustments, adaptation to locomotor demands and interlimb coupling), followed by a review of studies in which the partial spinal lesion approach was used to asses the contribution of supraspinal information to the locomotor control. Initially, however, I will provide an outline of the spinal generation of the basic stereotypic movement and discuss the importance of peripheral inputs in the control of locomotion as this information is important in our predictions, as summarized at the end of the introduction, and in the interpretation of the results.

1. Central generation of the stereotypic locomotor movement:

The step cycle:

During each step cycle, each one of the limbs performs a repetitive sequence of stereotypic movements. A representative example of the intact hindlimb step cycle is illustrated in Fig. 1 (adapted from Rossignol, 1996), showing the kinematic (A to C) and the related EMG activity (D) of selected hindlimb muscles. During one step cycle the limb is in contact with the ground during the support period, or stance, and is moved forward in the air during the swing period as was illustrated for the hindlimbs in (E), using a foot fall diagram in which the horizontal heavy

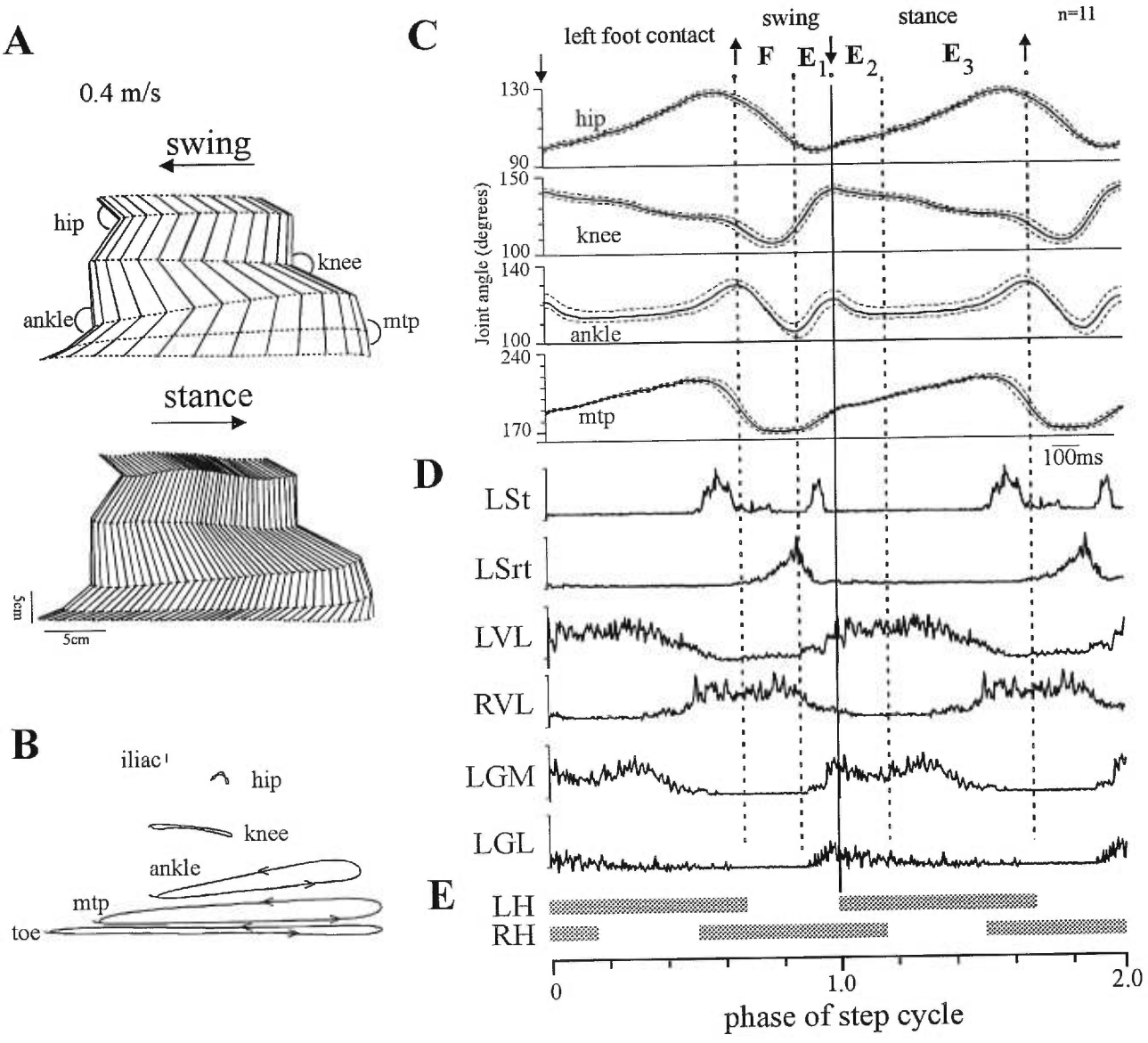
lines represent the stance and the empty spaces between them, represent the swing.

The movement of the hindlimb during the swing and stance is illustrated in Fig. 1A, using stick figure diagrams, and the trajectory of each joint is shown in Fig 1B. According to Philippson (1905), swing and stance can each be further subdivided into two phases: swing into F and E_1 and stance into E_2 and E_3. These are indicated in Fig. 1C, on the average joint angular displacement traces. During the F phase, all the joints are flexed to lift the foot of the ground and during E_1 the ankle and the knee extend in preparation to foot contact, while the hip continues flexing. Starting at foot contact, the limb flexes slightly (E_2 or yield) and then at E_3, all the joints extend backwards and the body is propelled forward to complete one step cycle.

In Fig. 1D, the related synchronized EMGs illustrate that, generally, the different muscles can be classified as flexors, which are activated during the swing, and as extensors, which are activated in preparation for foot contact and during most of the stance. The classification of muscles into extensors and flexors dates to the beginning of the century (Sherrington, 1910) and is based on observations showing that some muscles contract during activation of the flexor reflex in the spinal and the decerebrate cat, while others relax. Even so, Sherrington noted that in some cases this general classification into flexor and extensor muscles did not apply. For example biarticular muscles, such as Sartorious, act to flex the hip and

extend the knee (anterior part) while its medial part, acts to flex both the hip and the knee. The step cycle, although stereotypic, can be adapted to changes in locomotor requirements, as in the case of perturbations, when walking at different speeds or on an inclined surfaces. This subject will be addressed in more details later (see section 4, concerning the regulation and adaptation of locomotion).

Fig.1: Kinematics and synchronized EMG activity of the step cycle in an intact cat (adapted from Rossignol, 1996): A representative example of the normal hindlimb step cycle (cat EB2) taken during treadmill locomotion at 0.4m/s. (A) Stick figure diagrams of the hindlimb movement during the swing and the stance of one step. The arrows illustrate the direction of the movement and the semicircles indicate the direction of the angular measure. (B) Trajectories of the joint movement during one step cycle, reconstructed from the position of reflective markers placed on the bony landmarks of each joint. The arrow heads indicate the direction of movement. (C) Average angular displacement traces of 11 consecutive step cycles calculated for each one of the joints. The dotted envelope around the traces indicate 1 standard error of width. The up and down arrows with the related vertical dashed lines, indicate foot contact and foot lift, respectively. F, E_1, E_2 and E_3 refer to subdivision of the step cycle according to Philippson (1905). (D) The averaged and rectified EMG activity in selected extensor and flexor muscles. (E) Foot fall diagram of the left and the right hindlimbs (LH, RH respectively). The heavy horizontal lines illustrate the stance period, while the empty spaces illustrates the swing phase. All the events are repeated twice, on a scale from 0 to 2, to better appreciate the events occurring around foot contact.



adapted from Rossignol, S. 1996

Spinal generation of the stereotypic movement:

It is generally accepted that the basic stereotypic locomotor pattern is generated in the spinal cord by intrinsic spinal neuronal networks and can be expressed in the absence of descending or peripheral inputs. As proposed by Sherrington: "the seat of the rhythm is obviously not peripheral. It is not in the muscles or their motor nerves.....nor it can lie in the receptive organs of the skin or their afferent nerve trunks for direct stimulation of the cross section of the spinal axis itself provokes the rhythmic replay. The rhythm is therefore central in its seat (Sherrington, 1910)". This ability of the spinal cord to generate the locomotor rhythm was demonstrated by Brown (1911) in the spinal and deafferented cat and later in acute or chronic spinal cats in which all phasic inputs were eliminated by currarization (Grillner and Zangger, 1974,1979, Pearson and Rossignol, 1991). However, in order to express the alternating locomotor rhythm in these preparations, administration of drugs such as L-DOPA is needed. L-DOPA acts by releasing Noradrenaline (NE) from noradrenergic terminals (Anden et al. 1966). A locomotor rhythm in the absence of phasic inputs can also be induced by stimulation of the mesencephalic locomotor region (MLR) in decerebrate currarized cats (Jordan et al. 1979). In all of these experimental conditions, the expressed locomotor pattern resembles closely the intact pattern (Rossignol et al. 1993). It was suggested that L-DOPA in the spinal

preparation, and MLR stimulation in the decerebrate cats, acts to release the spinal locomotor rhythm by influencing common spinal interneurons constituting the central pattern generation (Grillner, 1973, Grillner and Shik, 1973, Jordan, 1991). The central pattern generator (CPG) is influenced by peripheral and by descending inputs from supraspinal centers, which act to adjust the locomotor rhythm according to the animal needs and to changes imposed by the environment, as will be detailed next.

2. Implication of peripheral inputs in locomotion:

Peripheral inputs are not necessary for the generation of the basic locomotor pattern which, as described in the former section, is intrinsic to spinal neuronal networks. However, the EMG pattern recorded during MLR evoked locomotion in the acute decerebrate cat after deafferentation showed that the basic locomotor pattern, although generally preserved, is sometimes less stable (Grillner and Zangger, 1975, Grillner, 1981). For example, it includes episodes in which the knee flexor (St) discharges during the extension phase. Such unusual patterns were also reported in currarized cats (Grillner, 1981). In addition, it has also been shown that an alternating rhythm can be induced by bilateral stimulation of cut dorsal root ganglions, both in acute walking kittens and under fictive conditions, suggesting that tonic afferent input can result in rhythmic discharge similar to locomotion (Grillner and Zangger, 1974).

Changes in the limb position or pressing the skin abolished rhythmic activity recorded in ventral root filaments during MLR stimulation in the decerebrate cat (Orlovsky and Feldman, 1972). Further, preventing or accelerating the hip flexion during the swing phase in the decerebrate preparation delayed or accelerated, respectively, the knee and the ankle flexion (Orlovsky, 1972c). However, delaying the ankle or the knee flexion had little effect on the movement of other joints, suggesting an important role for the hip in shaping the locomotor movements in other joints (Rossignol, 1996). The important influence of the hip position on the locomotor pattern was demonstrated in spinal kittens walking on a treadmill (Grillner and Rossignol, 1978). Gradually extending the hindlimb backward resulted in flexion when a certain angle was attained, an angle in which the swing phase is normally initiated. Manipulation of the leg position during fictive locomotion in the spinal cat also affected the locomotor rhythm. Moving the leg progressively from extension to flexion decreased the flexor burst duration and increased the cycle period (Pearson and Rossignol, 1991). The hip afferents responsible for signaling the limb position are not known but they probably included both joint receptors and muscle afferents (Grillner, 1979).

In addition to the inputs from the hip, signaling hip position, load signals can also contribute to determining the transition from swing to stance. Stimulation of the plantar surface of the foot in the thalamic cat, or direct stimulation of the sural nerve, mimicing an increased load on the

foot, caused prolongation of the stance phase if given during the extensor activity (Duysens and Pearson, 1976). Stimulation given during the swing phase prolonged the flexor activity and shortened the following extensor burst. Increase of load on the ankle extensor muscle in another set of experiments (Duysens and Pearson, 1980) caused the disappearance of the flexor burst, suggesting that during normal locomotion, an increased load will prevent the transition from stance to swing.

Similar results to those of Duysens and Pearson (1976) were obtained in fictive spinal cats by stimulation of group I afferents, mainly of group Ib afferents which are implicated in load signaling (Conway et al. 1987). Gossard et al. (1994, see also Pearson and Collins, 1993) further showed that the pathway transmitting excitatory information from Ib afferents is active only during locomotor activity and is phasically modulated during the step cycle. The pathway is oligosynaptic and is suggested to affect the CPG.

More recent studies (Guertin et al. 1995) extended the results of Conway et al. (1987), by showing that selective activation of muscle spindle afferents (Ia), especially of the ankle extensor, can enhance the extensor ENGs in other joints of the same limb during fictive MLR-evoked locomotion. In addition, it was shown (Angel et al. 1996) that a disynaptic pathway conveying information from Ia afferents appears during locomotion to further enhance extensor activity and force production. Peripheral input from group II afferents is also suggested to effect the

locomotor rhythm (Perreault et al. 1995). Therefore, from the above description, multiple parallel pathways can act to enhance extensor activity during locomotion.

Peripheral inputs can also entrain the locomotor rhythm. In fictive spinal cats the locomotor rhythm (evoked by Noradrenergic agonists) could be influenced in a 1:1 manner by applying sinusoidal hip movement at a certain frequency around the frequency of the spontaneous locomotion (Anderson and Grillner 1983), or by applying rhythmic stretches to extensor muscles (Conway et al. 1987, Pearson et al. 1992). Exceeding that range of frequencies caused a reset of the spontaneous locomotor rhythm. Below that range, the locomotor rhythm was not affected, but there was a concomitant increase in the amplitude of the extensor burst (Conway et al. 1987, Guertin et al. 1995). Repetitive stimulation of group I afferents had similar effects to those seen with muscle stretches (Pearson et al. 1992). Removal of muscle afferents by gradual denervation reduced the efficacy of the entrainment of MLR evoked locomotion in the decerebrate cat, and it was abolished after complete denervation (Kriellaars et al. 1994). The entrainment phenomenon suggests that afferent pathways can directly influence the CPG.

In the intact freely moving cat, peripheral inputs from cutaneous afferents are modulated is a phasic manner with the step cycle in a similar manner to these described for acute preparations (Forssberg et al. 1977,

Forssberg, 1979, Drew and Rossignol, 1987). It is interesting, however, that the intact cat subjected to deafferentation by removing the dorsal roots ganglions (Goldberger and Murray, 1988) or by cutaneous denervation of the distal hindlimb (Bouyer and Rossignol, 1998) can recover ordinary locomotion and, with time, can also adapt to more demanding tasks such as walking on a ladder or on a narrow beam. It should be emphasized, however, that the walking strategies are not necessarily the same as in the intact cat.

Taken together, it is suggested that peripheral information about hip position and load help assure an appropriate transition from swing to stance and proper integration of corrective responses to the ongoing locomotor rhythm.

Another important role of peripheral inputs during locomotion is to enhance the activity of extensors according to the locomotor demand (Pearson, 1995). The latter information may have a special importance after partial spinal lesions and is suggested, at least in part, to underlie recovery of weight support in these cats, as will be detailed in the first paper of the thesis.

3. The involvement of descending pathways in initiation of locomotion:

Descending inputs from several brain stem regions such as the subthalamic locomotor region (SLR), mesencephalic locomotor region (MLR) and pontomedullary locomotor strip or region (PLS, PLR) have been shown to be implicated in the initiation of locomotion in the cat and in other species. The involved structures and their pathways into the spinal cord are summarized in Fig. 2 (adapted from Rossignol, 1996).

MLR: Precollicular post mamillary decerebrate cats do not walk spontaneously (Shik et al. 1966). However, stimulation of the MLR, a region which coincides with the cuneiformis nucleus and the pedonculopontine nucleus (PPN) (Grillner, 1981, Armstrong, 1986), Garcia-Rill and Skinner, 1987b, can evoke episodes of quadrupedal walking on a moving treadmill, even in the absence of phasic inputs (Jordan et al. 1979). The evoked locomotor gait resembles the intact one and can be modulated as a function of stimulus strength and adapted to different treadmill speeds (Shik et al. 1966).

No direct projections have been found between the MLR and the spinal cord; however, it has considerable descending projections to the MRF (Steeves and Jordan, 1984, Garcia-Rill et al. 1983). Electrophysiological studies show that stimulation of the MRF itself (at the convergence site of MLR inputs), which coincides with parts of the nucleus reticularis gigantocellularis (NRGc) and magnocellularis (NRM), can evoke locomotion (Mori et al. 1977, Garcia-Rill and Skinner, 1987b).

Injection of chemical agents such as Acetylcholine and Cholinergic agonists or Glutamic acid in the MRF have the same effects (Garcia-Rill and Skinner, 1987a, Noga et al. 1988). Cooling of the MRF (Shefchyk et al. 1984) or injection of GABA (Garcia-Rill and Skinner, 1987a, Noga et al. 1988) abolishes spontaneous and MLR-evoked locomotion.

The MRF neurons activated by the MLR stimulation have been included within the reticulospinal pathways (Orlovsky, 1970b, Garcia-Rill and Skinner, 1987a, b, Peterson, 1984). These reticulospinal neurons, project through the ventrolateral funiculus of the spinal cord (VLF) (Orlovsky, 1970b, Garcia-Rill and Skinner, 1987b). This was demonstrated, functionally, by restricted lesions to the ventrolateral spinal quadrants in the acute decerebrate cat. These lesions abolished MLR-evoked locomotion (Steeves and Jordan, 1980), while lesions to the dorsolateral funiculi did not have a major effect on it (Noga et al. 1991). The descending MLR affects are probably mediated through interneurons (Shefchyk and Jordan, 1985, Jordan, 1991). The close proximity of the MLR to catecholaminergic neurons of the locus coeruleus (Steeves et al. 1975), together with the known effects of L-DOPA and Noradrenergic agonists on initiation of locomotion in the acute spinal cats, suggested the involvement of this pathway in initiation of locomotion. However, their contribution was not found to be essential to MLR-evoked locomotion, which persists after depletion of CNS Noradrenaline by 6-OHDA (Steeves et al. 1980).

PLR: The pontomedullary locomotor strip is a polysynaptic pathway composed of a chain of neurons (Mori et al. 1977, Shik, 1983, Noga et al. 1988, Garcia-Rill et al. 1983) which extends from the MLR region, along the lateral tegmentum, to the spinal cervical cord. At this level, its neurons are primarily located in the dorsal horn (Mori et al. 1977, Shik, 1983). Electrical stimulation along this pathway or application of Glutamic acid evokes locomotion in decerebrate cats, as does stimulation of the dorsolateral aspects of the cervical spinal cord, a zone that may coincide with Sherrington's stepping point (1910). In the spinal cord, these descending affects (Shik, 1983, Kazennikov et al. 1985) are conveyed primarily through interneurons located in the dorsal horn, where their axons travel along the border of the ventrolateral quadrants. Other studies (Shefchyk et al. 1984, Noga et al. 1991), suggest a second parallel pathway which conveys PLR descending inputs through the MRF and the VLF. The PLR and the MLR seem to be separate pathways for initiation of locomotion, as MLR-evoked locomotion is not abolished by cooling the PLR or after disconnecting the MLR from the PLR zone (Shefchyk et al. 1984, Noga et al. 1991). The medial MLR areas (Shefchyk et al. 1984, Jordan, 1991) perhaps together with other locomotor regions, such as the SLR and the corticobulbar afferents (Mori et al. 1977, Shik, 1983) are suggested to converge on the PLR. In addition, the close proximity of the PLR to the spinal nucleus of the trigeminal complex may indicate that this sensory system may be involved

in the initiation of locomotion of sensory origin (Noga et al. 1988, Rossignol, 1996).

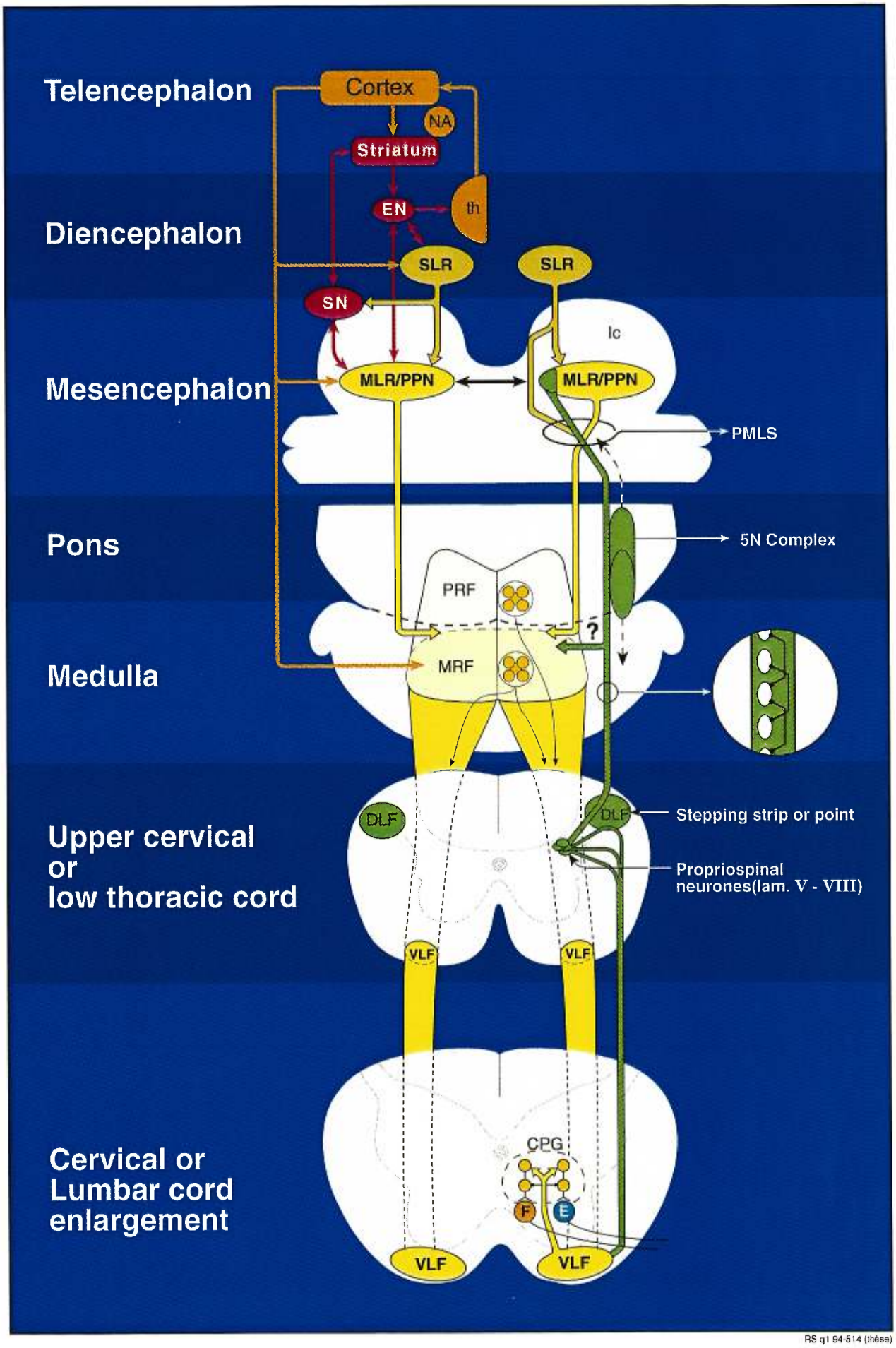
SLR: In contrast to the precollicular post-mamillary preparation, the precollicular pre-mamillary decerebrate cat can walk spontaneously. Lesion to the SLR impairs voluntary goal directed locomotion (Shik and Orlovsky, 1976). However, these cats are capable of walking with MLR stimulation indicating that their locomotor capacities are not abolished by the SLR lesion. On the other hand, SLR evoked locomotion does not depend on the integrity of the MLR, as it persists after lesions to the MLR (Orlovsky, 1969). The descending pathways conveying SLR inputs are not clearly established. However, injection of HRP into the SLR (Berezovskii 1984 cf. Armstrong, 1986) labells terminals in many brain stem structures, such as the MRF, which are implicated in locomotion. Reticulospinal neurons in the MRF are activated monosynaptically by SLR stimulation and show marked spontaneous activity relative to what is observed in the precollicular post mamillary cat (Orlovsky, 1970b), indicating that the SLR may exert some tonic effects on them.

Corticobulbar pathways: In the cat, the contribution of the motor cortex to the initiation of the locomotor pattern does not seem to be essential, as lesions to the motor cortex or pyramidotomy leads to only slight and transient locomotor deficits during ordinary walking (Liddell and Phillips, 1944 cf Armstrong, 1986). Stimulation of sectioned pyramids in the mesencephalic cat can, however, evoke locomotion, while stimulation

of the intact pyramids stop MLR-evoked locomotion (Shik et al. 1968). The locomotion evoked after stimulation of the pyramids does not depend on the integrity of the SLR or the MLR, suggesting that cortical afferents converge on more caudal structures in the brainstem (Shik et al. 1968, Grillner, 1981, Armstrong, 1986).

Taken together, several pathways descending in the ventral and in the dorsal quadrants of the spinal cord are implicated in initiation of locomotion. Their relative contribution to the locomotion of the intact cat is not known. However the possibility that they can function independently is of importance to explain recovery after partial spinal lesions as discussed in the first paper of the thesis.

Fig. 2: **The supraspinal structures implicated in initiation of locomotion and their pathways (adapted from Rossignol, 1996).** 5N, trigeminal nucleus complex; CPG, central pattern generator; DLF, dorsolateral funiculus, EN, entopeduncular nucleus; IC inferior colliculus; MLR, mesencephalic locomotor region; MRF medullary reticular formation, NA nucleus accumbens; PMLS, pontomedullary locomotor strip; PPN, pedonculopontine nucleus; PRF pontine reticular formation; SLR, subthalamic locomotor region; SN, substantia nigra; Str, striatum; Th, thalamus; VLF, ventrolateral funiculus



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4. The involvement of descending pathways in adaptation of locomotion:

Postural adjustments:

The full expression of locomotion depends on the appropriate integration of mechanisms which control the initiation of locomotion and those which control postural adjustments; "The execution of stepping movement by the limbs does not of course in itself amount to walking. For this latter act, the reflex stepping of the limbs has to be combined with reflex maintenance of the erect posture of the body" (Sherrington, 1910).

Stimulation of the MLR in the precollicular, post-mamillary, decerebrate cat evokes locomotion only if an adequate level of postural muscle tone has been developed (Mori et al. 1978). With MLR stimulation, an increase in the extensor muscles force and EMG activity is observed. At that time the cat can stand without external support and stepping can be evoked when the treadmill is put in motion (Mori, 1987). These results demonstrate that MLR stimulation can provide the necessary background excitability (Mori, 1987) in parallel to the initiation of locomotor rhythm (Noga et al. 1991)

In addition to the MLR and SLR, whose activation causes postural changes and initiates the locomotor rhythm, two other distinct midpontine regions, the dorsal tegmental field (DTF) and ventral tegmental field (VTF), have been found to be implicated in the control of locomotor-

related postural adjustments. The location of these areas and their pathways into the spinal cord are illustrated in Fig. 3 (taken from Mori et al. 1992). The DTF region contains descending fibers originating from the nucleus reticularis pontis oralis (NRPo) which terminate on reticulospinal neurons in the nucleus reticularis gigantocellularis (NRGc). The VTF area corresponds to the rostral part of the nucleus raphe magnus. The pathways from the DTF and VTF areas descend bilaterally in the ventral and ventrolateral spinal quadrants (Mori et al. 1992, Mori, 1989).

Stimulation of the DTF in a standing decerebrate cat, (Mori, 1987) decreases the level of extensor tonus to the point that the cat cannot support its weight. On the other hand stimulation of the VTF causes an increase in extensor tonus and results in an improvement of the maintenance of posture. In both cases, the postural changes outlast the stimulus. Thus, by activating these zones it is possible to "set" and "reset" the level of muscle force output (Mori et al. 1982, Mori, 1987). These brainstem areas can modify the efficacy by which MLR stimulation evokes locomotion. Stimulation of DTF with low intensities during MLR-evoked locomotion causes a decrease in extensor EMG amplitude and duration. Intense stimulation suppresses locomotion completely. Stimulation of the VTF during MLR evoked locomotion promotes gait changes such as transition from stepping of the hindlimbs only to quadrupedal walking. It is suggested (Mori et al. 1992) that the fibers from SLR and MLR regions

project to the VTF area. Intense stimulus in the VTF can evoke locomotion independently, accompanied by pronounced increase in extensor tonus.

Stimulation of the DTF and VTF in freely moving cats, using chronically implanted electrodes (Mori, 1987) caused sequential postural changes. Applying a stimulus to the DTF initially stopped the cat from walking. If the stimulus continued, the cat first sat and then laid down. Stimulation of the VTF evoked the opposite postural changes which led to walking. The stepping, however, resembled more that observed in the decerebrate than in the normal cat (Mori, 1987).

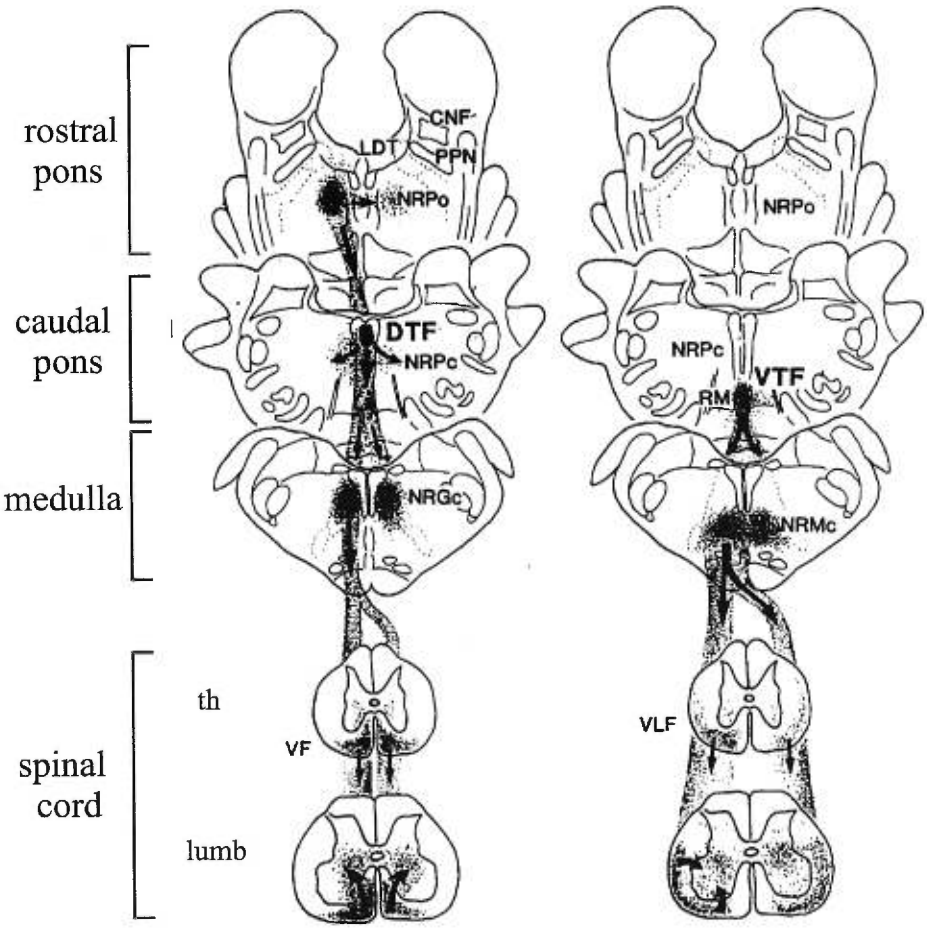
It was found that DTF and VTF effects on muscle tonus are mediated by setting the excitability level of α-MNs (flexors and extensors). Thus, α-MNs were hyperpolarized upon DTF stimulation and depolarized with stimulation of the VTF. Both effects also outlasted the stimulus duration. α-MN hyperpolarization upon DTF stimulation or chemical activation of NRPo, is of tonic postsynaptic origin and also is caused by disfacilitation, a result of Ia excitatory inputs withdrawal (Mori, 1989). The long lasting depolarization after stimulation of the VTF area is suggested to be mediated by 5-HT. 5-HTP was shown to initiate long lasting excitability increase in extensor motor neurons, "plateau- potentials" (Hounsgaard et al. 1988, Conway et al. 1988). This phenomenon could be suppressed by stimulation in an area which corresponds to the DTF

and expressed by stimulation of an area corresponding to the VTF (Crone et al 1983 cf Mori, 1987).

In summary, Mori's studies suggest that the expression of locomotion requires regulation of postural muscle tone and that the systems for initiation of locomotion and for postural control probably affect some shared neuronal structures at the level of the brain stem and spinal cord (Mori, 1987).

Fig. 3: **Reticulospinal pathways implicated in adjustment of posture (taken from Mori, 1989):** (A) pathways exerting postural suppression (B) pathways exerting postural augmentation. NRGc: nucleus reticularis gigantocellularis, NRMc: nucleus reticularis magnocellularis, VLF: ventrolateral funiculus, DLF: dorsolateral funiculus, Th: thoracic, Lumb: lumbar, LDT: laterodorsal tagmental nuclei, RM: nucleus raphe magnus, CNF: nucleus coneiformis.

Descending pathways relating to
A: Postural suppression **B**: Postural augmentation



Mori 1992

Another pathway, implicated in regulating the level of extensor activity, is the lateral vestibulospinal pathway (Orlovsky, 1972a, Armstrong,1986), originating from Deiters' nucleus (lateral vestibular nucleus) and descending in the ipsilateral ventral quadrants of the spinal cord (Kuypers, 1981, Brodal, 1969) as illustrated in Fig. 4

Stimulation of the lateral vestibulospinal nucleus (LVN) in the decerebrate cat (Orlovsky, 1972a) increased extensor activity. The increase was much more pronounced during evoked hindlimb locomotion compared to rest, especially if the stimulus was given at the end of the swing or at the beginning of the stance. Destruction of the LVN markedly decreased ipsilateral extensor activity or even abolished it. In addition, the activity of most LVN neurons (67%) during locomotion was found to be modulated in relation to extensor activity (Orlovsky, 1972b). Immobilization of the limbs abolished or greatly reduced this phasic activity which occurred only if the cerebellum was intact (Orlovsky, 1972b), suggesting that the modulation of the activity of LVN neurons may be of peripheral origin. In the absence of afferent input under fictive locomotion conditions, stimulation of the LVN could reset the locomotor rhythm (Russell and Zajac, 1979); however there is no direct evidence to show that LVN is essential for the initiation of locomotion (Jell et al. 1985, Armstrong, 1986).

In the decerebrate cat, walking quadrupedally, the majority of LVN neurons (Udo et al. 1976, 1982) show frequency modulation. In this

preparation the increase in discharge frequency occurs twice during the step cycle, in E_1 or E_2 phase and in F or E_3 phase of the ipsilateral limb. It is suggested that both peaks of activity are correlated to extensor activity. The first relates to activity before paw placement and the second one provides background activity in case of perturbation which will help to maintain the alternating stepping activity (Udo et al. 1982). This study shows as well, that hindlimb immobilization decreased the rhythmicity in LVN neurons. The same effect could also be achieved by restraining the forelimbs, suggesting that the activity of LVN neurons can be influenced by activity of limbs in both girdles (Udo et al. 1976 Armstrong, 1986).

Mori et al. (1988), have further shown that the phase-locked activity of LVN to extensor activity persists with changes in treadmill speed and in different walking gaits. The largest number of neurons was activated during gallop compared to walking or to trot and there was also a group of cells that were recruited upon transition from one gait to another. These results suggest that LVN neurons are activated when there is a need to increase postural muscle tone in relation to the step cycle phase.

Modulation of EMG activity by the vestibulospinal pathways was also shown during recording of LVN neurons in the freely moving cats (Matsuyama and Drew, 1996). The neurons were found to discharge twice in each step cycle during level walking, while an increase in the depth of modulation was observed during walking on an inclined treadmill. Bilateral destruction of the LVN in chronic cats initially caused severe ataxia and

then their walking on the treadmill was characterized by a marked (albeit temporary) reduction in extensor activity, especially in the hindlimbs (Yu and Eidelberg, 1981). The strong excitatory effects of vestibulospinal pathways on extensors are exerted monosynaptically on αMNs and γMNs. Polysynaptic interactions (excitatory or inhibitory, ipsi- or bilateral) are found with interneurons of segmental reflex pathways, as well on cells of ascending pathways (Grillner and Hongo, 1972).

Regulation and adaptation of the locomotion rhythm:

The evidences implicating the reticulospinal pathways in the initiation of locomotion (see section 3) and in locomotor related postural adjustments (see the pervious section) were presented earlier in the introduction. In addition, there is abundant evidence suggesting that the reticulospinal pathway may be implicated in the regulation of the locomotor rhythm on a step by step basis (Drew et al. 1986).

Reticulospinal neurons were found to be rhythmically active during evoked locomotion (Orlovsky, 1970a). According to single unit recordings in the intact cat (Drew et al. 1986) or during fictive locomotion (Perreault et al. 1993) their discharge is correlated to the periods of activity of flexors or extensors of more than one limb. Such interlimb influences were also demonstrated by microstimulation of these brain stem neurons at rest. The stimulation evoked compound, reciprocally organized

movements in limbs of the same girdle (Drew and Rossignol, 1990a,b). During locomotion of the thalamic cat (Drew and Rossignol, 1984), as well as of the fictive preparation (Perreault et al. 1994) and the intact cat (Drew, 1991b), the evoked responses, excitatory or inhibitory, were organized with respect to the step cycle and caused changes in the amplitude as well as in the duration of the muscle or in the related nerve activity, including resetting of the existent locomotor rhythm. Therefore, a small group of reticulospinal neurons can carry information simultaneously to more than one muscle in more than one limb to reinforce the locomotor rhythm on a step by step basis, or even to reset it. Further, the discharge pattern of reticulospinal neurons during locomotion can be modified by cutaneous afferent information arising in each one of the four limbs, suggesting the possible implication of the reticulospinal system in producing a coordinated responses in reaction to external unexpected perturbations (Drew et al. 1996a).

Two other important structures involved in the adaptation of locomotion to changes in the environment are the motor cortex, the origin of the corticospinal pathway, and the red nucleus, the origin of the rubrospinal pathway. Both pathways, share functional similarities and are thought to reinforce each other's action (Kuypers, 1981, Massion, 1967).

The motor cortex and the corticospinal pathway:

In the cat, the corticospinal axons descend through the medullary pyramids and pass, mainly, into the contralateral dorsolateral funiculus of the spinal cord (Kuypers, 1981, Brodal, 1969, for location see Fig. 4). In addition to this main pathway, anterograde WGA-HRP injections (Satomi et al. 1989) demonstrated the existence of other crossed and uncrossed corticospinal tracts. These additional pathways, composed of a small number of axons, are located bilaterally in the lateral, ventral, and the ipsilateral dorsal funiculus. Histological and electrophysiological studies (Armand et al. 1974, Armand and Aurenty, 1977, Armand et al. 1985) showed that the origin of the corticospinal pathway is mainly in area 4 of the motor cortex. Studies of collateral distribution of single axons in the spinal cord (Shinoda et al. 1986) show that a single corticospinal axon gives off collaterals at different levels of the spinal cord. About one third of the cortical fibers, recorded in the forelimb representation of area 4, send collaterals also to the upper thoracic level, while cortical cells recorded in the hindlimb representation of area 4 distribute their collaterals in the lumbosacral cord, but not to the cervical cord. The distribution of terminals in the spinal cord includes interneurons in the dorsal horn and lamina V-VII. In lamina VII they are distributed bilaterally.

Lesions to the motor cortex or to the medullary pyramids result in only minor deficits during unobstructed walking (Armstrong, 1986). During treadmill locomotion, the observed deficit after such lesions

(Eidelberg and Yu, 1981b) was an increase in the extension of all the joints during the stance phase, a deficit from which the cats recovered within 2 weeks. More recent studies (Jiang and Drew, 1996) in which the corticospinal and the rubrospinal pathways were severed at the thoracic level, show that the cats suffered from sustained locomotor deficits, including paw drag of the hindlimbs during the swing phase of the step-cycle, a deficit which was accompanied by changes in the temporal coupling of the burst onset of the hip and knee flexors. Changes were also observed in the intralimb joint coupling during the transition from stance to swing. These results suggests that the corticospinal and rubrospinal pathways are implicated in maintaining an appropriate intralimb coupling and EMG pattern, as well as in the control of the transition between stance and swing.

The implication of the motor cortex in simple locomotion is also supported by studies of the discharge pattern of single units in motor cortex of cats during treadmill locomotion (Armstrong and Drew, 1984, Drew, 1991a). Eighty percent of the motor cortex neurons, projecting their axon through the medullary pyramids, discharge rhythmically during locomotion. The discharge of the majority of these neurons attains a maximum once in a step cycle, in the swing or the stance phase, as well as at the transition between the stance and swing (Armstrong, 1986, Drew, 1991a). Further, as mentioned before, stimulation of the medullary pyramids during evoked locomotion in the decerebrate cat (Orlovsky,

1972a) or stimulation of the motor cortex of the intact cat (Armstrong and Drew, 1985) could lead to resetting of the locomotor rhythm, suggesting that the corticospinal pathways can influence the central pattern generator (Drew 1991a, Kalaska and Drew, 1993).

It should be emphasized that even cats that showed only transient deficits during ordinary walking after lesions to the motor cortex or to the medullary pyramids, showed severe locomotor deficits when they were tested in more demanding tasks such as walking on an horizontal ladder or crossing obstacles (Armstrong, 1986). Such deficits were also observed after temporary inactivation of the motor cortex with Muscimol, a GABA agonist (Drew et al. 1996b).

These findings suggest a major importance of the motor cortex in the control of skilled locomotion and in adapting the locomotion to changes in the environment (Armstrong, 1986, Drew, 1991a). This suggestion is supported by related changes in the discharge pattern of cortical units of freely moving cats during tasks such as walking on an horizontal ladder, walking over barriers (Armstrong, 1986, Beloozerova and Sirota, 1993) or when crossing over obstacles attached to a treadmill belt (Drew, 1988, 1991a, 1993). In all these tasks, an increase in the discharge frequency was observed compared to simple, control, walking as well as an increase in the depth of modulation (Beloozerova and Sirota, 1993). A more detailed analysis of the discharge pattern of these cortical units (Drew, 1993, Widajewicz et al. 1994) revealed a correlation

between the unit discharge and the changes in EMG amplitude and duration (especially in flexors), which occur while stepping over the obstacle (Drew, 1993, Widajewicz et al. 1994).

It is therefore suggested that the motor cortex controls the timing, the duration and the amplitude of the EMG, to ensure the needed changes in the limb trajectories during gait modifications. It is also implicated in determining the interlimb coupling and postural adjustments required to execute the task (Drew, 1993, Widajewicz et al. 1994, Drew et al. 1996b).

The rubrospinal pathway:

The rubrospinal pathway of the cat originates mostly from neurons in the magnocellular (caudal) portion of the red nucleus (RN). The axons cross in the mesencephalon and descend to the contralateral dorsolateral funiculus (see Fig. 4); the distribution of their terminals closely resembles the terminal distribution of the corticospinal pathway. The rubrospinal neurons are organized somatotopically, so that axons descending to the lumbosacral spinal cord originate in the ventral- and ventro-lateral parts of the magnocellular portion while those innervating the cervical spinal cord originate mainly from the medial and dorsal parts (Kuypers, 1981, Massion, 1967, Brodal, 1969). Rubrospinal axons show more limited collateralization in the spinal cord compared to corticospinal axons (Kuypers, 1981).

In the decerebrate cat (Orlovsky, 1972c), the discharge of rubrospinal hindlimb- related neurons during locomotion is increased

compared to rest. In the majority of these neurons the discharge was modulated in a phase-dependent manner, and was maximal during the swing phase of the contralateral limb. Such phase dependence was also observed in the responses to stimulation of the RN during locomotion, which caused an increase in flexor activity when applied during swing (Orlovsky, 1972a). Decerebellation, or preventing the movement of the contralateral limb, decreased the discharge frequency of the neurons and abolished their modulation. The source of the modulation is suggested to be of central spinal origin, (mostly via the cerebellum) as it can be recorded even in the fictive preparation, in which phasic peripheral inputs are absent (Arshavsky et al. 1988, Orsal et al. 1988, Vinay et al. 1993).

Lesions to the red nucleus in decerebrate cats do not interfere with MLR evoked locomotion suggesting that it is not essential for production of the basic locomotor synergy. However, after such a lesion, instability was observed in the movements of the distal joints (Shik et al 1968, cf armstrong 1986). These findings support earlier observations which showed that bilateral lesions to the RN, in otherwise intact cats, resulted in knuckling of the fore- and the hind-feet (cf Armstrong, 1986, Massion, 1967). These results suggest the involvement of the RN in control of the distal joints (Armstrong, 1986). A correlation between the discharge pattern of RN neurons and paw placement, induced by light touch to the unsupported limb in awake cats was demonstrated by Amassian and Batson (1988). Further, in the intact cat, RN neurons were

found to be involved in gait modifications during locomotion (Lavoie and Drew, 1997). The discharge pattern of these neurons (related to the contralateral forelimb) was not only modulated during locomotion but also increased when stepping over an obstacle. Many of these cells had cutaneous receptive fields which included the forepaw. Microstimulation of the RN during locomotion in the intact cat evoked twitch responses during swing while it inhibited extensor activity during stance. Prolongation of the stimulus enhanced these effects but did not cause prolongation or resetting of the step cycle (Rho et al. 1997). Intracellular recordings in lumbar αMNs, in response to stimulation of RN (decerebrate cat) mainly excited flexor αMNs and at the same time inhibited polysynaptically extensor αMNs (Massion, 1967, Hongo et al. 1969). However, according to Jankowska (1988) the number of direct contacts on αMNs is limited (see also McCurdy et al. 1987) and the main influence of the rubrospinal pathway is through interneurons. These interneurons participate in different spinal reflexes and are also affected by other descending pathways. Rubrospinal terminals were also found on interneurons that mediate presynaptic inhibition of primary afferents.

In summary, several descending pathways in the dorsal and the ventral quadrants of the spinal cord are implicated in adaptation of the locomotor rhythm and of posture during ordinary or obstructed walking. A special "overall" implication is attributed to the reticulospinal pathways.

Of a major importance to our study are Mori's results that the expression of locomotion requires proper postural muscle tone.

5. Implication of different pathways in the control of interlimb coupling:

Adequate quadrupedal walking requires coordination between the fore- and hindlimb movements. According to gait analysis, based on kinematics and or on electrophysiological recordings (Halbertsma et al. 1976, Hildebrand, 1976, English, 1979), it is possible to identify several basic patterns of interlimb coupling. During alternate locomotion (walk, trot) the movement of the limbs of the same girdle occurs strictly out of phase. During walking, the homolateral limbs (fore-and hindlimbs of the same side) will contact the walking surface with a phase interval of about 0.25 of the step cycle, while during pacing the homolateral limbs are placed almost simultaneously, i.e. with zero phase interval. During trot, the diagonal fore- and hindlimb are tightly synchronized. The number of supporting limbs during one step cycle depends on the walking speed. At moderate speeds the cat will frequently alternate between 3 and 2 supporting limbs (Hildebrand, 1976, Grillner, 1975, Gorska et al. 1993c). In gallop, and in jumping, there is a strong tendency of both hindlimbs to act in phase. In this situation, the coupling of the movement between the homolateral limbs is asymmetric and they are acting out of phase. Since these basic patterns of interlimb coupling can be generated in the acute

cat, spinalized at the cervical level (Miller et al. 1975, Miller and Van der Meche, 1976) it was suggested that the basic interlimb patterns can be generated at the spinal level. It was further proposed that the interactions between the two girdles are mediated by the long propriospinal pathways which connect the two spinal enlargement. The implication of the propriospinal pathways in the control of interlimb coupling was studied by Kato et al. (1984). In these experiments, adult cats were subjected to a series of hemisections, damaging to a different extent the propriospinal pathways. Since the deficits in interlimb coupling after complete or partial damage to the propriospinal pathways had similar consequences, Kato et al. (1984) had suggested that propriospinal pathways play little role in the coordination between the fore- and the hindlimbs.

English (1980) also tested the possible role of the dorsal columns in interlimb coupling. Bilateral lesions of the dorsal columns at the thoracic level, but not at the cervical level, increased the occurrence of homolateral coupling (pacing) which is not commonly observed in intact cats walking overground (English, 1979). Because the lesion only shifted the coupling pattern towards pacing and did not disrupt the pattern completely, it was suggested that the contribution of dorsal columns to interlimb coupling is only minor. The implication of the ascending dorsospinocerebellar pathway was also excluded (English, 1985). Lesions to the ascending ventrospinocerebellar pathway caused, however, a step by step variability in the coupling pattern and was

suggested to play an important role in the control of interlimb coupling. Its action is probably mediated via the cerebellum (English, 1989).

Kato et al. (1984) suggested the involvement of supraspinal descending pathways in the control of interlimb coupling. Rossignol et al. (1993) proposed that the medullary reticulospinal pathway whose, neurons are suggested to be involved in regulation of muscle activity in different limbs and its stimulation during locomotion exerts phase dependent responses in all four limbs (see section 4).

According to the results of the studies reviewed above it is difficult to attribute the control of interlimb coupling to any specific pathway. It was proposed that interlimb coupling is a result of the combined contribution from different sources, peripheral, segmental, propriospinal and supraspinal, on to the stepping generators (Grillner, 1975, Miller and Van der Meche, 1976, English and Lennard, 1982, Rossignol et al. 1993, Rossignol, 1996) which may explain observations of permanent decoupling between the walking rhythms of the forelimbs and the hindlimbs after extensive spinal lesions to the dorsolateral funiculi (Jiang and Drew, 1996, Gorska et al. 1993b) or to the ventral and ventrolateral quadrants (Afelt, 1974, Eidelberg et al. 1981a, Gorska et al. 1993a, Bem et al. 1995).

6. The involvement of descending Noradrenergic and Serotoninergic pathways in the control of locomotion:

Retrograde histofluorescence and HRP studies show that noradrenergic and serotoninergic pathways in the spinal cord originate in the brain stem (Dahlstrom and Fuxe, 1964, 1965). The main source of descending noradrenergic axons to the spinal cord is the nucleus locus coeruleus which projects primarily through the ventro-lateral funiculus. In addition, nucleus subcoeruleus, Kolliker-Fuse and cell bodies in A5, send fibers through both the ventro-lateral and the dorsolateral funiculi. The noradrenergic fibers innervate the lumbar spinal cord bilaterally, although the ipsilateral contribution dominates (Stevens et al. 1985, Kuypers and Maisky, 1977, Kuypers, 1981, Marshall, 1983). The serotoninergic axons originating in the raphe nuclei, such as raphe pallidus and obscurus, descend mainly in the ventral and ventro-lateral funiculi while axons from raphe magnus descends primarily through the DLF (Martin et al. 1978, Kuypers, 1981).

The noradrenergic (NE) precursor L-DOPA, was shown to evoke considerable effects on spinal reflexes (Jankowska et al. 1967a,b). Stimulation of flexor reflex afferets (FRA), in the presence of L-DOPA, released long latency and long duration alternating discharge in flexor and extensor nerves, in MNs and in interneurons, suggesting that L-DOPA affected neurons implicated in the generation of spinal locomotor rhytmicity, probably by releasing noradrenaline from terminals of

descending noradrenergic pathways (Anden et al. 1966). It was further shown that i.v. injection of L-DOPA or of the α2 noradrenergic agonist, Clonidine, in acute spinal cats improved weight support and allowed locomotion of the hindlimbs on a treadmill (Forssberg and Grillner, 1973). The direct involvement of NE was demonstrated by administration of NE itself, through an intrathecal cannula in the acute spinal cat which, as for L-DOPA, evoked long latency reflexes following stimulation of high threshold afferents. Intrathecal application of NE to the spinal cord was also capable of inducing and maintaining locomotion (Kiehn et al. 1992). In early chronic spinal cats, i.p. or i.t. injection of Clonidine initiated locomotion and later, when the spinal pattern was already established (late spinal cat), it modulated the step duration and increased flexor and extensor burst duration, although there was no change in their mean amplitude (Barbeau and Rossignol, 1991, Barbeau et al. 1993). Preliminary information shows that Methoxamine, an α1-noradrenergic agonist, is not as effective as Clonidine in the initiation of locomotion early after complete spinalization and in the late spinal cat (which recovered hindlimb locomotion), Methoxamine only slightly modulates the step cycle duration (Chau et al. 1998b).

In contrast to NE and Clonidine, the 5-HT precursor (5-HTP) did not evoke locomotor rhythm either in the low spinal decerebrate cat, (Grillner and Shik, 1973), or in the complete chronic spinal cat during the first week after spinalization, when Clonidine or L-DOPA caused a

dramatic change in kinematics and EMG pattern (Barbeau and Rossignol, 1990, Barbeau and Rossignol, 1991, Barbeau et al. 1993). However, in both preparations there was a marked increase in the tonic activity in all muscles (Grillner and Shik, 1973) or at least in the flexor activity (St) with treadmill activation (Barbeau and Rossignol, 1991). A marked increase in the amplitude of extensor and flexor muscles, as well as in their duration, was observed in the chronic late spinal cat after i.p. injection of the 5-HT precursor, 5-HTP and of Quipazine and 5-MeODMT, which are 5-HT agonists (Barbeau and Rossignol, 1990, Barbeau and Rossignol, 1991). 5-HT was also implicated in the control of posture and muscle force during locomotion in the decerebrate cat and probably as well in the intact cat (Mori, 1987, see also the section about "the involvement of descending pathways in postural control during locomotion").

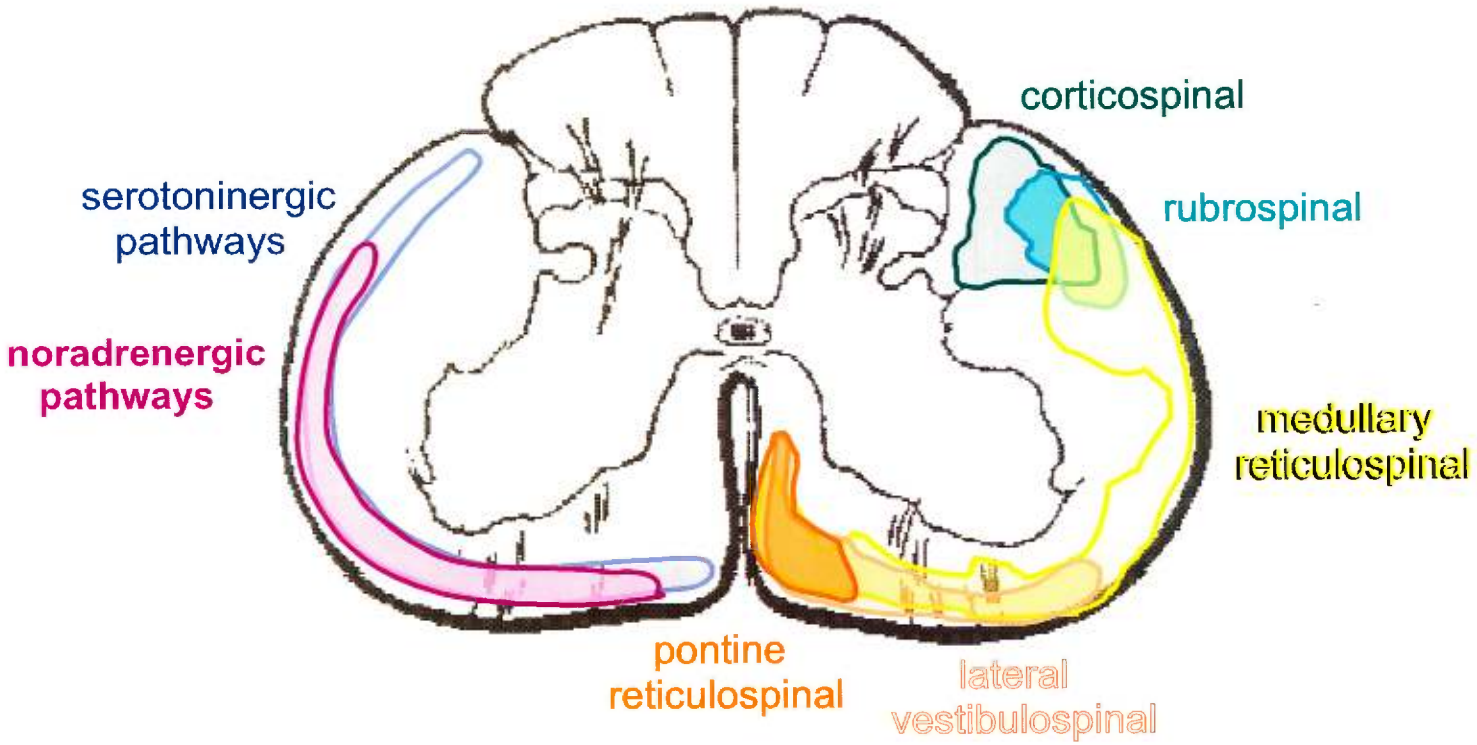
The implication of NE and 5-HT in locomotion was also tested by depletion of NE and 5-HT in the brain stem and the spinal cord of otherwise intact cats. NE levels were reduced by intraspinal and intraventricular injection of 6-hydroxydopamine and of the NE synthesis inhibitor, alpha-methyltyrosine. 5-HT was depleted by intraventricular injections of 5,6-dihydroxytryptamine and i.p. injection of p-chlorophenylalanine (Steeves et al. 1980). The depletion of NE and 5-HT was not found to affect the MLR evoked locomotion in the cat after decerebration, suggesting that these neurotransmitters are not essential for MLR evoked locomotion. It is important to emphasize that prior to the

acute experiment the cats subjected to the depletion showed severe locomotor deficits such as ataxia and attempts to walk resulted in their loosing balance and falling. Stepping movements were, however, observed when the cats were layed on their sides.

Taken together, the studies reviewed here implicate the noradrenergic and serotoninergic pathways in the modulation of the locomotor pattern in decerebrate cats and in the late spinal cat. Noradrenergic drugs can also initiate locomotion in the acute and the early spinal cat. It is suggested (Grillner, 1981, Mori, 1987) that NE and 5-HT set the level of background excitability in αMNs, permitting the expression of variety of locomotor movements. L-DOPA, Clonidine and 5-HTP were found to induce long lasting increase in the excitability of αMNs ("plateau potentials") in acute spinal cats (Hounsgaard et al. 1988, Conway et al. 1988); so does Methoxamine in the decerebrate cat preparation (Lee and Heckman, 1996, Lee and Heckman, 1997). The effect of 5-HTP on the bistable properties of the MNs have many similarities to the effect of L-DOPA. However, they differ in their targets and strength of action. 5-HTP strongly affected extensor MNs, while L-DOPA effect both extensors and flexors but to a lesser extent (Conway et al. 1988).

Fig 4. **The location of major descending pathways in the cat spinal cord (C-8):** The location of the cortico- rubro-, vestibulo- and the pontine and medullary reticolospinal pathways are illustrated on the right side of the diagram only, (according to Petras, 1967). For the corticospinal pathway only the main crossed component is illustrated, while the ipsilateral components were not included. The rubrospinal pathway is crossed, while the vestibulospinal pathways descend through the ipsilateral spinal cord. The reticulospinal pathways descend in the spinal cord bilaterally, however only the main ipsilateral component is illustrated. The noraderenrgic and serotoninergic pathways are bilateral with a main ipsilateral component. This part of the figure was reconstructed by scanning several illustration of the individual pathways and superimposing them on a standard spinal section. The location of the noradrenergic and serotoninergic pathways was added only on the left side of the diagram, according to diagrams of Kuypers and Maisky (1977), and of Holstege and Kuypers (1987).

Location of major spinal descending tracts in the cat (C-8)



adapted from: Holstege, J.C. and Kuypers, H. G. J. M., 1987,
Kuypers, H.G.J.M. and Maisky, V. A., 1977 and Petras, J. M., 1967.

7. The effects of subtotal spinal lesions on motor function and control of locomotion:

The partial spinal lesion approach (excluding hemisections) has been used in only a few studies to try and understand the contribution of descending pathways to motor function and, more specifically, to the control of locomotion (Eidelberg, 1981, Vilensky et al. 1992, Rossignol, 1996).

Schäfer (1910) systematically studied the effects of specific funicular lesions in the monkey by applying electrolytic lesions to the bulbar pyramids and to the ventral and ventrolateral quadrants at the midthoracic level. Pyramidal lesions were followed by a quick recovery, while ventrolateral lesions caused paralysis of the hindlimbs in 2 of 3 monkeys. In the third monkey in which the lesion included "almost exactly" the ventral half of the spinal cord, some weak voluntary movements could be observed. Unfortunately these interesting observations were limited to 2-3 weeks post surgery. Motor recovery was observed in the monkey after bilateral lesions to any spinal funiculi (Mattler and Liss 1959, cf Vilensky et al. 1992). The correlation between subtotal lesions and the return of motor function in the monkey was studied as well by Lawrence and Kuypers (1968a,b). The applied lesions were based on their anatomical findings, which divided the descending brain stem pathways into medial and lateral systems according to the

terminal distribution of their axons in the spinal gray matter. The medial pathways included the pontine and medullary reticulospinal pathways as well as the vestibulospinal pathway, which terminate in the ventromedial parts of the internuncial zone. The lateral pathway included the rubrospinal axons which terminate in the dorsal and lateral internuncial zone. Bilateral lesions to the lateral system caused deficits in the control of distal extremities, similar to lesions to the pyramidal tract. In contrast, lesions to the medial pathways produced severe postural deficits and deficits in the proximal movement of the limbs. It took monkeys 10-40 days to start righting their body and even later their gait was unsteady. It must be emphasized that these results were obtained in monkeys that had first recovered from bilateral pyramidotomy. Monkeys that were subjected only to medial system lesions showed postural deficits that were less pronounced. For example, body righting was observed immediately after the surgery. Monkeys that were first trained to walk on a treadmill and then were subjected to partial spinal lesions, were reported to recover locomotion only if intact tissue was found in the ventrolateral quadrants (Eidelberg et al. 1981b).

In adult cats, Windle et al (1958) reported the return of some locomotor function after sparing 1-10% of axons restricted to the ventral quadrants at a low thoracic level. However no details are given. Afelt, (1974) observed the overground behavior of adult cats subjected to partial spinal lesions at thoracic level. According to her observations,

cats, in which at least one ventral quadrant was spared, recovered locomotion, albeit with postural and interlimb coupling deficits. Another cat, in which only the DLF on one side was spared, behaved as a complete spinal cat.

Treadmill locomotion of cats subjected to subtotal lesions was tested by Eidelberg at al. (1981a). In this study, HRP was injected to try and correlate the extent of the damage and the return of locomotor capacities. Eidelberg's results showed correlation between recovery of locomotion and sparing of axons in the vestibulospinal and in the medullary and pontine reticulospinal pathways. Similarly, in the acute decerebrate cat (Steeves and Jordan, 1980, Noga et al. 1991) it was demonstrated that initiation of locomotion by stimulation of the mesencephalic locomotor region (MLR) depends on the integrity of ventrolateral tracts containing the reticulospinal pathways. According to these findings (Afelt, 1974, Windle et al. 1958, Eidelberg et al, 1981a,b) it was concluded by Eidelberg (1981) that pathways in the ventral half of the spinal cord are "necessary and sufficient" to sustain locomotion. These conclusions are, however, mainly based on spinal lesions, designed to spare only small patches of tissue in the ventral and the ventrolateral quadrants and the ability to walk in the absence of these pathways was not actually directly addressed (Vilensky et al. 1992).

More recent studies in the cat (Gorska et al. 1990, Gorska et al. 1993a,b, in the monkey (Vilensky et al. 1992) and in human (Nathan, 1994) imply,

however, that long term recovery of voluntary locomotion is possible despite extensive lesions of the ventral and lateral quadrants, even if there are major locomotor deficits such as disrupted interlimb coupling (Gorska et al. 1993a). However, in these studies, the observations were limited to the long term period post spinal lesion and the analysis focused on the deficits in the coupling between the fore- and the hindlimbs. In addition, evaluation of the extent of the spinal lesion was limited to inspection of cross sections from the site of lesions, a method which may limit the interpretation of the extent of the lesion.

Therefore the **first objective** of this thesis was to investigate the contribution of descending pathways in the ventral and ventrolateral quadrants of the spinal cord to the control of locomotion, by extensively damaging the reticulo- and vestibulospinal tracts (see Fig. 4). The results of the lesions can provide knowledge on the role of these pathways in the control of locomotion in the adult freely moving cat. Furthermore, such lesions also permit us evaluate whether other descending pathways in the dorsolateral quadrants (see Fig. 4), such as the cortico- and rubrospinal tracts, can be sufficient to sustain locomotion in the absence of these important ventral and ventrolateral pathways.

From the data reviewed in the introduction about the possible implication of pathways in the ventral and the ventrolateral funiculi such as the reticulospinal pathways in initiation of locomotion, we expected

that, if these pathways are indeed "necessary and essential" for locomotion as proposed before, then our cats would not be able to initiate locomotion with the hindlimbs and would behave as complete spinal cats even in the presence of descending pathways in the DLF. On the other hand, if initiation of locomotion was still possible, due to the remaining pathways, but if the ventral and ventrolateral pathways have a major importance in the control of the ongoing locomotor rhythm and in mediating the posture-related adjustments, then we would predict that these cats would walk but would express severe locomotor deficits such as irregularity and poor interlimb coupling. These deficits should be accompanied by severe postural deficits as a result of damaging the vestibulospinal pathways and the reticulospinal pathways implicated in posture. The deficits expressed in the early days after the lesion are suggested to reflect primarily the lack of the normal contribution of the lesioned pathways to locomotion, while the long term deficits would probably reflect more the compensation that can be achieved by the remaining pathways and their limits.

To answer these questions, adult cats were implanted with chronic electromyographic (EMG) electrodes and their ability to walk on a treadmill was compared before and after lesions of the ventral and ventrolateral spinal cord, at low thoracic level (T11-T13), which left the dorsolateral quadrants intact. In addition the time course of locomotor recovery was followed in each one of the cats. This was aimed at

identifying and comparing the early and long term deficits. The description of the recovery period and the long lasting locomotor deficits are detailed together with proposed adaptive mechanisms, in ***the first paper of the thesis; "Recovery of treadmill locomotion after bilateral chronic ventral and ventrolateral spinal lesion in the adult cat: deficits and adaptive mechanisms"*** (revised version, J. Neurophysiol)

We considered it important to try and improve the residual locomotor capacities as a useful animal model for patients with partial spinal lesions. Therefore, the partial spinal cat was found to be a useful animal model in which we attempted, as the **second objective** of this thesis, to try and improve the residual locomotor capacities using pharmacological tools. Two groups of drugs were chosen, the Noradrenergic (NE) and Serotoninergic (5-HT) drugs. These drugs, as detailed in a previous section, are known to be involved in the initiation or the modulation of locomotion in complete spinal cats and have also been used in clinical assays with patients (Barbeau and Rossignol, 1994, Tator et al. 1993). Accordingly, it was expected that noradrenergic agonists such as Clonidine would improve the locomotor rhythm and serotoninergic agonists such as quipazine would improve weight support.

The drugs were injected through a chronically implanted intrathecal cannula and the treadmill locomotion was compared before and after the drug application in the early stage and long term post spinal lesion. The

results of these experiments are described in the second paper of the thesis; ***"The effects of Noradrenergic and Serotoninergic drugs on treadmill locomotion in adult cats subjected to bilateral chronic ventral and ventrolateral spinal lesion"*** (submitted version, J.Neurophysiol)

PAPER #1

Recovery of treadmill locomotion after bilateral chronic ventral and ventrolateral spinal lesion in the adult cat: deficits and adaptive mechanisms.

by:

Edna Brustein

Serge Rossignol

Abstract:

The recovery of treadmill locomotion of 8 adult cats, subjected to chronic ventral and ventrolateral spinal lesions at low thoracic levels (T11 or T13), preserving at least one dorsolateral funiculus and the dorsal columns, was documented daily using electromyographic (EMG) and kinematic methods. The data show that all cats eventually recovered quadrupedal voluntary locomotion despite the extensive damage to important pathways (such as the reticulospinal and the vestibulospinal) as verified by injection of WGA-HRP caudal to the site of lesion. Initially, (in the early period after the spinal lesion), all the cats suffered from pronounced locomotor and postural deficits and they could not support their hindquarters or walk with their hindlimbs. Gradually, during the recovery period, they regained quadrupedal walking although their locomotion was wobbly and inconsistent and they suffered from poor lateral stability. EMG and kinematic data analyses showed a tendency for an increase in the variability of the step cycle duration but no major changes in the step cycle structure or in the intralimb coupling of the joints. However, the homolateral fore- and hindlimb coupling was highly perturbed in cats with the largest lesions. Although the general alternating pattern of extensor and flexors was maintained, there were various changes in the duration and amplitude of the EMG bursts as well as a lack of amplitude modulation during walking uphill or downhill on the

treadmill. In cats with larger lesions, the forelimbs also seem to take a greater propulsive role than usual as revealed by a consistent increase of the activity of the triceps. In cats with smaller lesions, these deficits were transient, but for the most extensively lesioned cats they were pronounced and lasted long term post lesion even after reaching a more or less stable locomotor behavior (plateau-period). It is concluded that recovery of quadrupedal locomotion is possible even after a massive lesion to ventral and ventrolateral quadrants, severing the vestibulospinal pathway and causing severe, although incomplete, damage to the reticulospinal tract. The quick recovery in the less lesioned cats can be attributed to remaining pathways normally implicated in the locomotor function. However in the most extensively lesioned cats, the long period of recovery and the pronounced deficits during the plateau-period may indicate that the compensation that may be attributed to remaining reticulospinal pathways is not sufficient and that other pathways in the dorsolateral funiculi ,such as the corticospinal, can sustain and adapt, up to a certain extent, the voluntary quadrupedal walking.

Introduction:

It is of major clinical importance to identify and understand the adaptive locomotor capacities of the nervous system after spinal cord injuries if one is to design and implement rehabilitation strategies for locomotion (Barbeau and Rossignol 1994). After a complete spinal transection at the low thoracic level, adult cats can walk with the hindlimbs on a moving treadmill, with weight support of the hindquarters and proper plantar foot placement. This indicates that the spinal cord, together with peripheral afferents, but in isolation from descending supraspinal inputs, can generate hindlimb locomotion (Grillner 1981, Rossignol 1996, Rossignol et al 1996, Barbeau and Rossignol 1987, Belanger et al. 1996). With the addition of an intensive training program and/or injection of noradrenergic agonists, these locomotor capacities can be expressed earlier and better (Barbeau et al. 1993, Barbeau and Rossignol 1987, Barbeau and Rossignol 1991, Chau et al. 1998). However, the hindlimbs capacities remain limited. it is not initiated voluntarily and it cannot obviously be expressed in coordination with the forelimbs. In addition there are often deficits such as paw drag during swing (Belanger et al. 1996).

Since in humans, the percentage of partial versus complete spinal lesion is increasing in the population (Tator et al 1993) there is a need for a better understanding of such conditions. The work of colleagues (Jiang and Drew 1996), has already shown that lesions limited to the

dorsolateral pathways result in prolonged steps, paw drag and an inability to step properly over obstacles, but otherwise, there are only transient deficits in posture and interlimb coupling, suggesting that ventral and ventrolateral pathways are capable of controlling quadrupedal voluntary locomotion.

The present complementary work aims at describing the consequences of lesions to the ventral and ventrolateral parts of the spinal cord which carry pathways such as the vestibulo-and the reticulospinal, while leaving the dorsolateral ones intact. Former studies in chronic adult cats (Windle et al. 1958, Afelt 1974, Eidelberg et al. 1981, Eidelberg 1981) have suggested that pathways in the ventral and ventrolateral quadrants are essential for the recovery of voluntary quadrupedal locomotion (for more detailed review see Rossignol et al 1996). These conclusions are, however, mainly based on spinal lesions, sparing only small patches of tissue in the ventral and the ventrolateral quadrants which were found to be sufficient to sustain locomotion. Similarly, in the acute decerebrate cat (Steeves and Jordan 1980, Noga et al. 1991) it was demonstrated that initiation of locomotion by stimulation of the mesencephalic locomotor region (MLR) depends on the integrity of ventrolateral tracts containing the reticulospinal pathways. However, there are now mounting evidence in the cat (Gorska et al. 1990, Gorska et al. 1993a, b), in the monkey (Vilensky et al. 1992) as well as in human (Nathan, 1994) that long term recovery of voluntary locomotion is possible

despite extensive lesions of the ventral and lateral quadrants, even if there are major locomotor deficits such as disrupted interlimb coupling (Gorska et al 1993a). In these studies, however, the period of gradual recovery of locomotion was only briefly mentioned and none have analyzed and compared the deficits observed during the recovery period to those observed long term post lesion, after reaching a stable locomotor behavior (plateau-period), or followed their evolution with time, nor have appropriate histological analysis been done to evaluate the extent of the spinal lesion. The locomotor deficits expressed in early days after the lesion probably reflect primarily the lack of the normal contribution of the lesioned pathways to locomotion, whereas the long term deficits reflect the limit of the compensation that can be achieved by remaining pathways to the recovery of locomotion.

The evaluation of such a recovery process requires chronic recording methods. Therefore, cats were implanted with chronic EMG electrodes which allows one to identify changes in EMG activity under constant recording conditions in the same cat before and after a lesion restricted to the ventral and ventrolateral pathways and document the locomotion characteristics both during the recovery period and long after achieving a stable locomotor behavior. The electromyographic (EMG) activity was recorded daily and was synchronized to video images to allow detailed kinematic analysis. It also seemed important to evaluate more precisely the extent of the spinal lesion. Since remaining viable

axons of descending pathways cannot be identified by inspection of histological sections only, WGA-HRP was injected caudal to the site of lesion at the end of the recovery period and labelled cells were counted in the brain stem nuclei and in the motor cortex, the origin of descending corticospinal pathway.

It will be shown that, after small lesions, the locomotor deficits are minimal, but that, after large spinal lesions, cats can still initiate voluntarily quadrupedal locomotion. The step cycle structure of the individual limb is minimally affected, but there are severe locomotor deficits such as perturbed fore- and hindlimb coupling. There are also major changes in the adaptation of the hindlimb locomotion to more demanding situation such as walking on slopes. However, the cats can still perform such task through a greater use of the forelimbs .

Methods

General experimental protocol:

Experiments were carried out on 8 adult cats (2.5-4.8 kg) which were trained to walk on a treadmill at different speeds (0.2-0.7m/s) and with different inclines (10 degrees uphill and downhill). When the cats could consistently maintain regular locomotion for periods of about 20 minutes, they were chronically implanted with electrodes to record electro-myographic activity from fore- and hindlimb muscles. After recovery from surgery, 7-12 control experiments were made over a 1-6 weeks period to determine the control (intact) EMG and kinematic values. Thereafter, the cats were submitted to the ventral-ventrolateral spinal lesion at thoracic level (T11 or T13) and their locomotor recovery was followed and documented daily. No special training program was applied and the recordings on the treadmill started when the cat could walk voluntarily with all 4 limbs at a minimal treadmill speed of 0.1m/s. When the cats did not show any further locomotor improvement (48-343 days post lesion, depending on the extent of the lesion), WGA-HRP was injected caudal to the site of the spinal lesion (L2). Then, 3 days later, the cats were perfused and the spinal cord, i.e, the site of HRP injection and the site of lesion were taken for histological processing, as well as, the brain stem and the motor cortices. All the surgical procedures and

experimental protocols were reviewed and approved by the University Ethics Committee.

Surgical procedures:

EMG implantation:

The bipolar EMG electrodes were chronically implanted under sterile conditions. First, the cats were premedicated with acepromazine maleate (Atravet, 0.1mg/kg sc), Atropine (0.05mg/kg sc) and Penicillin G (40000 IU/kg, im) and then anaesthetized with pentobarbital sodium (Somnotol 35mg/kg i.v). Two 15 pin connectors (TRW Electronic Components Group, Elk Grove Village, IL) were fixed to the skull and 14 pairs of Teflon- coated stainless steel wires (AS633, Cooner Wire, Chatsworth, CA), soldered to the connector pins, were directed subcutaneously to different muscles acting around the fore- and hindlimb joints. The portion of the wire which was inserted into the muscle belly was exposed for 1-2mm and then fixed in situ by a silk thread. The EMG electrodes were implanted in the following muscles, listed according to their main function: in the hindlimbs, the hip flexors-Iliopsoas (Ip) and Sartorius (Srt), the knee flexor- Semitendinosus (St) and the ankle flexor-Tibialis Anterior (TA), the knee extensor-Vastus Lateralis (VL) and the ankle extensors-Gastrocnemius Medialis (GM) and Lateralis (GL); in the forelimbs: the

elbow flexor-Cleidobrachialis (ClB) and the elbow extensor (lateral head)-Triceps Brachii (TriL).

All muscles were implanted both on the left (L) and on the right (R) side of the animal. Usually the left side of the animal faced the video camera, except for cat EB5 which faced the camera from the right side. After the implantation, the cats were monitored closely. They were placed in an incubator to maintain body temperature and an analgesic, buprenorphine hydrochloride (Temgesic, 0.005-0.01mg/kg sc), was given every 6-8 hours for 24-28 hours. In addition, lactate ringer dextrose 5% was administered i.v. Twenty-four to forty-eight hours later, the cat was returned to its individual cage and was given amoxicilline (Amoxil 22mg/kg) orally twice a day for 10 days. In addition to implantation of EMG electrodes, two bipolar nerve cuffs were placed around the Superficial Peroneal nerve on each side and secured using a thin layer of impression material (light body\low viscosity Hydrophilic Vinyl Polysiloxane, Reprosil HF). These were used to stimulate the nerve at rest and during locomotion. In cats EB7 and EB8 a Teflon cannula (Teflon tube- thinwall, size 24 Gauge) was inserted into the intrathecal spinal space at C1 and lowered so that the tip was located at L4-5. The rostral end of the cannula was fixed into dental acrylic, next to the EMG connectors, and served to administer drugs intrathecally (these effects will be reported in the companion paper). The presence of the cannula is quite conspicuous in EB8 (see Fig. 1) and it may have compressed part of

the adjacent tissue, although there were no signs of necrosis in the tissue surrounding the imprint of the cannula.

Ventral and ventrolateral spinal lesions:

After the 7-12 control experiments (following the EMG implantation) the ventral and ventrolateral spinal lesion was performed. The procedure was done under general anesthesia as described above. A low thoracic vertebra was exposed, T11 for cats EB1-6 and T13 for cats EB7-8 and two small openings were drilled through the vertebral pedicles to approach the spinal cord laterally on both sides. After making a small incision in the dura, a microknife and/or a small forceps were inserted through the openings to lesion the ventral half of the spinal cord while leaving the dorsal portion intact and protected by the overlying laminae. Afterwards, the incision was closed in anatomical layers. The post operative care was the same as after the EMG implantation. It must be noted that all cats, in contrast to the complete spinal cats whose bladder had to be expressed manually (Belanger et al. 1996), controlled their micturition and did not need manual voiding.

Recordings and data analysis:

EMG activity and the video images used for kinematics analyses were recorded simultaneously and were synchronized using a SMPTE time-

code (time-code generator Skotel TCG-80N and time-code reader TCR-80N).

EMG: The amplified and filtered (100Hz-3kHz) signals were recorded on an analog VHS tape recorder (Vetter 4000A PCM Recording Adaptor with cut-off frequency of 1.25KHz) and later played back and printed out using an electrostatic polygraph (Gould ES-2000). Walking sections, representing the locomotor capacities of the cat at that day, were chosen for detailed analysis, were then digitized at 1KHz using an AT/486 computer. Custom made programs were used to determine each burst onset and offset and then to calculate their duration and amplitude. The amplitude was calculated as the integral of the rectified burst of EMG activity divided by the burst duration. Then, it was averaged and presented as percent of the control values.

Kinematics:All the walking sections selected for EMG analysis were also used for limb movement analysis as well as for defining the interlimb coupling. Six reflective markers (3M reflective tape) were placed over the skin of the following bony landmarks: the rostral tip of the iliac crest, the femoral head, the knee joint, the lateral malleolus, the metatarsophalangeal (MTP) joint and the tip of the 3rd toe.

The cats were videotaped walking on the treadmill using a Panasonic digital 5100 shutter camera (resolution of 16.7 ms/field) and a videotape recorder (Panasonic AG 7300). The camera was adjusted to get a clear image (shutter between 1/500 and 1/1000). The position (X,Y

coordinates) of the reflective markers was digitized off line using a 2D PEAK Performance System. The coordinates of the position of each one of the markers were used to calculate the joint angular displacement and to reconstruct stick diagrams of single or several consecutive step cycles. In addition to digitizing the position of the relective markers, the SMPTE time-codes engraved on the video film were also used to determine the exact time of paw lift and paw contact of each one of the 4 limbs. The time of each of these kinematics events was then used to calculate the duration of the step cycle, the swing and the stance and to construct foot fall diagrams for each one of the consecutive steps in the walking sequence. The foot fall diagrams were grouped according to interlimb coupling types and then normalized and averaged. From the averaged and normalized foot fall diagram the percentage of time the cat supported its weight with 2,3, or 4 limbs was calculated and presented in a table form (see Table 3)

All the recordings were reviewed and analyzed to determine the evolution of the recovery. However, we illustrate mainly representative data taken during the recovery period and from the plateau-period when the cats were already walking at their best. These data were compared to those obtained in the intact state.

Histological analysis:

Histological evaluation of the spinal site of lesion

The damage of the spinal lesion was evaluated by inspecting, under light microscope, the physical damage and the extent of cellular and fibrous necrosis in consecutive cross sections of spinal cord stained with Kluver-Barrera (cats EB6-EB8, 8μm thick, 1 in 50 saved) or with Cresyl violet methods (EB1-EB5, 40μm thick 1 in 5 saved). Whereas with the first method myelinated axons can more easily be identified, in the latter it is very well possible under the microscope to identify regions of intact axons that appear as regular, densely-packed profiles in areas of the white matter. Following these observations the total extent of the damage was reconstructed and its severity in each region was indicated using different graphic patterns as illustrated in Fig.1.

WGA-HRP labelling and cell distribution analysis:

The method of WGA-HRP injection and the related histological procedure have been described earlier (Jiang and Drew 1996). Briefly, after a recording period of 48-343 days, when the cat showed no more locomotor improvement, WGA-HRP (2%) was pressure -injected, a few segments caudal to the site of the spinal lesion, usually at L2. The penetrations were done in two parallel frontal planes which permitted the whole cross section of the spinal cord, to be covered with the WGA-HRP solution (total of 20-

30μl). Three days later, the cat was perfused with 2L of phosphate-buffered saline 0.9%, 2 L fixative (glutaraldehyde 1.25%, paraformaldehyde 1%, sucrose 1% in phosphate-buffer at pH 7.4) and 1L buffered sucrose solution 4%. The whole brain was removed and the brain stem and motor cortices were cut sagitally (40μm) and every 3rd section (1/3) was taken for precipitating the HRP reaction products using the Tetramethylbenzidine (TMB) method (Mesulam 1978). The reaction was carried as well on cross sections (1/5 saved) taken from the site of injection to verify that it was completely covered with HRP reaction products. Then, the HRP labelled cells in the red nucleus and the lateral vestibular nucleus were counted in all the sections. Moreover, in the motor cortex and the reticular formation the location of the labelled cells was digitized as well (adapted from Matsuyama and Drew 1997). Briefly, the contours of histological sections were traced using a pantograph and then digitized using Autocad. The digitized sections were centered and oriented according to anatomical coordinates (Berman 1968) using a custom-made software. The digitization of the cells' position, directly onto the computer image, extended in the brain stem from the midline to 2.5mm, on each side and was done on alternating sections (every 240 μm). Then the cells were divided into two major groups according to stereotaxic coordinates (adapted from Matsuyama & Drew 1997). The first group (A0 to P6), corresponds to the pontine reticular formation (PRF) which includes the nucleus reticularis pontis oralis and nucleus reticularis

pontis caudalis while the second group (P6 to P11), corresponds to the medullary reticular formation (MRF) which encompassed the nucleus reticularis gigantocellularis and the nucleus reticularis magnocellularis. For the motor cortex, digitization was done in all the sections (every 120 μm) up to 10mm from the midline (Jiang and Drew 1996). The values were compared to data from 3 intact cats (one-tail student t-test), after verification of the normality of the distribution of the intact values using Kolomogorov-Smirnov test for goodness of fit with Lilliefors correction (Zar, J.H. 1996, Stephens, M.A. 1986). Missing values, resulting from technical problems in processing the perfused tissue, were identified in Table 1 as "not available" (NA)

In this paper only counts are presented, whereas the detailed cells distribution in the brain stem nuclei and in the motor cortex will be presented in a forthcoming paper.

Results:

Evaluation of the extent of spinal lesions:

To facilitate the description of the results, we have defined two groups of cats; EB1-EB4 and EB5-EB8. This grouping was based both on the extent of the spinal lesion (evaluated histologically) and on the cat's locomotor capacities during the recovery period and the plateau-period when they were already walking at their best .

The reconstructed lesion sites for the 8 cats used in this study (Fig. 1) are based on inspection of consecutive spinal cord cross sections stained with Kluver-Barrera (EB6-EB8) or Cresyl violet methods (EB1-EB5) for physical damage at the site of the lesion, as well for the extent of cellular and fibrous necrosis. The related counts of WGA-HRP labelled neurons are summarized in Table 1, and the total counts are illustrated next to each lesion site in Fig. 1. The sections are presented in an order (EB1-EB8) which reflects the gradual augmentation of the extent of the damage applied and the area it encompassed in the spinal cord. The first group (EB1-EB4) had incomplete lesions, restricted to the ventral or lateral spinal cord, while the second group (EB5-EB8) showed bilateral damage to both ventral and ventrolateral funiculi. It is important to note that in some of the cats (EB1, EB6, EB7, EB8) a cavity (syrinx) developed so that the damage was eventually larger than initially intended.

----------Insert Table 1 near hear----------

The counts of HRP labelled cells in the brain stem nuclei and the motor cortex completed the histological evaluation of the spinal lesion. The number of labelled cells in the pontine nuclei was taken as an indicator of the extent of the lesion to the pontine reticulospinal pathways whose axons descend mainly ipsilaterally and are located around the ventral median fissure. The number of HRP labelled cells in the medullary reticular formation nuclei was taken as an indicator of the extent of lesion to the medullary reticulospinal pathways. This pathway descends bilaterally in the spinal cord, however the ipsilateral contribution dominates (Brodal 1969, Kuypers 1981, Kuypers and Maisky 1977, Kuypers and Maisky 1975). Most of the MRF axons descend in the ventrolateral funiculi, however they are found as well in the dorsolateral funiculi (Petras 1967, Kuypers and Maisky 1977).

The cell counts in the PRF and MRF confirm the histologically based division of the cats into two groups. For example, cat EB2 representing the first group (EB1-EB4), was subjected to a moderate lesion, damaging the ventral funiculi, the ventrolateral funiculus on the left and only parts of the right ventrolateral funiculus. The number of HRP labelled cells found in the right PRF were normal (97% of the control values), while on the left a moderate reduction is observed (84%), indicating a preserved pontine reticulospinal pathway on the right and a mostly preserved one, on the left. This fits with the histological observations of the lesion, showing that the tissue around the upper

ventromedian fissure is largely intact. The HRP labelled cell counts in the MRF are the same on the right and on the left, 64%, despite of the asymmetric lesion to the ventrolateral funiculi, suggesting also the existence of spared axons in the mass of necrotic tissue observed on the left ventrolateral funiculus. The second group includes the most lesioned cats (EB6-EB8) in which a significant decrease ($p<0.01$) in MRF cells is observed on both sides, together with a significant reduction ($p<0.05$) in PRF cell counts to levels below 30% of control values, at least on one side. In cat EB6, for example, intact tissue was observed in the dorsal columns, in the left dorsolateral funiculus (DLF), as well as some patches in the right DLF. However, the ventral half of the spinal cord is highly deformed and occupied by fibrotic tissue and a large syrinx. Despite the massive deformation and fibrotic tissue, the number of labelled cells in the right PRF was at the level of control value. On the left, however, the lesion was much more severe as only 14% HRP labelled cells were found in the PRF. In cat EB7, with a very extensive spinal lesion sparing the dorsal columns and parts of the left DLF, the decrease in cell counts is striking, (up to total of 18% and 23% of the intact values on the left and on the right brain stem, respectively) suggesting severe damage to most of the reticulospinal axons. It should be emphasized that the design of our lesion aiming at damaging ventral and ventrolateral quadrants and keeping the dorsolateral quadrants intact, will preserve a certain population of reticulospinal axons descending in the dorsolateral funiculi

of the spinal cord (Petras 1967). The HRP analyses is not available for cat EB5 which died unexpectedly. However, based on the physical damage at the site of the spinal lesion and its locomotor capacities during the recovery and the plateau periods, it was included in the second group.

In addition to damaging the reticulospinal tracts, a more pronounced damage was observed, in all cats, to the lateral vestibulospinal pathways. The axons of this pathway pass ipsilaterally in the ventromedial aspect of the ventral funiculus and originate in the lateral vestibular nucleus (LVN) (Kuypers 1981, Brodal 1969). The number of HRP labelled cells in the LVN decreased remarkably to 0-33% of the intact values. In cat EB2 we have found only 13% and 10% of labelled cells in the right and left LVN, respectively. In cat EB7, however, 0.5% labeled cells were identified in the right LVN while, only 0.1% were counted in the left LVN. The very low number of labelled cells in LVN corresponds to a real disruption of this pathway and was not related to a methodological artifact, since the spinal cord cross section at the site of injection was completely filled with HRP reaction products and there is a considerable number of heavily labelled cells in the PRF, whose axons descend in adjacent spinal cord areas.

As already mentioned, the aim of this study was to lesion the ventral and ventrolateral pathways while preserving important ascending and descending pathways in the DLF such as the rubrospinal and the

corticospinal. As both rubro- and corticospinal tracts are mainly crossed (Kuypers 1981, Brodal 1969), the HRP labelling in the red nucleus and motor cortex were taken as markers of the integrity of the contralateral DLF. In all cats, both the rubrospinal and the corticospinal pathways were somewhat affected by the lesion, as indicated in Table 1. For example, 1486 HRP labelled cells were counted in the right red nucleus of cat EB2, while on the left, 1420 labelled cells were found. These values correspond to 63% and 60% of the intact values. In cat EB6, which had a very extensive lesion (including extensive damage to the right DLF) 2013 labelled cells were found in the left cortex corresponding to 66% of the intact values. However, on the right cortex 4155 cells were counted, i.e, to 136% of the control values. Such a phenomenon, of pronounced increase in the number of labelled cells was also observed in the right motor cortex of cat EB7 which showed an even more outstanding increase to 192% of control values. The increase in the total number of labelled cells in the cortex of EB6 and EB7 was also accompanied by changes in the distribution of the cells. In the normal cat (Jiang and Drew 1996, Brustein et al. 1997) the maximal number of cells is found around the lip of the caudal bank of the cruciate sulcus and more laterally as well on the rostral bank. In cat EB7, however, in addition to the normal cell distribution, labelling appeared as well more laterally around the fundus of the cruciate sulcus and, in cat EB6, labelling appeared more medially on the rostral bank.

Recovery of locomotion (general remarks):

Surprisingly all the cats eventually recovered voluntary quadrupedal locomotion overground and on the treadmill. However, early after the lesion, the cats could not walk or even support their hindquarters and walked around using their forelimbs only. For the group of cats, EB1-EB4, with relatively moderate lesions, this period was limited to 1-3 days. However, for the group of cats EB5-EB8, with most extensive lesions, it extended over more than 3 weeks. After, during the recovery period the cats gradually regained some weight support of the hindquarters and the ability to stand and walk voluntarily with no external help. Yet, they all suffered from poor lateral stability and often fell on one side resulting in a wobbly, inconsistent walking for a few steps at a time. It is interesting to note, however, that the cats did not have problems negotiating obstacles placed in their way overground, or on a treadmill.

We have found that a good general indicator of the locomotor recovery with time after the lesion was the maximal treadmill speed the cat could attain and maintain at least for a few step cycles, as illustrated in Fig. 2. The group of cats EB1-EB4, with less extensive lesions, recovered quickly, as noted from the steep slope of the curves and could achieve treadmill locomotion speeds between 0.5-0.7m/s within 10 days of the lesion. On the other hand, cat EB7, as an extreme example of the most lesioned group (EB5-EB8), had a lesion damaging all areas except

the dorsal columns and parts of the left dorsolateral funiculus (see Fig. 1 and Table 1), started walking quadrupedally (with no external support) only after 34 days and followed a slow recovery as indicated by the more gradual increase of the curve. Furthermore, it could never walk at speeds higher than 0.4m/s and even later, its performance regressed with time, maybe due to the progression of a syrinx. However, it should be remembered that, even then, treadmill walking in cats with the largest lesions (group EB5-EB8) was irregular and wobbly, sometimes with a wide base of support of the hindlimbs, a diagonal orientation of the trunk and occasional stumbling. These deficits were mostly transient for cats EB1-EB4; however lateral swaying of the hindquarters was still evident for a few weeks post lesion even when they were already walking at their best.

The irregularity of the hindlimb stepping is illustrated in Fig. 3 using stick figures of the right hindlimb (cat EB5) constructed for several consecutive steps at 0.4m/s, before the lesion, during the recovery and the plateau periods. In the recovery period the steps are highly variable in duration and the cat tends to stumble and struggle to maintain the imposed speed. The cat often lifts the feet higher than normal, as seen in the increased paw trajectories. During the plateau-period, however, stability is much better as indicated by more regular steps and smoother trajectories.

The step cycle duration and its variability (average ± SD), measured from successive onsets of St (EB2, EB3, EB6) or Srt (cat EB7), pre- and post lesion, are illustrated in Fig. 4. For cats with a moderate lesion, represented by EB2 and EB3, the average cycle duration is not changed post lesion but there is a tendency of increase in its variability as reflected by larger SD values relative to the control, see especially cat EB2 tested with variance ratio test. The more severely lesioned cats such as cat EB6, show a sustained decrease in the cycle duration long term post lesion (see as well Fig. 7A and B for cat EB5) and the most severely lesioned cat, EB7, showed as well a day to day variability in its locomotor performance reflected by very large SD. However, because of the small number of steps performed (6-13) in each trial, the post lesion values were not always found to be statistically different from the control ones. A comparison between the average cycle duration of the left and the right hindlimb showed no statistical difference even in cats with an asymmetrical lesion such as cat EB7 (see Fig. 4, cat EB7). Despite the variability of the cycle duration, regression analysis showed that there are no major changes in the relationship between the duration of the step cycle subcomponents (swing and stance) and the overall cycle duration, obtained however in a more limited range of walking speeds, as illustrated in Fig. 5 for the most lesioned cats, EB5-EB7. Some changes were observed in the slopes and their correlation values (see Table 2 for slope values and coefficients) but they affected mainly the swing (see

especially cat EB6) while those of the stance stay generally high as before the lesion. However, a comparison between the slopes of the intact situation and the recovery period or the plateau-period were not found to be statistically different in the studied cats, neither for the stance nor for the swing.

----------Insert Table 2 near hear----------

Intralimb coupling:

The calculated joint angular displacement of the first step cycles illustrated in Fig. 3 for cat EB5 (representing the most lesioned group), from an intact and plateau-period (88 days post lesion) trials, is presented in Fig. 6A and Fig. 6B, respectively, with the averaged angle-angle plots (Fig. 6C, D). The related raw EMGs are presented in Fig. 7. Notice that after the lesion, the cat's step cycle is shorter (see as well Fig. 4) resulting in a greater number of steps for the same period of time. Some changes were observed in the magnitude of the angular excursion traces. For example, before the lesion, the hip excursion ranged, on the average, between 99±1 to 130±1.5 degrees, while 88 day post lesion, it was reduced (84± 4.1 to 105±3.7). Such changes were observed as well for the knee, ankle and MTP joints and results in a general down displacement of the related angle-angle plots. Despite the excursion

changes described above, the joint coupling is largely preserved. The only coupling change, which is specific to this cat, was observed for the knee relative to the ankle. A somewhat earlier extension of the knee relative to the ankle at the end of the swing results in an eight shape angle-angle plot.

Another important observation illustrated in Fig. 6, is the lack of paw drag at the onset of swing. Similarly, paw placement at the beginning of the stance was always on the plantar surface even during the recovery period (see Fig. 3).

Inspection of the related EMGs (Fig. 7) completes and supports our observations from the angular displacement analysis. The phase relations between the flexors RSt and RSrt are illustrated by heavy lines, aligned on the onset of RSt. The dashed lines in A and B are aligned on the onset of the bursts of the extensors RVL and RGM, to demonstrate their phase relationships. The burst relations of the extensors are not changed after the lesion. No changes were observed, as well, in the relations between the lift and the RSt burst onset which always precedes the lift. However, the relations between the onset of the flexors RSt and RSrt is modified. In the control (intact) there is a constant delay in the onset of RSrt burst relative to RSt, whereas, after the lesion in cats EB5-EB7, they are activated almost at the same time. Yet this change in the bursting phase is not reflected as any detectable change in the coupling relations between the hip and knee as already described above for their angle-angle plot. No

such changes were observed for cats with smaller lesions (EB1,EB2 and EB4), which maintained the normal St-Srt burst onset relations after the lesion. This measure was not available for cats EB3 and EB8. The noticeable increase in the EMG amplitudes will be discussed separately (see *EMG activity*).

Interlimb coupling:

It was demonstrated that the step cycle duration on the left and on the right hindlimbs is the same and varies together in most extensively lesioned cats (see Fig. 4). Not only was the cycle duration the same but, in addition, the lesion did not affect the alternating coupling pattern between the hindlimbs. This is demonstrated by the raw EMGs of the left and the right VL and the related foot falls illustrated for cat EB5 in Fig. 8 A, E and I. In this example, the averaged phase difference between the right and left VL burst onset in the intact situation is maintained at 0.52, whereas for the recovery period and the plateau period it is 0.49 and 0.48, respectively. One should notice however that the individual phase onset values vary considerably after the lesion. This is easily seen from the consecutive phase values of the RVL burst onset (empty symbols) graphed in Fig. 8F.

One of the most pronounced deficits observed post lesion was a step by step inconsistent homolateral fore- and hindlimb coupling. The more extensive the lesion, the more perturbed were the coupling relations.

Two examples are given to illustrate the most pronounced coupling changes. The first one is taken from cat EB5 (Fig. 8) which is also representative of the coupling deficits seen in cat EB6. Both of these cats improved their coupling deficits over time. The second example, taken from cat EB7 (Fig. 9), shows a perturbed interlimb coupling which did not improve even long term after reaching the plateau-period. The coupling relations between the homolateral fore- and hindlimb are illustrated by comparing the phase of burst onset (relative to St) of the hindlimb extensor (VL) and the forelimb extensor (TriL) in the intact situation, during the recovery and the plateau-periods, for up to 30 consecutive step cycles at 0.4m/s. The intact cat maintains a phase difference of 0.2 on average (Fig. 8B). These constant phase relations are also indicated by the dashed lines connecting the related foot fall (Fig. 8A). During the recovery period, cat EB5 (Fig. 8F) shows perturbed step to step interlimb coupling which is manifested in phase shifts ranging between 0 to 0.64.

To further describe the gradual and cumulative phase difference during the stepping sequence, a "cumulative difference" value was used (Fig. 8D,H,L). First, the phase of VL burst onset was calculated relative to TriL. The value obtained for the first step cycle was used as the reference, which was then subtracted from each of the values of the following step cycle. Thus, a value of 0 is expected when the phase relations of VL versus TriL in one cycle is the same as for the reference. A value of 1 means that the hindlimbs deviated from the forelimbs by a whole step

cycle. As predicted, the "cumulative phase difference" for the intact cat, with constant limb coupling, shows small fluctuations around 0 (Fig. 8D). However, post lesion, this value varies much more, but always remains in the range of 1 phase i.e., within one cycle (Fig. 8H). Thus, for each step of the hindlimb, there is one step of the forelimb and the 1:1 step cycle relationship is preserved. During the plateau-period, when the cat is already walking at his best , it adopts a preferential homolateral in-phase coupling in which the fore- and the hindlimb of the same side are placed at the same time on the walking surface, as illustrated by the dashed lines in the foot falls of Fig. 8I. This is reflected as well in the "cumulative phase difference" values which stabilizes around 0 (Fig. 8L).

Cat EB7 (see Fig. 9) shows a more perturbed fore-hindlimb coupling and it does not recuperate even long term post lesion (>300 days). There is a gradual phase deviation between fore- and hindlimb, which is illustrated by the "cumulative difference" (Fig. 9D) and the related EMGs and foot falls in Fig. 9E. For about every 5 hindlimb step cycles, the forelimb drifted from the hindlimbs for a whole step cycle. Thus, after 10 step cycles performed by the hindlimbs, the forelimbs have made 2 more steps as illustrated in Fig. 9E by the step cycle numbers on the foot falls. There is up to 300ms difference between fore- and hindlimb step cycle duration (see Fig. 9C), i.e., the hindlimbs and the forelimbs are walking at a different mean frequency (1Hz and 1.4Hz respectively). In contrast to cats EB5-EB7, cats EB1-EB4 as well as cat EB8 did not demonstrate such

severe step to step fluctuations in fore- and hindlimb coupling. However, they all adopted some degree of homolateral in-phase coupling at least short term post lesion (see Table 3)

To further understand the gait modifications observed after the spinal lesion foot fall diagrams were constructed for each one of the cats for several consecutive step cycles, using kinematic events. After normalization and averaging, these foot fall diagrams were compared pre- and post lesion for the number of limbs supporting the cat at each moment as well as to identify the predominant limb coupling pattern. Such a foot fall diagram is shown in Fig. 10 (cat EB6) and the results are summarized for all cats in Table 3. In the control state, cat EB6 is supported mainly by 2 and 3 limbs (96% of the time) and by 4 limbs, in only 4% of the step cycle time. The associated coupling pattern between the homolateral fore- and the hindlimb, as already described using EMGs (see Fig. 7, 8), is on the average 0.2 in phase difference. After the lesion, there is a major increase in the time the cat is supported by 4 limbs: 17% (recovery period, day 77) and 33% (plateau-period, day 141). At first, this is associated with a diagonal-like coupling type, in which the forelimb is placed almost at the same time as the hindlimb of the opposite side and later, during the plateau-period, with homolateral in-phase coupling. Cat EB5 shows the same tendency of increased time supported by 4 limbs at a time. However, during the recovery period, it spent just 50% of the time in a diagonal coupling while in the rest of the steps in the sequence, the

coupling pattern was as in the control. The coupling pattern of cat EB7 was more variable. This cat spent one third of the steps in the sequence in diagonal couplings, another third in homolateral in-phase couplings and the rest as in the control. However, during the plateau-period, only diagonal and in-phase type of coupling were observed. Cats EB1-EB3 as well as cat EB8 did not use the diagonal coupling pattern during the recovery period but adopted homolateral in-phase coupling, which, again, was associated with an increase in the time spent on 4 limbs. However, during the plateau-period only cat EB1 kept walking in homolateral in-phase pattern while cats EB2,3 and 8 returned to control coupling and supporting limb pattern. This analysis was not applicable for cat EB4.

----------Insert Table 3 near hear----------

EMG activity:

Comparison of the raw EMG activity, before and after the lesion (see Figs. 7,8, 9) shows that, generally the alternating activity between extensors and flexors of the same limb (see RSt and RGM of cat EB5 in Fig. 7 and LSt and LVL of cat EB7 in Fig. 9E) is maintained as well as the alternating activity between muscles of contralateral limbs such as RVL and LVL or RTriL and LTriL (see Fig. 8A, E, I). However, various changes were observed in the amplitude and burst duration of the hindlimb muscles after the lesion. These differed from one cat to another as well as with time post lesion. Two examples are given in Table 4 to demonstrate the variability of the pattern. The first example is taken from cat EB2 which represents the moderately lesioned cat group (EB1-EB4) in which the changes were mostly restricted to the recovery period. The second example is taken from cat EB6 and mostly demonstrates the changes after a more extensive lesion (EB5-EB8) which are sustained and observed also during the plateau-period .

During the recovery period there was a significant decrease in the normalized amplitude of EB2 flexors to 38-71% of control values. A reduction was observed as well in the amplitude of the extensor LGM (48%), while VL amplitude on both sides increased. However, during the plateau-period, only the increase in VLs amplitude and the decrease in LGM amplitude are maintained. The bursts duration, though, are less

effected and these changes are noted only during the recovery period (see, LGM, RVL). In contrast, in cat EB6, all the recorded extensors and flexors of the hindlimbs show a significant increase in amplitude 77 days post lesion (122-433%) as well as 141 days post lesion, 110-141% (except LSt) and their burst duration is shorter. The variable changes observed for the different cats in the burst amplitude and duration are associated with an increased C.V. or SD values which reflects the wobbly walking and possibly the step to step corrections to avoid falling.

----------Insert Table 4 near hear----------

Contrary of the variable changes in amplitude and duration of the bursts of activity seen in the hindlimb muscles, marked and consistent changes were observed, across all the cats, in the normalized amplitude of the forelimb extensor TriL. Table 5 presents these values for all the cats expressed as percent of the control. In general, a significant increase ($p<0.01$) is observed during the recovery period (ranging between 124% and up to 337%) and is maintained long term post lesion, during the plateau-period (117%-330%). One exception is LTriL of cat EB7 which is significantly decreased both during the recovery and the plateau-periods. Other than that, EB8's LTriL is reduced but only long term post lesion and the TriL of EB5 shows no change during the recovery period (although on the long term it is significantly increased).

----------Insert Table 5 near hear----------

An important long term deficit was observed in the amplitude modulation of the hindlimb muscles activity when walking over a tilted treadmill (10 degrees). Cats in the intact state had no difficulty adapting their walking to slopes (up or down) of the treadmill, even at speeds of up to 0.7m/s, and their stepping was as regular and sustained as during level walking. During uphill or downhill walking, the activity in the fore-and the hindlimbs muscles was modulated up and down, respectively, as illustrated in Fig. 11 for cat EB5 (see intact experiments). The normalized amplitude of RVL, RSrt as well as of RCIB, during uphill walking increases by about 5-10%, compared to their amplitude measured during level walking, while downhill, a decrease of the same magnitude is observed . For this cat, however, the uphill modulation of RTriL amplitude is more pronounced, up to 20% from its activity at level. Notice that for RCIB only the EMG amplitudes recorded at the uphill and level situation are presented because of a major change in its activity pattern during downhill walking.

After the lesion, all the cats had major difficulties walking on a tilted treadmill even at very low speeds. All the deficits, such as poor lateral stability, inconsistent stepping, occasional stumbling, etc., described for their walking at level were more pronounced walking on a tilted treadmill

and were observed even when their level walking was much improved. After the lesion, a major deviation from the normal modulation pattern was found, both in the fore- and the hindlimb muscles. As seen in Fig.11, early on in the recovery period, RVL and especially RSrt amplitude at level is higher relative to the uphill one, a result of a wobbly and irregular walking during which the cats are producing large EMG bursts. Later, after day 40, there is no modulation in RVL amplitude, neither during uphill nor during downhill walking, while for RSrt, no modulation is observed for uphill walking but there is a pronounced reduction (50%) during downhill walking. At the same time, the forelimb muscles, in addition to a general increase of their amplitude, express a more pronounced amplitude modulation. RTriL as well as RCIB, double their amplitude during uphill walking, while during downhill walking, RTriL amplitude decreases to control values. The changes in the modulation of the hindlimb and forelimb muscle amplitude seems to depend on the extent of the spinal lesion, as cats with smaller lesions (EB1-EB4) recover the ability to modulate their hindlimb muscle activity when walking on an inclined treadmill and with it, the modulation of TriL activity is set back to control levels as illustrated in Fig. 12 for cat EB3.

Discussion:

Recovery of locomotion as a function of the extent of the spinal lesion:

Recovery of locomotion in adult chronic cats was reported to depend on the integrity of the ventral and ventrolateral spinal quadrants (Afelt 1974, Windle et al 1958, Eidelberg 1981). However, our experiments as well as those of others (Gorska et al 1993a, b, Bem at al 1995) show that cats with extensive lesions severing the ventral and ventrolateral quadrants, which eliminate the vestibulo- and severely damage the reticulospinal pathways (see cats EB6, EB7), but generally preserve pathways in the dorsolateral funiculus such as the corticospinal pathway, can recover quadrupedal voluntary locomotion. Our approach thus allowed us to establish that voluntary quadrupedal locomotion is still possible after severe damage to the ventral and ventrolateral pathways provided important ascending and descending tracts in the dorsolateral quadrants are present. This approach and conclusion are in contrast to that of others (Windle et al. 1958, Afelt 1974, Eidelberg et al. 1981) who concluded that even minimal patches of intact tissue in the ventral quadrants in absence of all the other pathways, was essential for hindlimb locomotion. Some of the lesions described in their studies have some similarities to ours. For example, Eidelberg et al. 1981, describe two cats in which only the dorsal columns were left intact and the HRP labelling

was confined mainly to the nucleus gracilis; both these cats behaved as complete spinal cats. Afelt (1974), describes a cat in which a patch of tissue remained in the medial DLF. This cat also behaved as a complete spinal cat. However, the extent of the lesion was evaluated only under light microscope and was not assessed by the HRP method. Thus, it is possible that the number of functional axons in the patch was much smaller than could be evaluated, which could explain the spinal behavior of the cat.

Gorska et al. 1993a and Bem et al. 1995 reported recovery of locomotion in cats with intact tissue only in the dorsal columns. This is in contrast to the above mentioned study of Eidelberg. We believe, in light of our HRP experiments, as well as on Eidelberg`s comments (Eidelberg et al 1981), that examining the site of lesion with light microscope only may not be sufficient to estimate the surviving axons, especially in a highly necrotic and deformed tissue as we have found for cat EB6 on the right PRF (see Fig. 1 and Table 1). Therefore, it is probable that more axons have survived elsewhere in the preparation of Gorska et al. 1993a and of Bem et al. 1995, which could explain their recovery.

In our cats there was a good correlation between the extent of damage to vestibulospinal, pontine reticulospinal and to the medullary reticulospinal pathways as evaluated by WGA-HRP labelling (see Table 2), the time for recovery of quadrupedal locomotion (Fig. 2) and the severity of the locomotor deficits. Such correlation justifies grouping the

cats into two major groups, moderately lesioned (EB1-EB4) and extensively lesioned (EB5-EB8) despite the variability in the extent of spinal cord lesions in each of the cats. Severely lesioned cats (EB5-EB8) such as cat EB7 with the most extensive lesion including the majority of the lateral and ventral funiculi eliminating the vestibulospinal and severely damaging the reticulospinal pathways, walked quadrupedally after 34 days. However, its walking was limited to low treadmill speed, and was highly variable with severe and permanent interlimb deficits. Cats in which more reticulospinal cells have been spared (EB1-EB4) recovered more quickly and their locomotor deficits were transient, even though there was a major loss of vestibulospinal cells.

In summary, although others have shown that small amount of intact tissue in the ventral and ventrolateral quadrants may be sufficient to maintain quadrupedal locomotion, we show that even after very extensive lesions of those pathways quadrupedal locomotion is possible, albeit with deficits which correlated with the extent of the lesion, as documented by HRP labelling of cells with descending spinal axons.

Recovery of weight support and lateral stability:

In line with the observations of others (Gorska et al. 1993a, Bem et al. 1995) we report a correlation between time of recovery of quadrupedal voluntary locomotion and the extent of the spinal lesion. Cats with

extensive lesions started walking after 10 days and up to a month post lesion (see Fig.2). Before that, as we have reported, the cats dragged their hindquarters and suffered from poor weight support and lateral stability. However, Gorska et al. 1993a and Bem et al. 1995 observed poor lateral stability mainly during the period of recovery. The only cats which showed these long term deficits (5-6 months), were the ones described to have only the most dorsal parts of the DLF and the dorsal columns intact. Our cats, however, even the ones classified as less extensively lesioned (EB1-EB4) exhibited swaying of the hindquarters also long term post lesion when they were walking at their best. This differences can not be attributed to a difference overground versus treadmill locomotion, as our cats showed the same deficits daily overground, as documented in preliminary observations of ground reaction forces (Brustein et al. 1995). It is interesting to note that the progressive recovery of weight support and walking was also observed after lesions sparing only the ventral quadrants (Eidelberg et al.1981, Afelt 1974) and after lesions restricted to the DLF (Jiang and Drew 1996). However, these were transient, and independent walking over the treadmill at speeds of 0.35m/s was reported in the latter paper, as soon as 3 days post lesion.

The observations on the correlation between recovery of weight support and walking is in line with Mori's concept about the importance of postural control to the full expression of locomotion. The reduction of weight support probably resulted from the damage to reticulospinal

pathways which descend in the ventral and ventrolateral funiculi and were suggested to regulate muscle tone (Mori 1989, Mori et al. 1992). In addition, the decrease in weight support may also result from destruction of the vestibulospinal tract which normally provides strong excitatory drive to extensors (Orlovsky 1972b). The quick recovery of weight support (2-3 days) in cats EB1-EB4, despite the elimination of most of the vestibulospinal pathway, could be due to the spared reticulospinal axons which may be sufficient to maintain the control of muscle tone after the lesion. However, in more extensively lesioned cats, such as EB7, in which a much smaller number of reticulospinal axons have survived, the time needed for recovery (up to 3 weeks), may suggest that this low number of neurons (about 20%) is not sufficient to maintain normal function (Sabel 1997) even after allowing for a long term reorganization.

One explanation for the recovery of weight support in these cats could be an increased dependence on segmental reflexes to set the muscle tone as suggested for the recovery of weight support in complete spinal cats (Grillner 1972, Pratt at al 1994, Guertin et al. 1995) However, this by itself may not be enough. Prentice and Drew (1996) suggest the existence of two alternative pathways for postural control during locomotion. One is active during unobstructed walking and the other functions during gait modifications such as stepping over an obstacle. It is interesting to note that our cats showed no problems in executing a visuomotor task such as stepping over obstacles (unpublished

observations) indicating that these skills were not affected. Implication of the motor cortex during execution of skilled locomotor tasks and gait modifications was shown by many (Armstrong 1986, Beloozerova and Sirota 1993, Drew 1991a , Widajewicz at al. 1994). It is possible that when the regulation of posture (during level walking) through the reticular formation is insufficient, alternative pathways such as the one active during skilled gait modifications may take over and together with enhancement of segmental reflexes can reestablish some of the postural functions during locomotion, although this is not sufficient for the recovery of lateral stability (Brustein et al. 1995).

Initiation of locomotion:

The involvement of pathways descending in the ventral and ventrolateral quadrants in the initiation of locomotion by MLR and PLR stimulation were studied in acute decerebrate cats which were subjected to different spinal cord lesions at C2-C3 or the brain stem (Steeves and Jordan 1980, Noga et al. 1991). It was found that lesions to the ventrolateral funiculi abolish MLR initiation of locomotion while any lesion in the dorsal half of the spinal cord had no effect. These findings suggested that MLR initiation of locomotion is conveyed through ventrolateral pathways (for reviews of brain stem centers involved in initiation of locomotor see Armstrong 1986, Jordan 1991, Rossignol 1996). The inability of MLR stimulation to initiate locomotion after ventrolateral

lesions observed in the acute experiments could actually correspond to the situation seen here in the early days post lesion in which no hindlimb locomotion was observed even up to several weeks after the largest lesions, as illustrated in Fig. 2. However, the chronic lesioned cats recover the ability to initiate voluntary locomotion. In the less lesioned cats the quick recovery, as discussed before (see "recovery of weight support"), the spared reticulospinal axons may be sufficient to regain this function, however for the most lesioned cats which started walking after more than 3 weeks post spinal lesion, the participation of alternative pathways in the DLF cannot be excluded. After an extended dorsal hemisection, it was found that stimulation of the pontine locomotor region (PLR) could still evoke locomotion although a high strength of stimulation was needed, suggesting that normally the PLR exerts its effects at least in part through the dorsolateral pathways (Noga at al. 1991). Such an indirect polysynaptic dorsolateral pathway was proposed by Shik to be involved in initiation of locomotion (Shik 1983, Kazennikov et al. 1985). The locomotion evoked by stimulation along this pathway can not be differentiated from the one evoked through other locomotor regions and it is thought to result from the convergence of inputs from the cortex and the reticular formation (Beresovskii 1990).

Step cycle structure and intralimb coupling:

The voluntary and quadrupedal walking was, however, inconsistent as described by the variability of the hindlimbs step cycle duration (Fig. 4), as well from the increased C.V and SD values of EMGs amplitude and duration summarized in Table 4. This variability may reflect both the poor lateral stability of the cats as well as the step to step adaptations to avoid falling and maintain stepping.

Despite the sustained decrease in the duration of the step cycle for the most lesioned cats (125-250ms) and its variability, the lesions did not seem to affect the step cycle structure (Fig. 5), i.e. the swing and the stance varies with the step cycle duration as before the lesion. This is in line with others (Gorska et al. 1993a) results for 3 of their cats. However in one cat with bilateral lesions to the DLF, and in cats after lesions to the lateral funiculi (Gorska et al. 1993b) the step cycle duration of the hindlimbs increased and the stance varied less with the walking speed, much like the swing in intact cats. This discrepancy may result from a difference in the extent and location of the lesions or from a difference between overground and treadmill locomotion. However, this does not resolve the above differences, as even the complete spinal cat maintains normal relations between the step cycle duration and the swing or the stance (Barbeau and Rossignol 1987, Belanger et al 1996).

In addition to the preserved step cycle structure, even the most lesioned cats in our study generally maintained intralimb coupling as

illustrated in Fig. 3, 6 and 7 using kinematic and EMG data. Even, in cat EB5 which showed somewhat earlier extension of the knee during the stance relative to the ankle, the change may be interpreted as a compensating one to maintain the step length. Another change noted in cats EB5-EB7 was a decrease in the delay between the burst onset of Srt relative to St. However the St maintained its relation to the paw lift. A change in the St-Srt bursting onset relations was observed in the complete spinal cat (Belanger at al. 1996) as well as in cats subjected to lesions restricted to the DLF (Jiang and Drew 1996). However in these cats the St activity is delayed relative to the lift and in the complete spinal cat it is accompanied by an earlier onset of TA and a paw drag. Although TA recordings were not available in our cats, the observed St-Srt relations was not accompanied by a paw drag at any time. In this case a tighter Srt-St bursting relations may be beneficial for the cat, assuring by a synchronous strong flexion of both the hip and knee at the beginning of the swing, that the hindlimb is cleared of the walking surface to avoid stumbling.

Interlimb coupling:

One of the most important deficits observed post lesion was a step by step inconsistent coupling between the homolateral fore- and hindlimb, calculated from the onset of EMG activity in the related extensors (Fig. 8, see as well English 1979 for the intact cat). The larger the lesion, the more

severe were the deviations of the fore- hindlimb coupling from the stable intact pattern. The deviations could be classified into 3 major classes in line with the observations of others (Bem et al. 1995), during overground locomotion. In the first class, the phase differences between the fore- and the hindlimbs vary in the limit of one step cycle and the step cycle duration in the homolateral fore- and hindlimbs is maintained the same all along the stepping sequence. In the second class, the fore- and the hindlimb of the same side are walking at a slightly different frequency as illustrated by an up to 300ms difference in their step cycle duration. This uncoupling results in a continuous phase drift between the two girdles. The third type is an in-phase coupling of the homolateral fore-and hindlimb, a walking pattern that is rarely seen for a trained cat walking at a moderate or high speed (Hildebrand 1976, English 1979). The first type of perturbation was observed in cats EB5-EB6 while the second was observed for the most lesioned cat, EB7. All cats, adopt the third, in-phase, walking pattern except the most lesioned cat EB7. In cats EB1-EB4 and EB8, this occurs during the recovery period, while for cats EB5-EB6 it appears during the plateau-period. Adopting homolateral in-phase walking allows the possibility to increase the period during which all limbs are on the ground to support the weight of the animal, which may contribute to the improvement in walking stability after the lesion, and is therefore a compensatory mechanism (Hildebrand 1976, Blaszczyk and Loeb 1993).

Bem et al. 1995 observed such coupling changes during overground locomotion in cats with different ventral and lateral spinal lesions, using kinematics events for the analyses. However, the interpretation of the results is not the same as ours and the homolateral in-phase coupling is related by these authors to be a source of destabilizing oscillations in the walking rhythm. However, if this was the case, our cats could not maintain up to a 30 consecutive steps, most of them in in-phase coupling, during the plateau-period. The values of "cumulative phase difference" (see Fig. 8 D,H,I) supports our interpretation. One can see that when the cat uses homolateral in-phase coupling (Fig. 8I) these values stabilize around 0 and only 3 step cycles out of the 30 show extreme deviation, suggesting the return of a more stable coupling pattern.

Changes in the interlimb coupling were observed as well after lesions sparing the ventral and ventrolateral quadrants during overground (Afelt 1974) and treadmill locomotion (Eidelberg 1981) and also after lesions restricted to the DLF (Jiang and Drew 1996), after damage to long propriospinal neurons (English 1989, see however Kato et al. 1984) and to the ventral spinocerebellar pathway. No changes were observed after lesions to the dorsal spinocerebellar tract (English 1985), or to the dorsal columns (Gorska et al. 1993b, Jiang and Drew 1996, see however English 1980). Thus, the fore- and hindlimb coupling pattern is suggested to result from the contribution of many converging inputs, peripheral,

segmental, propriospinal and supraspinal on to the stepping generators (Miller and Van der Meche 1976, English and Lennard 1982, Rossignol et al. 1993, Rossignol 1996). This is in line, therefore, with the suggestion of others (Jiang and Drew 1996), that the deficits in interlimb coupling are related to the extent of the spinal lesion and not necessarily to a specific damage to one pathway.

However, a common aspect to all the extensive lesions in our studies as well as those reported here is the damage in the lateral funiculus. Kato et al. 1984 suggested that supraspinal descending tracts are involved in conveying information adjusting the timing of activity in the fore- and the hindlimb. One of the most prominent candidates is the reticulospinal tract. Reticulospinal neurons not only are active and modulated during locomotion (Orlovsky 1970) but single unit recordings in the intact cat (Drew et al. 1986, Drew and Rossignol 1990a) and in the fictive locomotion preparation (Perreault et al. 1993) have shown that reticulospinal neurons discharge in correlation with the activity of flexors or extensors of more than one limb. Furthermore, microstimulation of these neurons at rest, evoked compound movements including one limb or more as well as the neck (Drew and Rossignol 1990a, b). The responses were organized reciprocally between limbs of the same girdle and were simultaneous, as well in the forelimb. During locomotion, the evoke responses, excitatory or inhibitory, were organized with respect to the step cycle and in the thalamic cat (Drew and Rossignol 1984)and in the fictive

preparation (Perreault et al. 1994) or the intact cat (Drew 1991b) caused changes in the amplitude as well in the duration of the muscle or in the related nerve activity up to resetting the existent locomotor rhythm. It was therefore suggested that the signal coming from a single reticulospinal neuron could be carried by one axon to more than one muscle in more than one limb to reinforce, on a step by step basis, the on going locomotor rhythm or even reset it. Taken together these observations suggest implications of the reticulospinal pathways in the control of the coordination between the limbs (Drew 1991b, Rossignol et al 1993).

Thus, if the contribution of the reticulospinal pathways to the control of fore- and hindlimb coordination is of major importance, severe damage to its axons will produce coupling instability between the two girdles. In that context, it is most interesting that up to a certain extent of the lesions (see cats EB5-EB6), a stable in-phase coupling can still be expressed indicating preserved functional interactions between the girdles. However, after more extensive lesions, such as in cat EB7, the step to step inconsistencies in the fore- and hindlimb coupling is sustained even long term post lesion. In this cat, the different reticulospinal pathways are severely damaged (see Table 1) and the remaining DLF pathways such as the corticospinal or the propriospinal and the ascending pathways in the dorsal columns are not sufficient to keep the stability of the interlimb pattern. Gorska et al. 1995, have tested the effect of damage to the caudal pole of the nucleus pontis oralis and to the rostral pole of the nucleus

reticularis pontis caudalis on the fore- and hindlimb coupling. They reported a transient effect on the interlimb coordination. The transitory effect is probably due to the limited extent of the lesion, as we have noted that permanent perturbations in the interlimb coupling is associated only with damage to most of the reticulospinal neurons in the pontine and medullary reticular formation nuclei.

EMG activity:

Various changes were observed in the amplitude and duration of the EMG activity as demonstrated in Table 4. The EMG activity of the most lesioned cats were more affected and showed in general a pronounced and sustained increase in amplitude and as well a reduction in their duration, which together permitted the cat to produce quick steps at the same length, to maintain the imposed treadmill speed. Another possibility is that the increase in the EMGs amplitude results from permanent changes in the motor units population such as that observed after complete spinal lesion (Cope et al. 1986, Edgerton et al. 1983). However these changes are associated with muscle atrophy which was not apparent here.

An important change is related to the deficits in the amplitude modulation of EMG activity when walking uphill or downhill on a treadmill. The increase in the EMG amplitude during uphill walking (see as well Pierotti et al. 1989 for the intact cat) is absent for the most lesioned cats,

while for the less extensively lesioned animals, the loss of modulation is transient and the cats recover it with time. The lack of amplitude modulation of the hindlimb extensors can be attributed to the absence or reduction of inputs from vestibulo- and reticulospinal pathways, as well as to damage of ascending pathways such as the ventral spinocerebellar tract, which could lead to a reduction or alteration in descending inputs. The vestibulospinal pathway exerts strong excitatory influence on extensors MNs during locomotion (Orlovsky 1972b) while the reticulospinal pathways may also exert powerful excitatory effects on the extensors muscles when evoked in the appropriate phase of the step cycle (Drew and Rossignol 1984, Perreault et al. 1994), although its effects have often been associated with flexors (Orlovsky 1970, Orlovsky 1972a). Elimination of these inputs may result in a more restricted firing rate and modulation pattern of the individual motor neuron which will limit the muscle force production.

The direct implication of vestibulo- and reticulospinal pathways in the modulation of the EMG activity during locomotion on inclined surface has been suggested by Matsuyama and Drew 1996. They recorded vestibulo- and reticulospinal neurons in chronic cats during walking over a tilted treadmill. The vestibulospinal neurons showed increased depth of discharge modulation during inclined locomotion as well as changes in peak discharge. The latter, effects, together with an increase in discharge, were observed as well for reticulospinal neurons. Thus, in the case of the

most extensively lesioned cats in which both the reticulo- and the vestibulospinal pathways are severely damaged such a modulation is absent and there is no recovery, while in the less lesioned cats long term post lesion the larger number of surviving reticulospinal axons are probably sufficient to maintain that function. When the EMG modulation is absent for the hindlimb muscles, we observed a more pronounced modulation in Tri amplitude, which is sustained for the extensively lesioned cats and transient for the less lesioned cats. Together this implies that the observed increase in TriL modulation can be interpreted as an adaptive mechanism which is applied as needed. Thus, the cat, by exerting control over the forelimbs muscles can compensate for the deficits in the hindlimb muscle activation. Preliminary data obtained using force platform suggest that the forelimbs contribute to propulsion in these cats (Brustein et al. 1995).

Summary and conclusions:

Sparing the ventral and ventrolateral funiculi was shown by Windle et al. 1958, Afelt 1974 and Eidelberg 1981 to be sufficient for recovery of locomotion. Our results, as well as those of others (Gorska 1993a, 1993b, Bem et al. 1995) show that quadrupedal voluntary locomotion is possible also after extensive lesions to the ventral and ventrolateral pathways, yet sparing pathways in the DLF. However, the cats suffer from major locomotor and postural deficits. Immediately after the lesion and for

a few weeks, the most lesioned cats could not support or walk with their hindlimbs, indicating the importance of the ventral and ventrolateral pathways to the normal locomotion which probably depends heavily on their inputs for initiation of locomotion and for maintaining posture. The time for recovery of weight support and walking in the most extensive lesioned cats also imply some reorganization in the spared pathways suggesting that they may not be the main contributor or be directly involved in the normal control of these aspect of the locomotion in the intact cat. The deficits observed during the plateau-period indicate, however, that DLF pathways, including the residual population of reticulospinal neurons, can be sufficient to provide the necessary drive to initiate locomotion as well as for some locomotor related postural adjustments. Although the activity in these pathways is sufficient to maintain the intralimb coupling, they are not sufficient to maintain the interlimb coordination or modulate the EMG amplitude during locomotion on an inclined treadmill, nor to maintain lateral stability. These functions are probably normally executed mainly by the brain stem pathways such as the reticulo- and the vestibulospinal. The "functional taking over" after partial lesions was suggested by Alstermark et al. 1987 for the recovery of food reaching in cats, by Vilensky at al. 1992 in the monkey as well as in human (Nathan 1994) . The basis for such reorganization is suggested to be due to plastic changes such as sprouting (Jiang and Drew 1996, Kozlowski et al. 1996, Kimura et al. 1994, Goldberger and Murray 1978,

Aoki et al. 1988). We have observed such changes in the motor cortex of cats EB6-EB8, which show up to a two fold increase in the number of labelled cells, as well as appearance of labelled cells in regions other than those related to the hindlimbs (Brustein et al. 1996). The suggested innervation of the lumbar spinal cord by axons of cells originating in more rostral levels may explain some of the return of locomotor function. We cannot however exclude reorganization at the spinal level as well (Carrier et al. 1997). These findings have a special clinical interest as they suggests that some spared axons in the spinal cord may be sufficient to initiate stepping and some postural adjustments. It would therefore seen important to maintain and improve these residual functions for instance, by drug application, as will be described in a forthcoming paper.

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Tables and Tables footnotes:

Table 1: HRP cell counts

HRP cells counts in the pontine reticular formation (PRF), Medullary reticular formation (MRF) and their totals as well as in the lateral vestibular nucleus (VLN), red nucleus (RN) and in the motor cortex after bilateral injection caudal to the site of lesion (L2).The average and SD values are taken from 3 intact cats , for a total of 6 left and right brain stems. The labelled cells counted in the lesioned cats are presented in numbers as well as in percent of the intact values. NA= not available, *$p<0.05$, **$p<0.01$.

Table 1: HRP cell counts

		PRF		MRF		TOTAL		LVN		RN		MOTOR CORTEX	
		cell number	% of intact	cell number	% of intact	cell number	% of intact	cell number	% of intact	cell number	% of intact	cell number	% of intact
INTACT	AVG	913		3037		3950		842		2346		3064	
	SD	378		535		868		7.5		268		396	
	n	6		6		6		2		4		3	
EB1	Right	274	30*	2133	70*	2407	61*	53	6**	1109	47**	NA	
	Left	412	45	1030	34**	1442	36**	179	21**	1102	47**	NA	
EB2	Right	889	97	1957	64*	2846	72	108	13**	1486	63**	1261	41**
	Left	764	84	1936	64*	2700	68	84	10**	1420	60**	3038	99
EB3	Right	358	39	1475	48**	1833	46*	58	7**	860	37**	1489	48**
	Left	284	31	792	26**	1076	27**	NA		NA		NA	
EB4	Right	1254	137	678	22**	1932	49*	NA		NA		1895	62**
	Left	402	44	550	18**	952	24**	280	33**	NA		1478	48**
EB5	NA												
EB6	Right	905	99	1463	48**	2368	60*	1	0.1**	1300	55**	4155	136**
	Left	133	14*	145	5**	278	7**	16	2**	0	0**	67	66**
EB7	Right	267	29*	626	21**	893	23**	4	0.5**	1352	58**	5890	192**
	Left	170	19*	543	18**	713	18**	1	0.2**	95	4**	403	13**
EB8	Right	217	24*	957	31**	1174	30**	1	0.1**	1300	55**	860	28**
	Left	447	49	1050	34**	1497	38**	0	0**	1557	66**	4300	140**

Table 2: Results of the regression analysis between the cycle duration and the swing or the stance (see Fig. 4).

Results of the regression analysis between the cycle duration and the swing or the stance, during the recovery period or the plateau-period relative to the intact state (cats EB5-EB7). None of the values were found to be significantly different from control.

Table 2: Results of the regression analysis between the cycle duration and the swing or the stance (see Fig. 4).

		Intact	Recovery period	Plateau-period
EB5	Day		36	88
	Speed range (m/s)	0.3-0.5	0.2-0.5	0.2-0.5
	n	35	49	49
	Slope of swing	0.16	0.11	0.08
	Correlation (r)	0.80	0.27	0.28
	Slope of stance	0.83	0.87	0.91
	Correlation (r)	0.98	0.89	0.95
EB6	day		77	141
	Speed range (m/s)	0.3-0.5	0.2-0.4	0.2-0.4
	n	36	23	16
	Slope of swing	0.14	-0.012	0.33
	Correlation (r)	0.63	0.02	0.68
	Slope of stance	0.85	1.01	0.66
	Correlation (r)	0.97	0.92	0.87
EB7.	day		42	245
	Speed range (m/s)	0.2-0.4	0.1-0.3	0.2-0.35
	n	27	34	18
	Slope of swing	0.05	0.1	0.19
	Correlation (r)	0.28	0.4	0.7
	Slope of stance	0.94	0.89	0.80
	Correlation (r)	0.98	0.96	0.97

Table 3: The average percent of time spent on 2,3 or 4 supporting limbs.

The average percent of time spent on 2,3 or 4 supporting limbs in 7 of the cats, in the intact state, during the recovery and the plateau-period. The values were calculated from the averaged and normalized foot fall diagram, after grouping all the steps according to the various types of limb coupling.

Table 3: The average percent of time spent on 2,3 or 4 supporting limbs.

		Intact	Recovery period		Plateau-period	
			4 days		8 days	
EB1	Number of supporting limbs					
	2	23	37		43	
	3	77	46		36	
	4	---	17		21	
	coupling type		homolateral in-phase		homolateral in-phase	
	n	5	10		11	
			1 day		7 days	
EB2	2	16	24		34	
	3	80	62		58	
	4	4	14		8	
	coupling type		homolateral in-phase			
	n	8	10		5	
			1 day		63 days	
EB3	2	21	31		30	
	3	76	60		68	
	4	3	9		2	
	coupling type		homolateral in-phase			
	n	8	4		5	
			36 days		88 days	
EB5	2	27	31	19	31	
	3	65	44	76	54	
	4	8	25	5	15	
	coupling type		diagonal		homolateral in-phase	
	n	11	12	10	28	
			77 days		141 days	
EB6	2	26	18		42	
	3	70	65		25	
	4	4	17		33	
	coupling type		diagonal-like		homolateral in-phase	
	n	17	10		11	
			42 days		196 days	
EB7	2	32	26	41	39	28	24
	3	60	65	34	41	46	68
	4	8	9	25	20	26	8
	coupling type			diagonal	homolateral in-phase	diagonal	homolateral in-phase
	n	8	3	4	2	5	2
			14 days		168 days		
EB8	2	37	41		33		
	3	59	51		65		
	4	4	8		2		
	coupling type		homolateral in-phase				
	n	12	8		19		

Table 4: amplitude and duration of the hindlimb muscles (0.4m/s)

The normalized and averaged EMGs amplitude, presented as a percent of the intact (%± C.V.) and their average duration (ms± SD) taken from cats EB2 and EB6 (at 0.4m/s) from intact, recovery and plateau-period, trials (*$p<0.05$, **$p<0.01$)

. Table 4: amplitude and duration of the hindlimb muscles (0.4m/s)

		Intact		Recovery period		Plateau-period	
				1 Day		21 Days	
	MUSCLE	Normalized Amplitude (%±C.V.)	Duration (ms±SD)	Normalized Amplitude (%±C.V.)	Duration (ms±SD)	Normalized Amplitude (%±C.V.)	Duration (ms±SD)
EB2	LSt	100±26 (n=124)	86±24	79±42* (n=9)	76±19	107±13 (n=28)	80±25
	LSrt	100±39 (n=120)	194±49	78±18 (n=9)	155±38*	99±19 (n=28)	188±45
	RSrt	100±16 (n=118)	242±31	40±12** (n=9)	206±44**	NA	
	LVL	100±16 (n=124)	501±97	457±48** (n=9)	469±52	314±41** (n=27)	510±86
	RVL	100±13 (n=120)	441±89	112±7* (n=9)	518±48*	112±5** (n=28)	522±65
	LGM	100±15 (n=68)	463±87	48±15** (n=9)	352±56**	67±11** (n=16)	491±76
				77 Days		141 Days	
EB6	LSt	100±15 (n=63)	196±31	227±59** (n=20)	122±29**	77±28** (n=22)	126±35**
	LSrt	100±17 (n=63)	326±46	136±39** (n=20)	287±68**	150±23** (n=22)	272±35**
	RSrt	100±14 (n=68)	334±34	153±19** (n=20)	274±44**	146±15** (n=22)	307±47**
	LVL	100±12 (n=69)	614±64	140±49** (n=20)	409±64**	131±12** (n=22)	486±73**
	RVL	100±9 (n=38)	647±62	225±18** (n=20)	503±68**	225±20** (n=22)	486±50**
	LGM	100±13 (n=62)	566±80	238±61** (n=20)	433±84*	146±34** (n=22)	397±70**

Table 5: TriL amplitude (% of control ±C.V.) at 0.4m/s.

The normalized and averaged TriL amplitude presented as a percent of the intact values (%± C.V.), for each one of the 8 cats from intact , recovery and plateau- period trails. NA= not available. *p<0.05, **p<0.01.

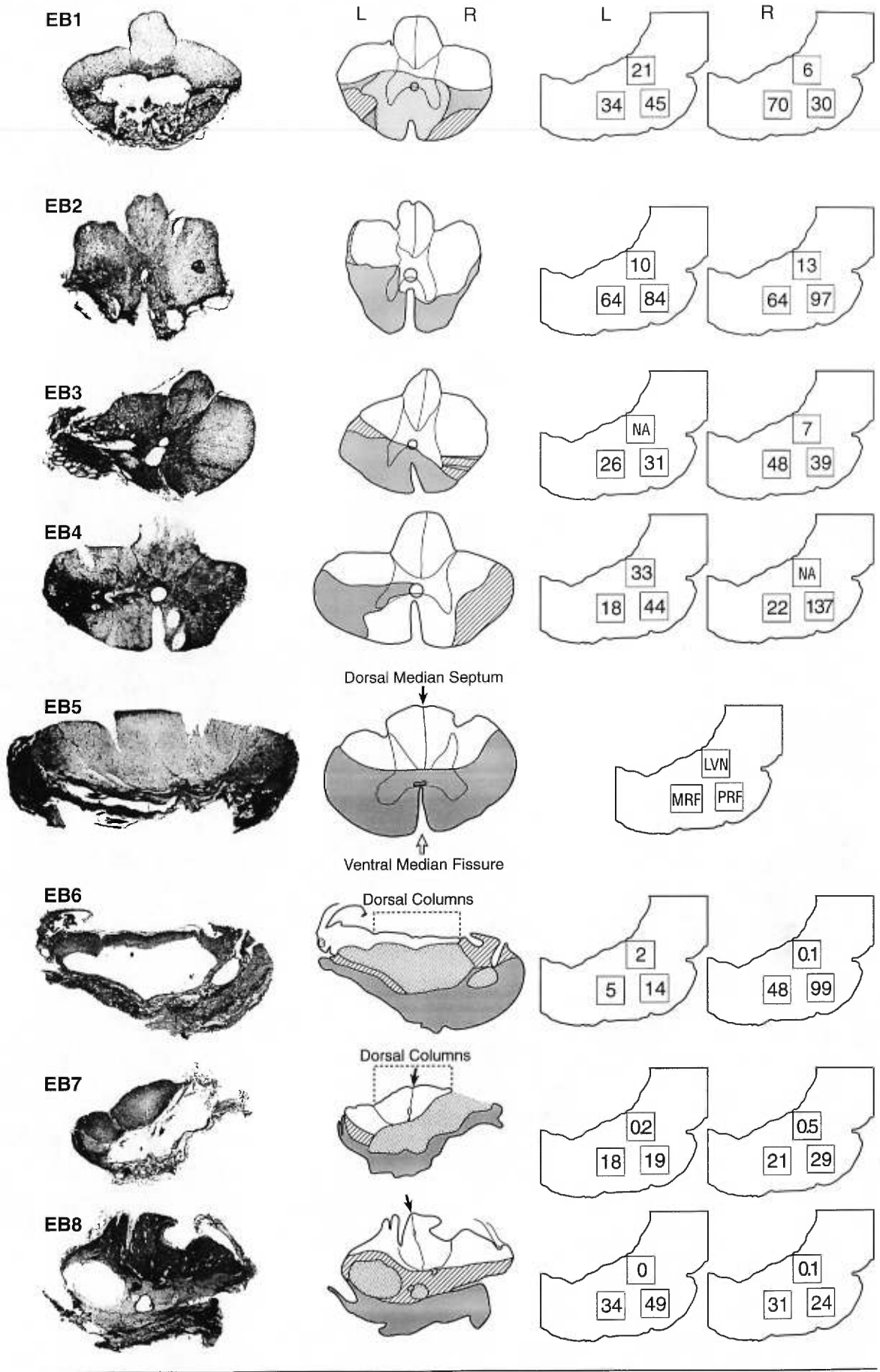
Table 5: TriL amplitude (% of control ±C.V.) at 0.4m/s.

	Intact		Recovery period		plateau-period	
	LTriL (%)	RTriL (%)	LTriL (%)	RTriL (%)	LTriL (%)	RTriL (%)
EB1	100±10 (n=20)	100±25 (n=31)	222±13** (n=10)	181±11** (n=14)	141±4** (n=10)	140±11** (n=11)
EB2	100±19 (n=26)	100±19 (n=26)	150±19** (n=9)	123±17** (n=9)	220±10** (n=27)	150±5** (n=28)
EB3	100±17 (n=40)	100±19 (n=44)	227±17** (n=15)	171±10** (n=17)	311±9** (n=34)	140±7** (n=36)
EB4	100±7 (n=20)	100 ±7 (n=22)	337±15** (n=16)	175±13** (n=16)	274±17** (n=15)	117±30* (n=15)
EB5	100±13 (n=24)	100±22 (n=25)	92±17 (n=25)	88±42 (n=25)	159±27** (n=31)	125±22** (n=31)
EB6	100±10 (n=28)	100±7 (n=29)	124±11** (n=19)	134±8** (n=19)	107±10* (n=22)	136±11** (n=22)
EB7	100±9 (n=16)	100±8 (n=17)	38±25** (n=19)	234±21** (n=19)	48±12** (n=10)	330±15** (n=10)
EB8	100±6 (n=14)	NA	93±10 (n=7)	NA	83±11** (n=19)	NA

Figure Legends:

Fig. 1: **Extent of the spinal lesions for all 8 cats:**

The histology of all the cats is displayed in four sets of columns. On the left , photomicrographs of the cross sections taken from the spinal site of lesion, stained with cresyl violet or with Kluver- Barrera, to demonstrate the maximal extent of the lesion. To the right of the photomicrographs, a schematic reconstruction of the site of lesion, evaluated using light microscope. For cats EB1-EB5, the total extent of the lesion is projected on a spinal cord section taken more rostrally, after inspecting several consecutive sections, while for cats EB6-EB8 the schemes illustrate the maximal site of lesion to emphasize the deformation of the spinal cord. The various texture identify the extent of damage. *Intact*: myelinated axons (or axonal profiles) appeared normal under the microscope; *Severe damage*: absent or highly fibrotic tissue; *Moderate damaged:* some myelinated axons (or axonal profiles) of normal appearance within fibrotic and gliotic tissue; *Syrinx:* cyst. L=left; R=right. The next two adjacent columns illustrate sagittal brain stem sections (left and right) and summarize the total values of HRP labelled cells (percent of control) found in the LVN, PRF and MRF for each cat when available (see Table 1).


EB1
L
R
L
R
21
34
45
6
70
30
EB2
10
64
84
13
64
97
EB3
NA
26
31
7
48
39
EB4
33
18
44
NA
22
137
Dorsal Median Septum
EB5
LVN
MRF
PRF
Ventral Median Fissure
EB6
Dorsal Columns
2
5
14
0.1
48
99
Dorsal Columns
EB7
02
18
19
0.5
21
29
EB8
0
34
49
0.1
31
24
Intact
Severe Damage
Moderate Damage
Syrinx
1 mm

RSq2 97-150-2

Fig. 2: **Recovery of treadmill locomotion following spinal lesion for each one of the 8 cats:** The graph illustrates the maximal treadmill speed each cat could attain and maintain at least for a few step cycles, as a function of days post spinal lesion

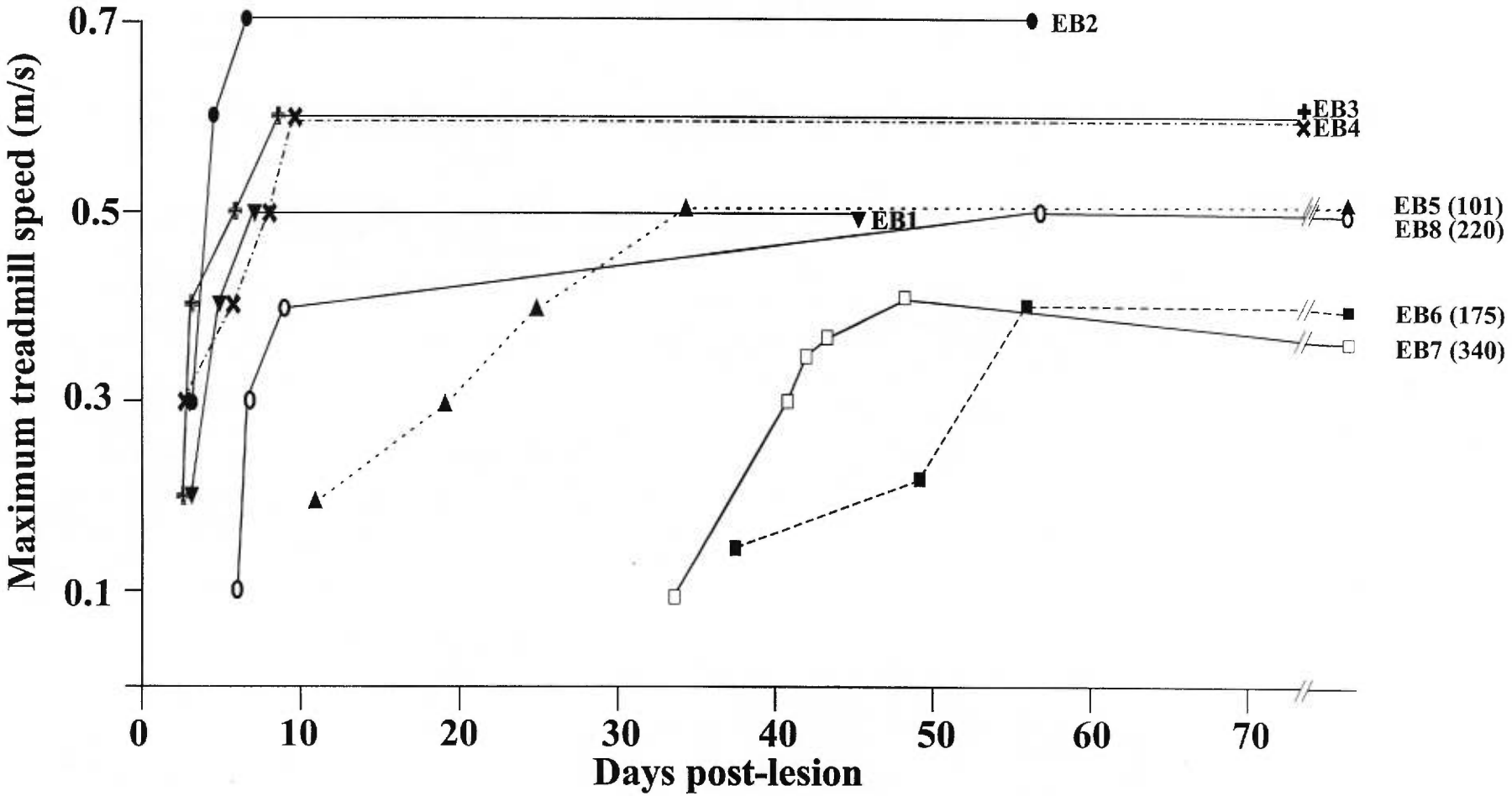
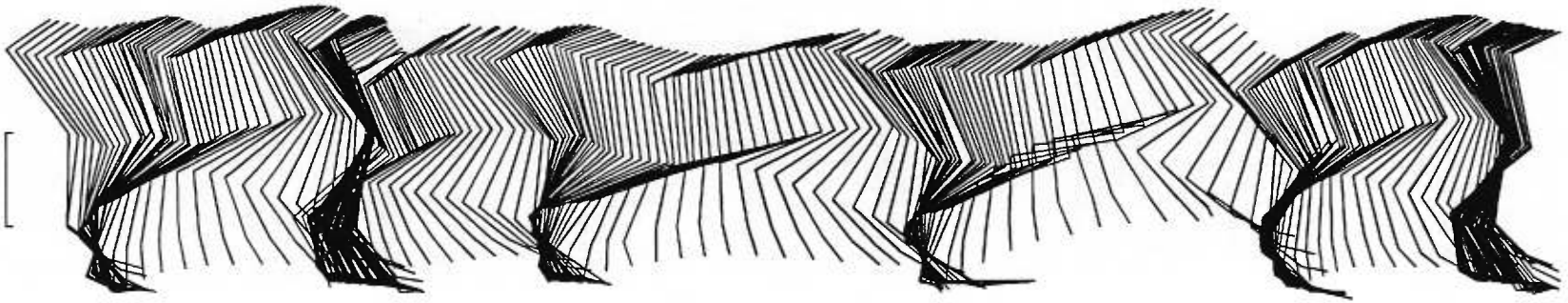

Maximum treadmill speed (m/s)
0.7
0.5
0.3
0.1
0
10
20
30
40
50
60
70
Days post-lesion
EB2
EB3
EB4
EB1
EB5 (101)
EB8 (220)
EB6 (175)
EB7 (340)

Fig. 3: **Stick figures of locomotion pre and post lesion**: The consecutive stick figure diagrams illustrates several steps over a period of 5s (0.4m/s), of the right hindlimb of cat EB5 to compare the walking before lesion (intact), short term post lesion (day 36) and long term post lesion (day 88). Each consecutive stick figure is displaced from the preceding one by 3 mm. Notice that in this kind of diagram, the X axis is respected which requires adjustments in the Y axis, explaining why the stick figure is shorter 88 days post lesion. The vertical bar indicates the unvarying length of the tibia (11cm).

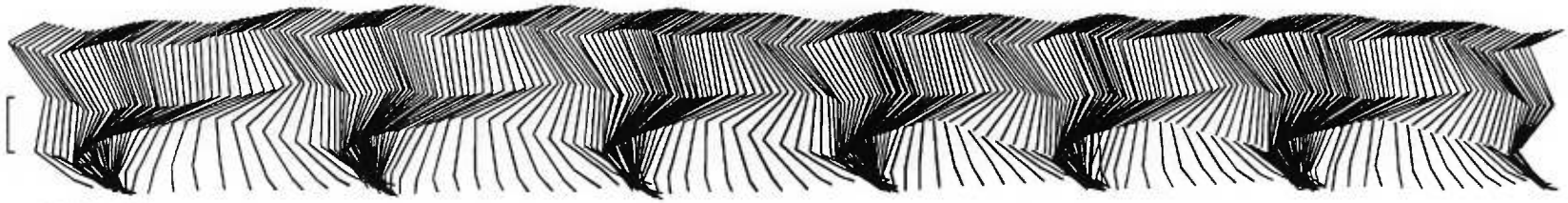
EB5 0.4m/s

Intact

Day 36 post-lesion



Day 88 post-lesion



da64,214,304

1s

Fig. 4: **Step cycles before and after lesions**: The graphs of the average step cycle duration (average ±SD), as a function of time after the spinal lesion are illustrated for cats EB2, EB3, EB6 and EB7. The shaded area represents the average cycle duration ± SD of 3 control (Intact) experiments. The number of steps used for the calculations varied between 68 and 127 in the control experiments and between 5 and 40 following the lesion, depending on the cat's walking deficits. L=left, R=right , * indicates the results of t-test between the average step cycle duration pre- and post lesion. ♦ indicates the results of variance ratio test between the variance pre-and post lesion. (♦, *= $p<0.05$, ♦♦, ** =$p<0.01$)

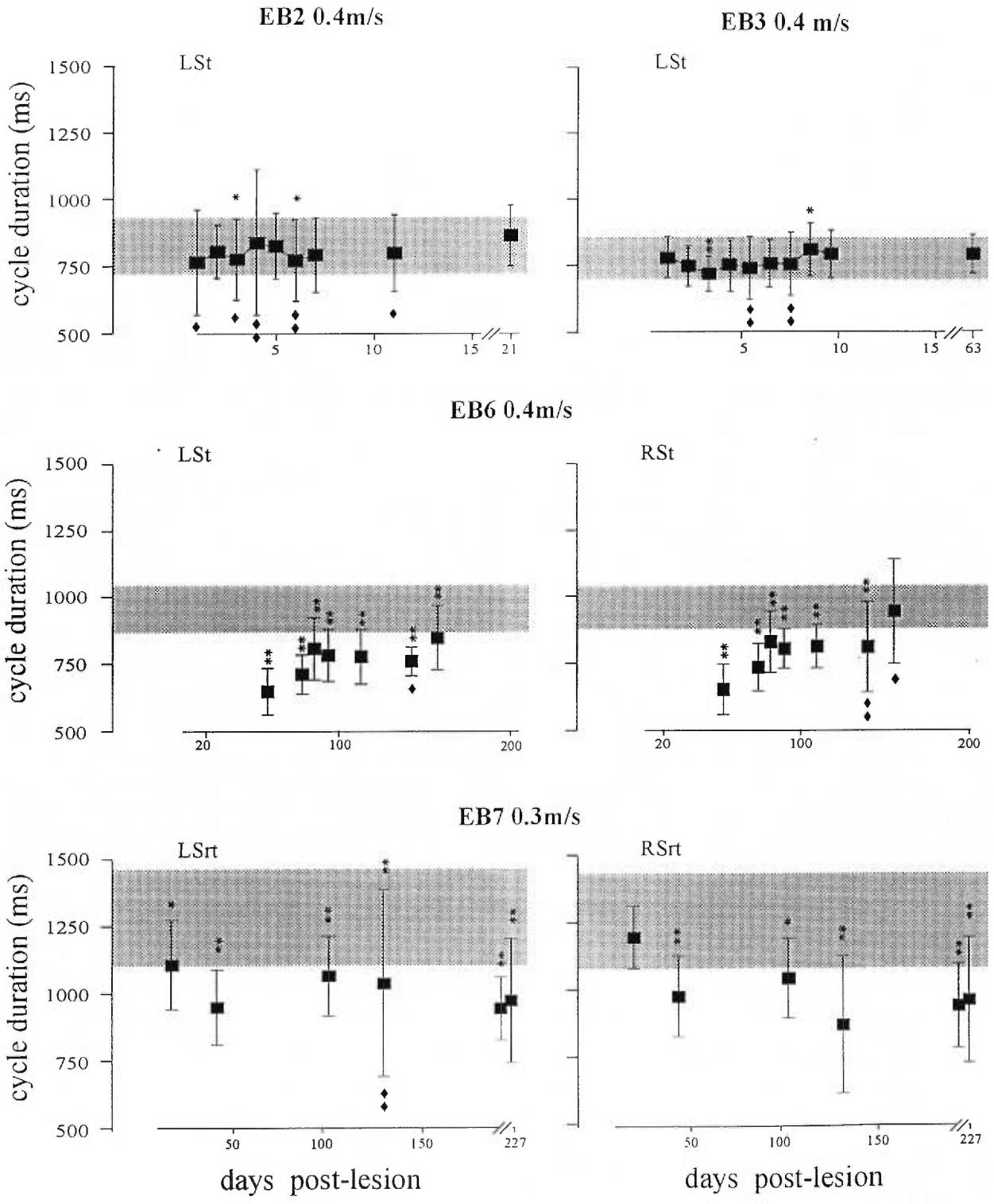

EB2 0.4m/s
EB3 0.4 m/s
LSt
LSt
cycle duration (ms)
1500
1250
1000
750
500
5
10
15
21
5
10
15
63
EB6 0.4m/s
LSt
RSt
cycle duration (ms)
1500
1250
1000
750
500
20
100
200
20
100
200
EB7 0.3m/s
LSrt
RSrt
cycle duration (ms)
1500
1250
1000
750
500
50
100
150
227
50
100
150
227
days post-lesion
days post-lesion

Fig. 5: **Correlation of swing and stance duration with the step cycle duration:** Linear regression relations between the stance or the swing and the step cycle duration in the intact cat and short term or long term post lesion, illustrated for the most lesioned cats: cat EB5 (A,D,G) , cat EB6 (B,E,G) and cat EB7 (C, F,I). The range of speeds, slopes values and correlation coefficients are summarized in Table 2.

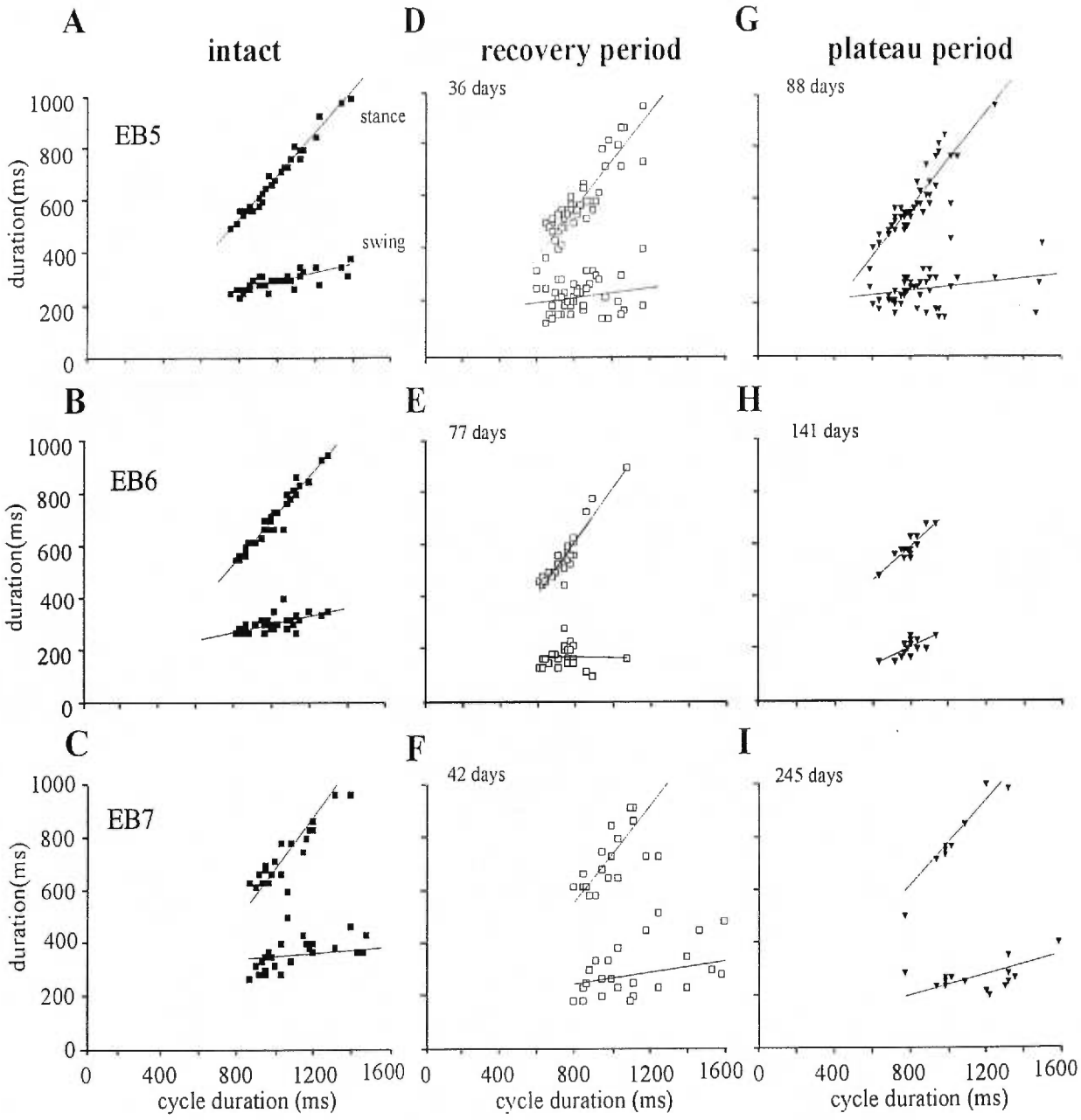

A
intact
D
recovery period
G
plateau period
EB5
stance
swing
36 days
88 days
B
EB6
77 days
141 days
E
H
C
EB7
42 days
245 days
F
I
duration(ms)
cycle duration (ms)
0
200
400
600
800
1000
1200
1600

Fig. 6: **Representative kinematic data recorded from the right hindlimb of cat EB5 in the intact state and 88 days post spinal lesion**. The angular excursions (A, B) are related to the first step cycles illustrated in the stick diagram of Fig. 3. Notice that before the lesion (intact), the cat makes 3 steps while after the lesion it makes 4 shorter steps for the same time interval. In the duty cycle traces the heavy line represents the stance while the down and up arrows represent foot contact and lift, respectively. The angle-angle plots in C (intact) and D (88 day post lesion) are the averages of the whole step sequence, n=9 (intact) n=11 (post lesion). The arrows around the plots indicate the direction of the movements in various subdivisions of the step cycle as defined by Phillipson (Phillipson 1905): Flexion (F) and first extension phase (E_1) in swing and the second (E_2) and the third (E_3) in the stance.

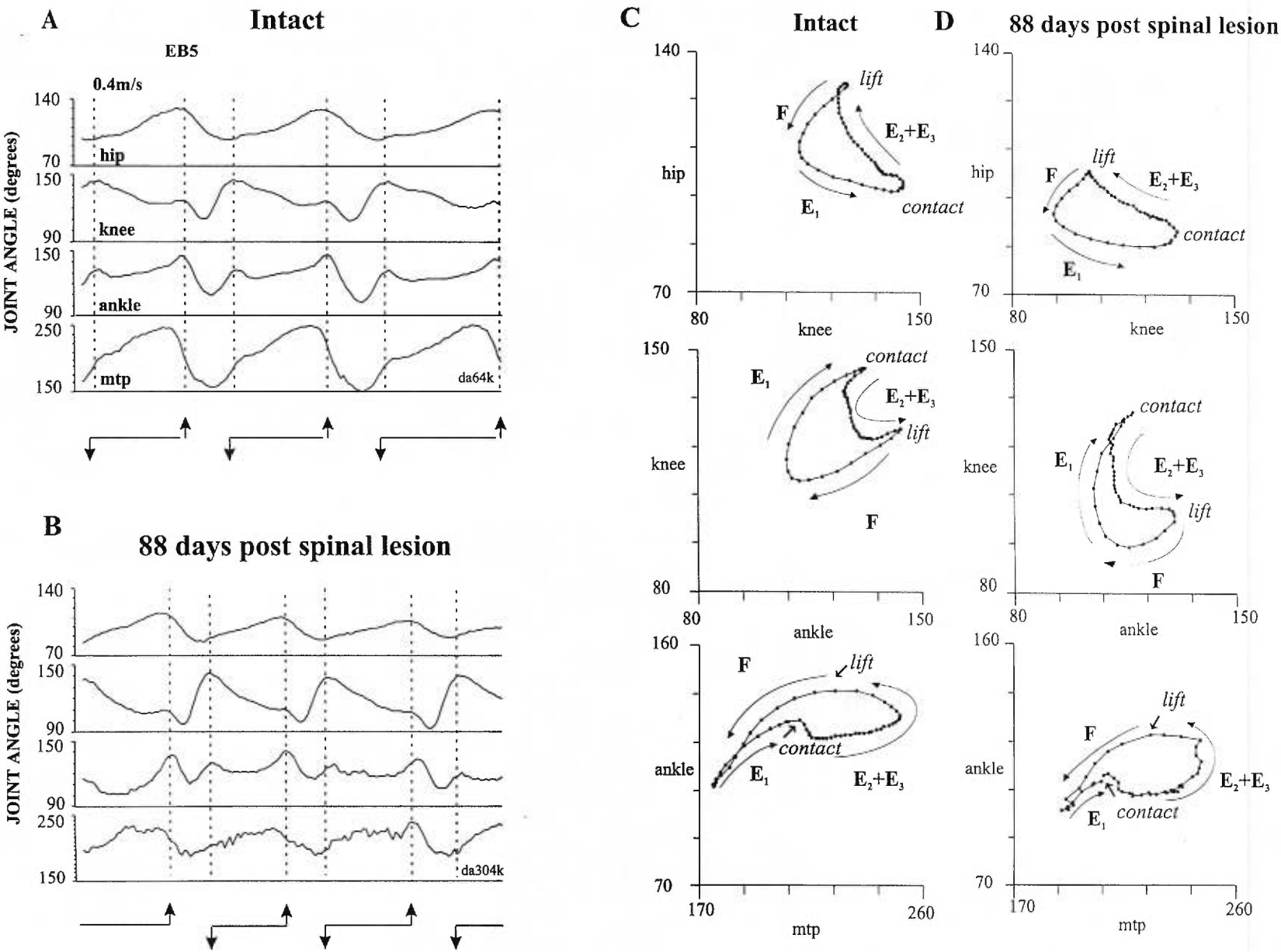

A
Intact
EB5
0.4m/s
JOINT ANGLE (degrees)
140
70
hip
150
90
knee
150
90
ankle
250
150
mtp
da64k
B
88 days post spinal lesion
140
70
150
90
150
90
250
150
da304k
C
Intact
D
88 days post spinal lesion
hip
knee
ankle
mtp
lift
contact
F
E1
E2+E3
80
150
70
140
160
170
260

Fig. 7: **Raw EMGs before and after the lesion**: A comparison of the raw EMGs before (A) and after the lesion (B) taken from cat EB5. Both are related to the kinematic data shown in Fig. 3. and in Fig. 6. The heavy vertical lines are aligned on the St burst onset while the dashed lines are aligned on the onset of RGM, to illustrate relations between phase onset of activity in flexors and extensors, respectively. In the duty cycle traces, the heavy line represents the stance while the down and up arrows represent foot contact and lift, respectively.

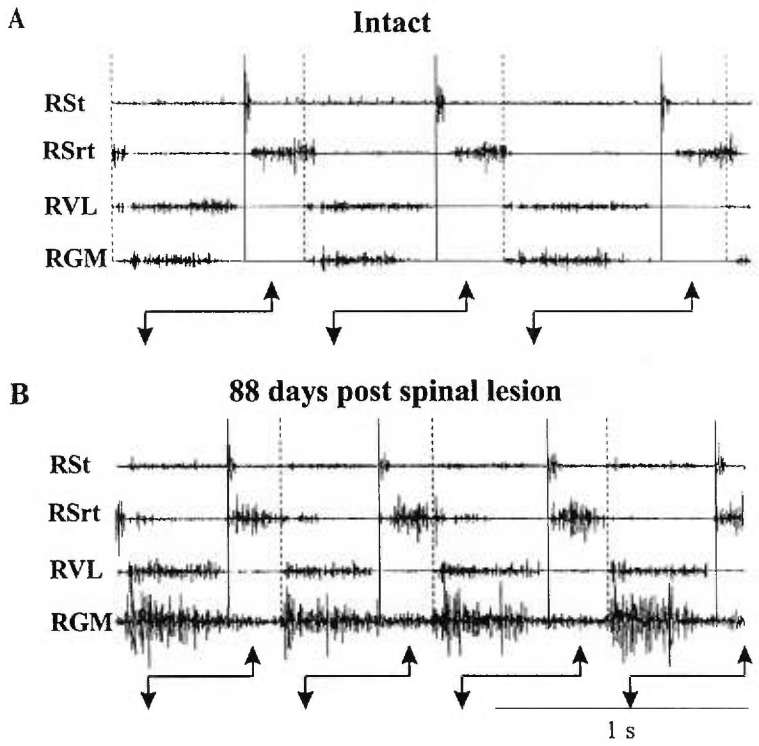

A
Intact
RSt
RSrt
RVL
RGM
B
88 days post spinal lesion
RSt
RSrt
RVL
RGM
1 s

Fig. 8: **interlimb coupling in cat EB5**: A comparison of interlimb coupling between the right hindlimb and the right forelimb of cat EB5; Intact, 36 and 88 days post lesion. A, E, I show representative raw EMGs and foot falls taken from the walking sequences used to calculate the phase of bursts onsets in graphs B, F, J , the cycle duration in graphs C,G,K and the cumulative phase difference in graphs D, H, L, for each one of the individual consecutive step cycles.

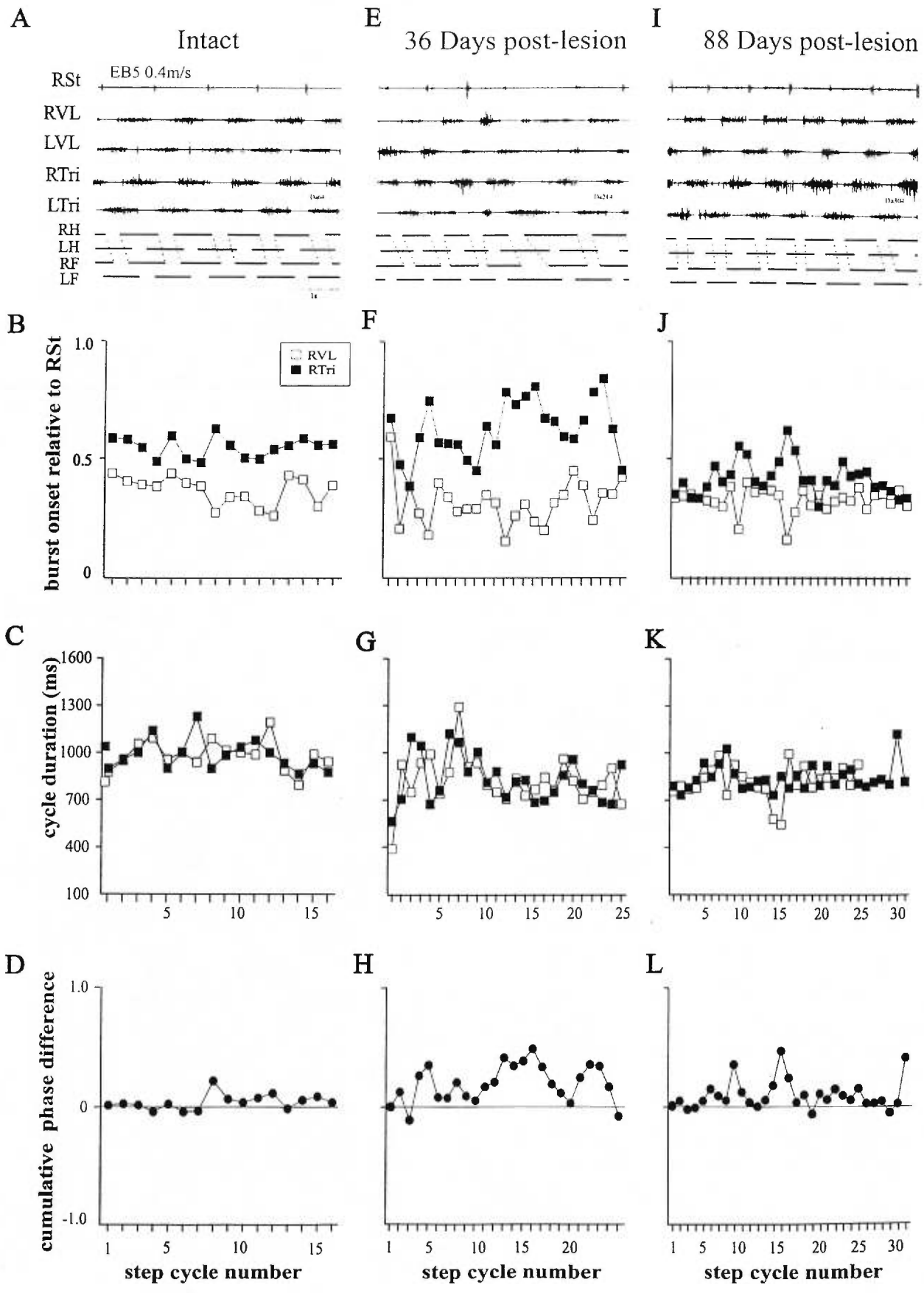

A
Intact
E
36 Days post-lesion
I
88 Days post-lesion
RSt
EB5 0.4m/s
RVL
LVL
RTri
LTri
RH
LH
RF
LF
B
F
J
burst onset relative to RSt
1.0
0.5
0
RVL
RTri
C
G
K
cycle duration (ms)
1600
1300
1000
700
400
100
5
10
15
20
25
30
D
H
L
cumulative phase difference
1.0
0
-1.0
1
5
10
15
20
25
30
step cycle number
step cycle number
step cycle number

Fig. 9: **Interlimb coupling in cat EB7:** Comparison of interlimb coupling between the left fore-and hindlimb of cat EB7 in the intact state and 51 days post lesion. A, B illustrate the individual values of left VL and TriL cycle duration in consecutive step cycles (as in Fig. 8 C,G, K). C,D illustrate the cumulative phase difference for the same sequence as in A and B. E: Raw EMGs and foot falls (51 days post lesion) from which the cumulative values in D were calculated, illustrate the different number of steps (indicated on the foot falls) done by the fore- and the hindlimbs.

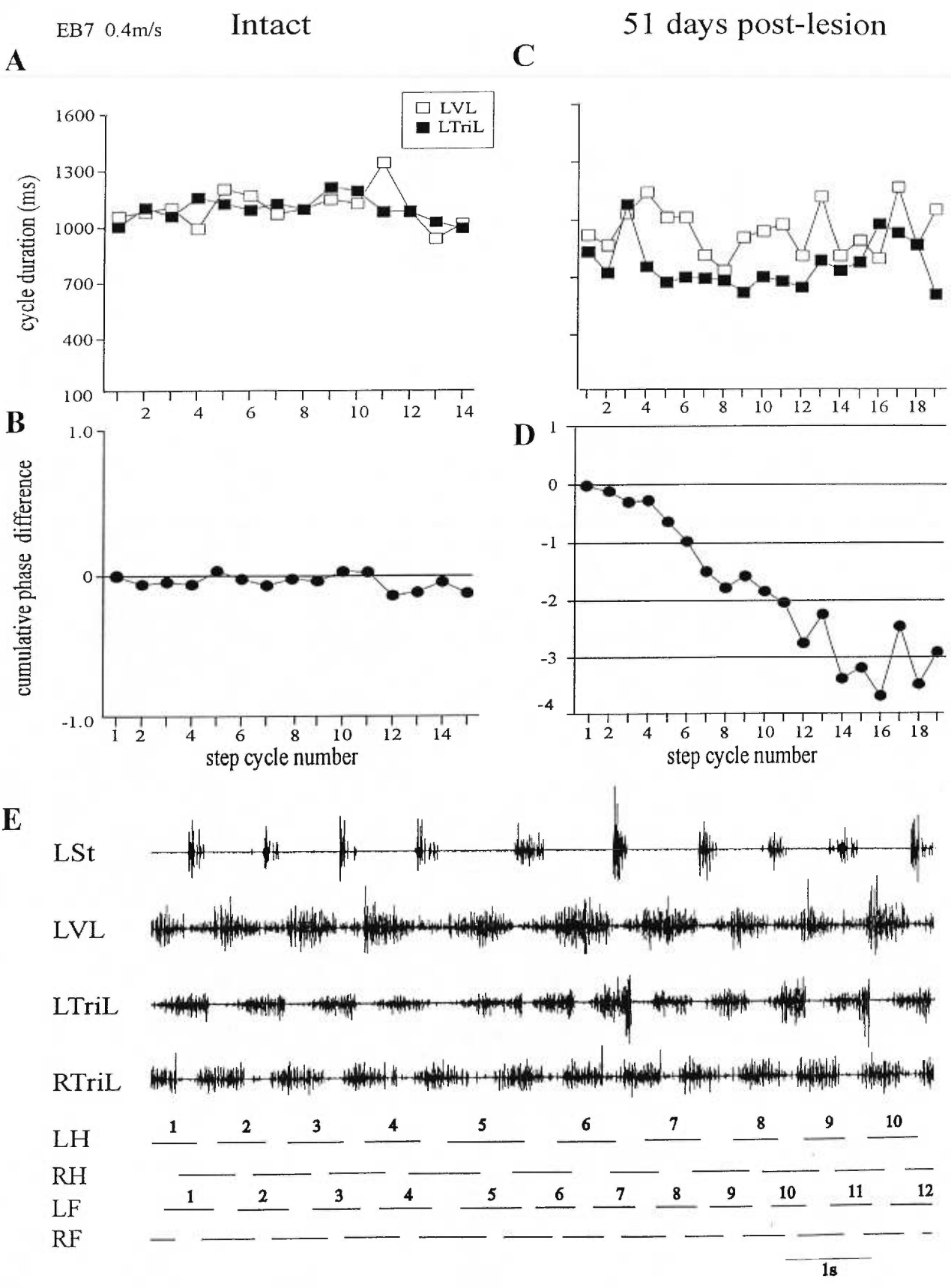

EB7 0.4m/s
Intact
51 days post-lesion
A
C
LVL
LTriL
cycle duration (ms)
1600
1300
1000
700
400
100
2 4 6 8 10 12 14
2 4 6 8 10 12 14 16 18
B
D
cumulative phase difference
1.0
0
-1.0
1
0
-1
-2
-3
-4
1 2 4 6 8 10 12 14
1 2 4 6 8 10 12 14 16 18
step cycle number
step cycle number
E
LSt
LVL
LTriL
RTriL
LH
RH
LF
RF
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10 11 12
1s

Fig. 10: **Foot fall diagrams**: Representative averaged and normalized foot fall diagrams taken from cat EB6 at intact, 77 and 141 days post lesion. The filled horizontal rectangles represent the stance of each one of the limbs; left (L) and right (R) hindlimb (H) and forelimb (F), respectively: LH, RH, LF, RF. The vertical lines divide the step cycle according to the number of supporting limbs, illustrated as well, in the bottom figurines in which the circles represent whether the limb is in contact with the treadmill (filled) or not (open). The total percent of the time the cat is supported by (2), (3), or (4) limbs is given by the stack bar diagram to the right.

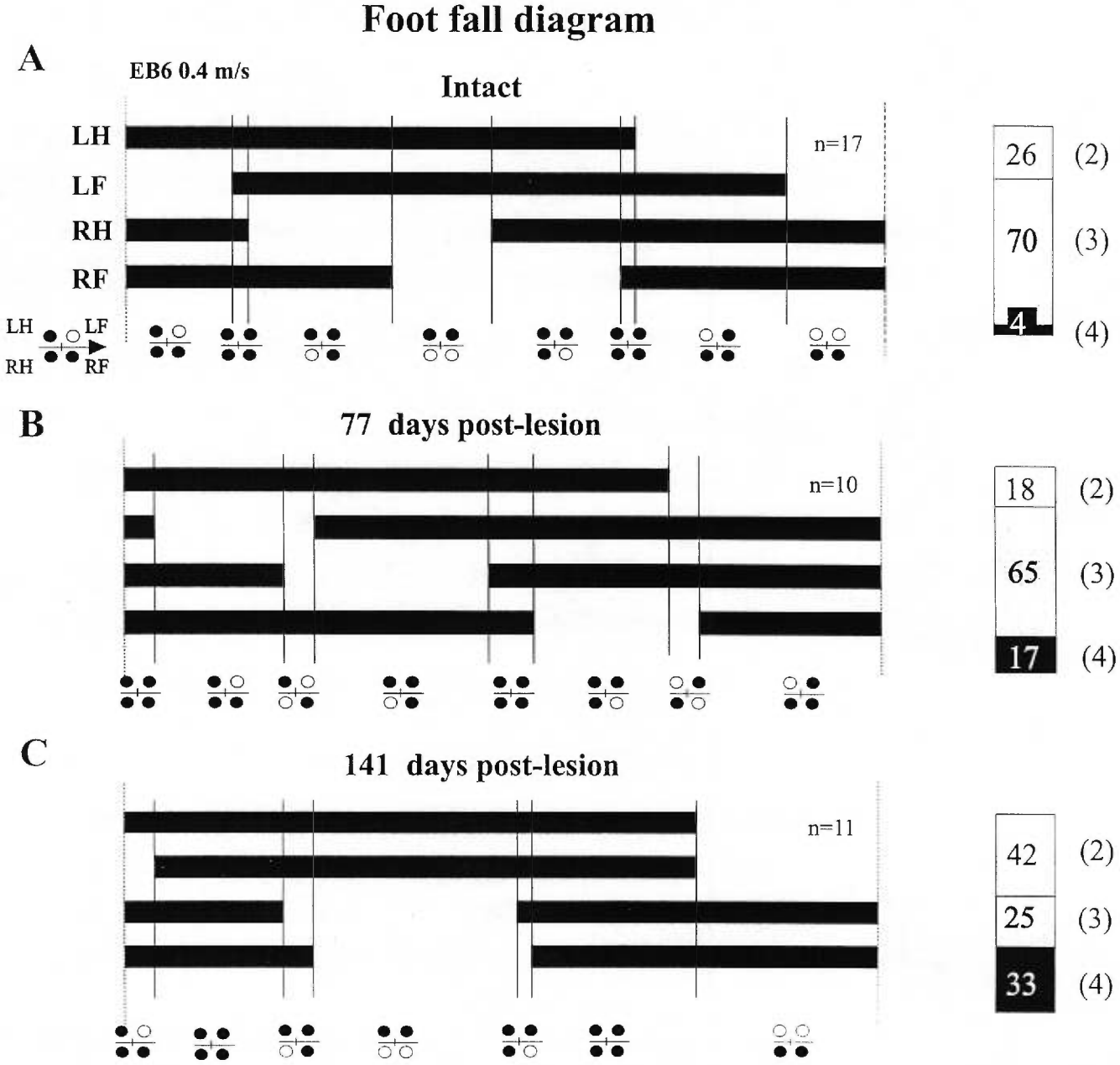

Foot fall diagram
A
EB6 0.4 m/s
Intact
LH
LF
RH
RF
n=17
26
(2)
70
(3)
4
(4)
B
77 days post-lesion
n=10
18
(2)
65
(3)
17
(4)
C
141 days post-lesion
n=11
42
(2)
25
(3)
33
(4)

Fig. 11: **EMG modulation during inclined walking on a treadmill (cat EB5):**

The averaged, integrated and normalized EMG amplitude calculated for cat EB5 during walking on inclined treadmill (10 degrees) before the lesion (intact) and as a function of days post lesion, to illustrate the changes in EMG modulation in right hindlimb and forelimb extensors (VL and TriL, respectively) as well in the flexors Srt and CLB. The number of steps cycles used for averaging ranged between 18-23 for the control experiments (indicated on the abscissa by their experiment number: e2, e5, etc.) while, after the lesion, 7-30 steps were used depending on the cat's walking capacities with time after lesion.

EB5 0.4 m/s

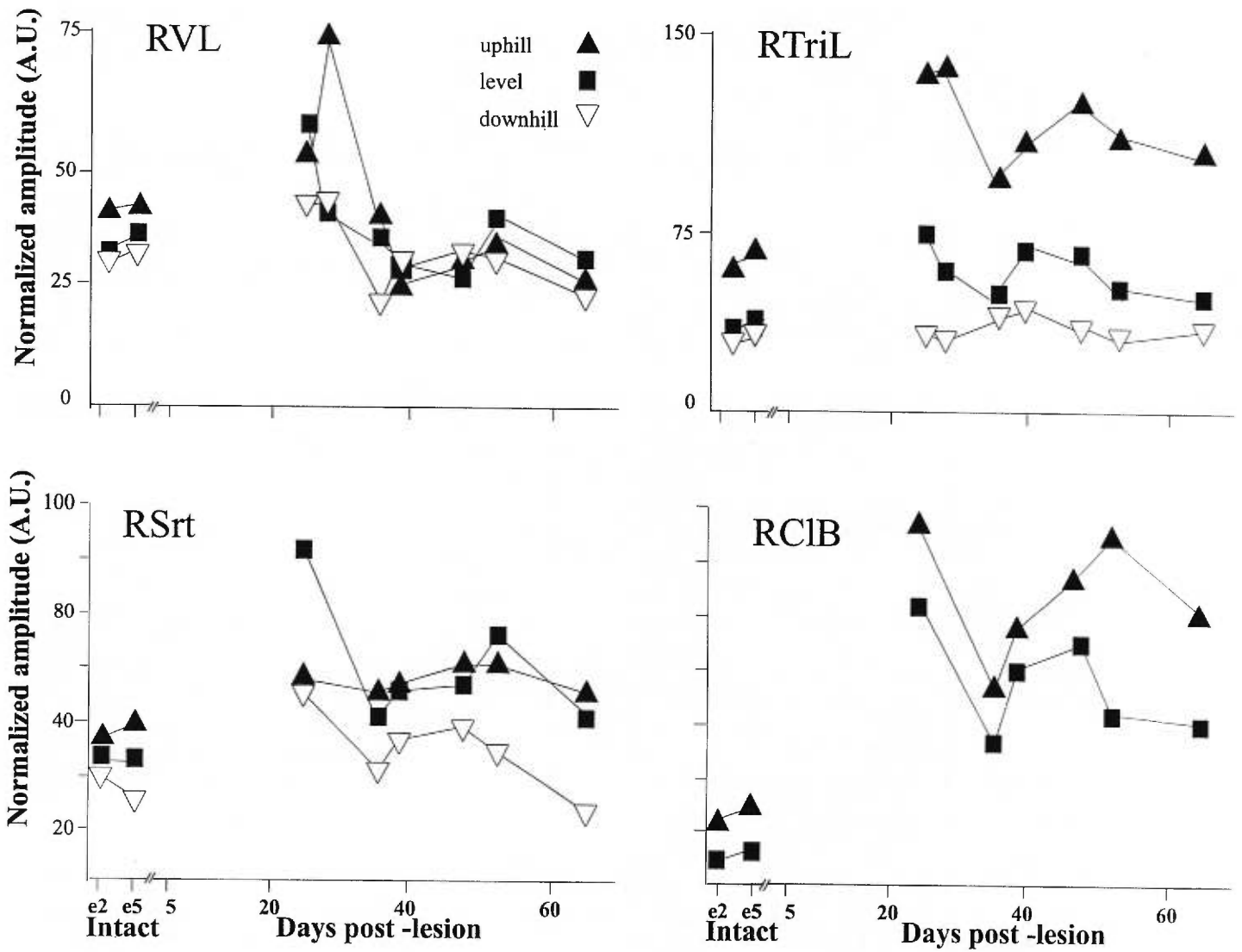
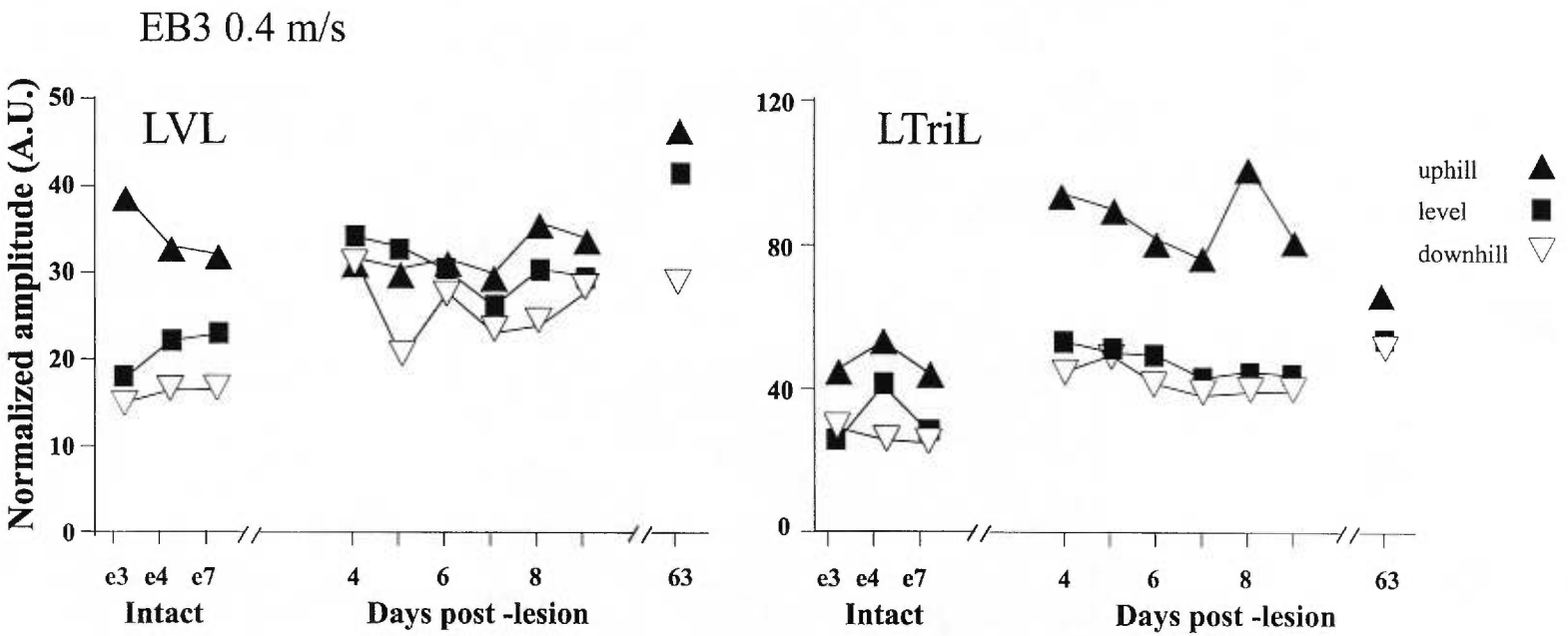

RVL
uphill
level
downhill
Normalized amplitude (A.U.)
75
50
25
0
RTriL
150
75
0
RSrt
100
80
40
20
RClB
e2 e5 5 20 40 60
Intact
Days post -lesion

Fig. 12: **EMGs modulation during walking on inclined treadmill (cat EB3):** The averaged, integrated and normalized EMG amplitude calculated for cat EB3 during walking on an inclined treadmill (10 degrees), before the lesion (intact) and as a function of days post spinal lesion, to illustrate the changes in EMG modulation in the left hindlimb and forelimb extensors (VL and TriL, respectively). The number of steps cycles used for averaging ranged between 35-40 for the control experiments (indicated on the abscissa by their experiment number: e3, e4, etc.) while after the lesion, 15-25 steps were used


EB3 0.4 m/s
LVL
LTriL
Normalized amplitude (A.U.)
50
40
30
20
10
0
120
80
40
0
e3 e4 e7
4 6 8 63
Intact
Days post -lesion
uphill
level
downhill

PAPER#2

The effects of Noradrenergic and Serotoninergic drugs on treadmill locomotion in adult cats submitted to bilateral chronic ventral and ventro-lateral spinal lesions.

by :

Edna Brustein

Serge Rossignol

Abstract:

The effects of serotoninergic and noradrenergic drugs (applied intrathecally) on treadmill locomotion were tested in two adult cats subjected to a ventral and ventro-lateral spinal lesion (T13). Despite the extensive spinal lesion, severely damaging, important descending pathways such as the reticulo- and vestibulospinal tracts, both cats recovered quadrupedal voluntary locomotion. The locomotor recovery occurred in 3 stages defined as, early stage, when the animal cannot walk with its hindlimbs, recovery period, when progressive improvement occurs, and plateau-period, when a more stable locomotor performance is observed. At the later stage, the cats suffered from postural and locomotor deficits, such as poor lateral stability, irregular stepping of the hindlimbs and inconsistent homolateral fore- and hindlimb coupling. The aim of this study was to evaluate the potential of serotoninergic and/or noradrenergic drugs to improve the locomotor abilities in the early and late stages. Both cats were implanted chronically with an intrathecal cannula and electromyographic (EMG) electrodes, which allowed determination under similar recording conditions of the locomotor performance pre- and post lesion, and comparisons of the effects of the different drugs. EMG and kinematic analyses show that Noradrenaline (NE) injected in early and plateau-periods improved quadrupedal locomotion. After NE, the regularity of the hindlimb stepping and of the

interlimb coupling increased and permitted the cats to maintain stable locomotion for longer periods of time. Methoxamine, the α1- agonist (tested only at the plateau-period) had similar effects. In contrast, the α2-agonist, Clonidine, deteriorated walking. Serotoninergic drugs, such as the neurotransmitter itself, 5HT, the precursor 5HTP and the agonist Quipazine improved the locomotion by increasing regularity of the hindlimb stepping and by increasing the step cycle duration. In contrast, the 5HT1A agonist DPAT caused foot drag in one of the cats resulting in frequent stumbling. Injection of combination of Methoxamine and Quipazine resulted in maintained, regular stepping with smooth movements and good lateral stability.

Our results show that the effect of drugs can be integrated to the residual voluntary locomotion and improve some of its postural aspects. However, this work shows clearly that the effects of drugs (such as Clonidine) may depend on whether or not the spinal lesion is complete. In a clinical context, this may suggest that different classes of drugs may be used in patients with different types of spinal cord injuries. The possible mechanisms underlying the effect of noradrenergic and serotoninergic drugs on the locomotion in the partial spinal cats are discussed.

Introduction:

Adult cats subjected to a massive bilateral ventral and ventro-lateral spinal lesion at the lowest thoracic level, severely damaging, among others, the reticulospinal and vestibulospinal pathways, can recuperate voluntary quadrupedal locomotion (Brustein and Rossignol, 1998, Rossignol et al. 1996, Gorska et al. 1993a, 1993b Bem et al. 1995). However, they suffered from transient as well as from long lasting postural and locomotor deficits, the severity of which depended on the extent of the spinal lesion. Early after the lesion (early period), none of the cats could walk or support the weight of their hindquarters, and they moved around using only the forelimbs most of the time. Gradually, during the recovery period, which lasted from 3 days and up to 3 weeks depending on the extent of the spinal lesion, the cats regained the ability to support and walk with their hindlimbs as well. However, even when reaching a plateau-period during which there was no further improvement, their quadrupedal stepping overground and on the treadmill was wobbly with poor lateral stability. The coupling phase between the homolateral fore- and hindlimb was highly variable, and, in the most lesioned cats, the fore- and the hindlimbs could walk at different frequencies which caused continual deviations in the coupling phase between the two girdles. These locomotor deficits often resulted in stumbling or falling on one side, which limited the cats to only a few consecutive steps at a time

and to only low treadmill speeds (0.1-0.35m/s). These locomotor deficits were more pronounced during walking uphill and downhill on inclined treadmill (10 degrees), and the amplitude modulation of the hindlimb EMG, usually seen when walking uphill, was absent.

The time course of the recovery and these locomotor deficits were described in detail and the possible adaptive mechanisms to the spinal lesion were discussed in Brustein and Rossignol (1998). The extensively lesioned cats did not improve their locomotor skills very much with time and expressed these deficits even 300 days post lesion. It was suggested that after functional and anatomical reorganization, the remaining pathways in the dorso-lateral funiculi such as the corticospinal and the remaining reticulospinal are sufficient to provide the necessary drive for initiation of locomotion as well as some postural control. However, their contributions are limited since the cats cannot maintain constant fore- and hindlimb coupling or adapt to more demanding situations such as inclined walking.

It is clinically relevant to try and improve the locomotor capacities in this animal model of incomplete spinal lesion, since the percentage of partial spinal lesions in the patients population is increasing (Tator et al. 1993). These patients exhibit residual locomotor capacities but suffer from locomotor and postural deficits such as difficulties in bearing weight, maintaining balance and adapting their walking speed (Barbeau and Rossignol, 1994). In the present study we have applied different

Noradrenergic (NE) and Serotoninergic (5-HT) drugs through a chronic intrathecal cannula to improve the residual voluntary locomotor function . These two groups of drugs were chosen because of their known involvement in initiation and/or modulation of locomotion in complete spinal cats (Barbeau at al 1993, Barbeau and Rossignol 1994, Chau et al 1998a, b)

The specific purpose of this study was then to investigate the effects of noradrenergic and serotoninergic drugs on the treadmill locomotion of cats subjected to extensive but partial spinal lesions of the ventral and ventro-lateral quadrants. We wanted first, to try and express locomotion in the early period after the lesion when there is practically no hindlimb locomotion and second, to improve the locomotor capacities (walking maintenance and regularity, coupling between the fore- and the hindlimbs and speed adaptation) at a later period when voluntary quadrupedal walking is reestablished but is deficient.

Therefore, two cats were implanted with an intrathecal cannula ending caudal to the spinal site of lesion and through which the different drugs were applied. The locomotor activity was compared before and after the drug application using chronically implanted electrodes to identify changes in EMG activity under constant recording conditions in the same cat as well as to follow its daily locomotor capacities during the recovery period and up to the point of achieving a stable locomotor behavior.

Our results show that Noradrenaline, injected early after the lesion, as well as, during the plateau-period, and the α_1-noradrenergic agonist methoxamine (tested only at the plateau-period), increased EMGs amplitude and improved step cycle and interlimb coupling regularity, resulting in a stable and maintained locomotor performance. On the other hand, clonidine (α_2 -adrenergic agonist), early after the lesion as well during the plateau-period caused a deterioration of the locomotor performance manifested in reduced apparent weight support of the hindlimbs, disorganisation of the movement and a decrement of the EMG activity and joint angular excursions. The serotoninergic drugs, 5-HT , 5HTP and Quipazine also improved the walking as reflected by better lateral stability, more regular and increased step cycle and EMG bursts duration, as well as some stabilisation of the fore- and hindlimb coupling. In contrast, injection of the $5HT_{1A}$ agonist, DPAT, caused a pronounced foot drag in one of the cats which impaired its walking abilities. After application of a combination of the α_1- noradrenergic agonist, Methoxamine and the 5-HT agonist Quipazine, cats could walk for a exceptionally prolonged time periods.

Methods

General experimental protocol:

Experiments were carried out on 2 adult cats (EB7 and EB8) weighing 4.1 and 2.8 kg, respectively, which were among the most lesioned cats as detailed in our previous study (Brustein and Rossignol, 1998). The cats were first trained to walk on a treadmill at different speeds (0.2-0.7m/s) until they could maintain a regular locomotion for periods of about 20 minutes. The cats were then anesthetized and implanted with electromyographic (EMG) electrodes and with an intrathecal cannula. Control experiments (n=7-12) were made over a 1 to 6 weeks period to determine the control (intact) EMG and kinematic values. Thereafter, the cats were submitted to the ventral and ventro-lateral spinal lesion at the thoracic level T13 and their locomotor recovery was followed and documented daily as well as long after no further recovery could be observed. Different adrenergic and serotoninergic drugs were tested and their effects on locomotion were followed for several hours and compared with the performance of the cat immediately prior to drug application.

Long after the cat has reached the plateau-period, WGA-HRP was injected caudal (L2) to the site of spinal lesion (at days 340 and 280 post lesion for cat EB7 and EB8, respectively). Then, 3 days later, the cats

were perfused and the spinal site of lesion, the spinal site of WGA-HRP injection, as well as the brain stem and the motor cortices were processed histologically. All the surgical procedures and experimental protocols were reviewed and approved by the University's Ethics Committee.

Surgical procedures:

During the first surgery, performed in sterile conditions and under general anesthesia, a cannula was inserted into the intrathecal (i.t) space of the spinal cord and electromyographic (EMG) electrodes were implanted in various fore- and hindlimbs muscles.

Intrathecal cannula insertion:

A Teflon cannula (Teflon tube- thinwall, size 24 Ga.) was inserted through an opening made in the atlanto-occipital ligament and gradually pushed through the intrathecal space, so that the tip was located at L4-5, evaluated before through external landmarks. The cephalic end of the cannula was inserted into a right-angle plastic port fixed to the skull by dental acrylic, next to the EMG connectors, and served for administration of drugs or sterile saline (0.9%). The inlet was capped to reduce contamination and was opened only at the time of drug or saline application. The dead space of the cannula was measured before the insertion and was determined to be about 100μl. In order to insure its

patency, the cannula was flushed with a bolus of 100µl sterile saline solution (0.9%) on non-experimental days.

EMG implantation:

The procedure was described in detail in a previous publication (Brustein and Rossignol, 1998). Briefly, two 15 pin connectors (TRW Electronic Components Group, Elk Grove Village, IL) were fixed to the skull using dental acrylic. Then, 14 pairs of Teflon- coated stainless steel wires (AS633, Cooner Wire, Chatsworth, CA) soldered to the connectors on previous days were led subcutaneously to the fore- and the hindlimbs and fixed *in situ* by a silk thread in selected muscles. The implanted muscles, listed according to their function, included in the hindlimbs: the hip flexors-*Iliopsoas* (Ip) and *Sartorius* (Srt), the knee flexor- *Semitendinosus* (St) and the ankle flexor-*Tibialis Anterior* (TA), the knee extensor-*Vastus Lateralis* (VL) and the ankle extensors- *Gastrocnemius Medialis* (GM) *and Lateralis* (GL); in the forelimbs: the elbow flexor-*Cleidobrachialis* (CIB) and the elbow extensor (lateral head)- *Triceps Brachii* (TriL). Although all muscles were implanted both on the left (L) and on the right (R) limbs only the left side of the animal facing the video camera was used to illustrate the kinematics.

In addition to implantation of EMG electrodes, two bipolar nerve cuffs were placed around the Superficial Peroneal nerve on each side and were used to stimulate the nerve at rest and during locomotion. However, the results of these measurements are not detailed in this paper.

Ventral and ventro-lateral spinal lesion:

The ventral and ventro-lateral spinal lesion was performed in a second surgical intervention, under general anesthesia, when recordings of the control period were completed. Two small openings were drilled through the vertebral pedicles of the thoracic vertebra (T13) which permitted an approach to the spinal cord laterally on both sides. To avoid damage to the cannula, it's location was first identified and then a microknife and/or a small forceps were inserted through the openings to lesion the ventral half of the spinal cord while leaving the dorsal portion intact and protected by the overlying laminae.

Recordings and data analysis:

EMG activity and the video images used for kinematic analyses were recorded simultaneously. They were synchronized using a SMPTE time code (time code generator Skotel TCG-80N and time code reader TCR-80N), recorded on both analog and video tape.

EMG: During the experiments, the amplified and filtered (100Hz-3kHz) EMG signals were recorded on a VHS tape recorder (Vetter 4000A). These recordings were printed on an electrostatic polygraph (Gould ES-2000) and representative sections chosen for detailed analyses allowing comparisons of locomotion before and after the drug applications. The selected sections were digitized at 1KHz using an AT/486 computer and custom made programs were utilized to determine the onsets and offsets

of the bursts and to calculate their duration and amplitude. The mean burst amplitude was calculated by dividing the rectified and integrated EMG bursts by their duration.

Kinematics: All the experimental sections used for EMG analysis were also processed to define the limb movement and the interlimb coupling pattern.

The joints of the left hindlimb were identified by six reflective markers (3M reflective tape) placed over the skin of the following bony landmarks: the rostral tip of the iliac crest, the femoral head, the knee joint, the lateral malleolus, the metatarsophalangeal (MTP) joint and the tip of the 3rd toe. The camera (Panasonic digital 5100 shutter camera with resolution of 16.7 ms/field) was adjusted to get a clear image of the markers as well as to ease the identification of foot lift and placement for each one of the limbs (shutter between 1/500 and 1/1000). The movements were video taped on a Panasonic AG 7300 recorder, and a 2D PEAK Performance System was used to determine the position (X,Y coordinates) of the reflective markers and to determine the timing of the foot lift and contacts on each successive video fields. The coordinates were used to calculate the joint angular excursions and to reconstruct stick diagrams of several consecutive step cycles. In these diagrams (see Fig. 1B and 1E), the X-axis is respected, which requires adjustments in the Y-axis, explaining why, in some of the illustrated diagrams in which the number of analyzed frames is equal, the vertical Y axis varies . The time of the various

kinematics events, such as paw lift and contact, were used to calculate the step cycle, swing and stance duration as well as to construct foot fall diagrams for each one of the consecutive steps in the walking sequence. The foot fall diagrams were then grouped according to interlimb coupling types and then normalized and averaged. The video tapes were reviewed frequently to get a better overall impression of the drug effects, since the kinematic analyses in one plane gives only a partial assessment of the locomotor improvement. In some cases the cats were also filmed walking from above, to better evaluate the body orientation during walking and its improvement after drug injections (not illustrated).

Drug application:

The drugs tested are listed in Table 1, together with their main action and the doses given. All the drugs were administered to both cats and were generally repeated in at least 3 separate experiments at different days following the partial spinal lesion (also indicated in the right most columns in Table 1). Before drug application, a walking sequence on the treadmill was recorded to determine the pre-drug baseline performance, which varied with time after the lesion (see Brustein and Rossignol, 1998). Then a bolus of 100μl of the drug solution, prepared with sterile saline (0.9%), was administered through the intrathecal cannula using an adapted syringe to fit the inlet. The syringe was fastened to the rostral end of the cannula to avoid leakage and to ensure that the

whole dose would be injected. Immediately after, a second bolus of 100μl sterile saline was injected and served to flush the drug out of the dead space of the cannula. Usually one dose of one drug was given, unless indicated otherwise, and consecutive drug experiments were separated by at least 48 hours. The doses were kept as small as possible to avoid central effects or any other discomfort to the cat. The reaction time to the application of a drug, its maximal effect and the fading time depended on the drugs. In order to cover the whole time course of the effects, recordings started as soon as 20-30 minutes after the application and was repeated each 30-45 minutes untill the drug effects wore off (between 2-5 hours). The results presented illustrate the maximal effects of the drug on any given day. The drugs were applied during the early, recovery and plateau-periods post lesion (see Table 1); however, the illustrate experiments were recorded in the early and the plateau-periods, when the cats exhibited a more or less stable locomotor behavior. The early period lasted almost 4 weeks for cat EB7 and only a week for cat EB8.

----------Insert Table 1 near hear----------

Evaluation of the extent of the lesion:

The histological procedures used to evaluate the extent of the spinal lesion were described in details in Jiang and Drew (1996), Matsuyama and Drew (1997) and in Brustein and Rossignol (1998). Briefly, consecutive cross sections taken from the spinal site of lesion were inspected for physical damage and for cellular necrosis after staining with Kluver-Barrera method. WGA-HRP (2%) was injected caudal to the site of the spinal lesion (usually L2) and labelled cells in the brain stem nuclei and motor cortices were counted and their location was digitized. In the brain stem, the first group of cells corresponds to the pontine reticular formation (PRF) which includes the nucleus reticularis pontis oralis and nucleus reticularis pontis caudalis while the second group corresponds to the medullary reticular formation (MRF) which encompassed the nucleus reticularis gigantocellularis and the nucleus reticularis magnocellularis (adapted from Matsuyama and Drew, 1997). Counts were performed as well in the lateral vestibular nucleus (LVN) and the red nucleus (RN). The values were compared to data from 3 intact cats.

Results

The extent of the spinal lesion and recovery of locomotion:

The results of the histological evaluation of the spinal lesion described in details previously (Brustein and Rossignol, 1998), are summarized for the two cats used in this study, EB7 and EB8, in Fig. 1A and D, respectively.

Briefly, examination of the site of spinal lesion of cat EB7 showed a major disruption of both ventral and ventro-lateral funiculi which is nicely correlated with a major decrease in the number of HRP labelled neurons found in brain stem nuclei corresponding to the origin of reticulospinal pathways descending in these quadrants. Only 19% and 29% HRP labelled cells (expressed as percent of the intact values) were found in the left and right PRF, respectively, while in the MRF only 18% and 21% HRP labelled neurons were found. The lateral vestibulospinal pathway was highly severed as well, resulting in 0.2%, 0.5% labelled cells in the left and right VLN, respectively. Intact tissue could be found only in the dorsal columns and in the left DLF (see cell counts in the right RN and motor cortex).

The lesion of cat EB8 encompassed almost all of the lateral vestibulospinal pathway as indicated by 0% and 0.1% of HRP labelled cells in the left and right VLN, respectively. The reticulospinal pathways

were severed as well, but to a lesser extent compared to cat EB7, resulting in 34% and 31% labelled cells in the MRF and 49% and 24% in the PRF, on the left and on the right, respectively. In cat EB8, the imprint of the cannula on the right DLF is quite noticeable. However, despite of the compression there were no signs of necrosis in the surrounding tissue nor any obvious locomotor deficits as seen in Fig. 1E. Further, the number of HRP labelled cells in the left and right RN are almost similar, while in the left motor cortex, a major increase in the number of HRP labelled cells (140%) was found. Such an increase in the total number of labelled cells was observed as well in the right motor cortex of cat EB7 (192%) which was also accompanied by changes in cells distribution (Brustein et al. 1997). In both cats the changes occurred in the motor cortex contralateral to the less affected DLF.

Despite the extensive lesion to the ventral and ventro-lateral spinal quadrants damaging important reticulo- and vestibulospinal pathways, both cats recovered voluntary quadrupedal locomotion. However, they suffered from long lasting locomotor deficits which their severity depended on the extent of spinal lesion, as illustrated in Fig. 1B to F (B and E, intact; C and F, post-lesion). From the consecutive stick figure diagrams and the angular excursion traces, it is noted that even at about 120 days post lesion in both cats (see Fig. 1C and F for cat EB7 and EB8 respectively), the hindlimb stepping and joint angular trajectories are highly irregular compared to the intact situation (Fig. 1B and E). The cats

suffered as well from poor lateral stability and sudden stumbling which are reflected in abrupt increases of the EMGs bursts amplitude (see LSrt of cat EB7 and RSrt for cat EB8). In addition to these sudden changes in EMG, there were, as well, permanent changes in the amplitude of discharge of some of the muscles (see increase in amplitude of knee flexors of both cats). Moreover, the homolateral fore- and hindlimb coupling was affected as illustrated for the most lesioned cat, EB7, in Fig. 3B and in the phase plots of Fig.11B. The fore-and the hindlimb coupling shows a gradual shift, resulting from different walking rhythms in the fore- and the hindlimbs. For example, in Fig. 1C, 6 bursts were counted in LTriL compared to only 5 bursts in LVL. In cat EB8 the fore- and the hindlimbs stepped at the same frequency and the coupling shifts were in the range of one step cycle duration. The ability to maintain a constant homolateral fore- and hindlimb coupling was permanently deficient for cat EB7 and transient for cat EB8. Cat EB8 not only maintained a more constant interlimb coupling but generally did better long-term post lesion compared to cat EB7. For example, cat EB8 could follow higher treadmill speeds of up to 0.5m/s, while cat EB7 could hardly walk at 0.4m/s and its daily performance was much more variable.

Drug applications:

Noradrenaline (NE) injection early post spinal lesion:

The effects of NE application early after the spinal lesion, are illustrated in Fig. 2 (cat EB7) and the changes in burst duration and amplitude are summarized in Table 2.

As already stated, early after the lesion (a week for cat EB8 and up to 34 days for cat EB7), the cats could not support or walk with their hindlimbs, and they moved around using the forelimbs only. Both cats suffered as well from poor lateral stability which resulted at that stage in difficulty in righting the body. However, when light weight support such as holding the tip of the tail was provided, a few highly disorganized quadrupedal steps could be performed at very low treadmill speeds (0.1-0.2m/s). Such a walking sequence is illustrated for cat EB7 in Fig. 2B. It is noticeable from the consecutive stick figures of the left hindlimb that the cat had difficulty in maintaining an upright position and even when the tail is held, the hindquarters tended to sag. This poor walking is reflected of course in irregular angular excursion traces and the highly disorganized EMG activity. Some episodes of exaggerated EMG activity, such as the one noticed at the end of the second step cycle, result from the cat's attempts to recover from a stumble. The disorganized stepping pattern of the hindlimbs is further demonstrated by the foot fall diagram. While the left hindlimb performed large steps, the right hindlimb performed short ones. The coupling between the fore- and the hindlimbs was also highly

perturbed; while the hindlimbs performed 3 consecutive steps the forelimbs performed 5, indicating that the hindlimbs and the forelimbs walked at different frequencies of about 0.5Hz and 1Hz, respectively.

Twenty minutes after the injection of a 3rd bolus of 1.6mM/100µl NE (Fig. 2C) there was a major improvement in the stepping pattern, which became more regular and sustained, although the experimenter still needed to help the cat by lightly holding the tail. The improvement in the walking is evident from the stick figure diagram, showing 5 regular consecutive steps of the left hindlimb at a treadmill speed of 0.4m/s. The angular displacement is much improved in all joints relative to the pre-drug condition and it is somewhat exaggerated compared to the intact pattern in Fig. 2A. For example, the range of angular displacement in the ankle after the NE application is between 142±4 and 83±5 (degrees±SE), compared 134±4 to 98±3 in the intact situation. This pronounced increase in angular displacement is accompanied by a marked increase in the normalized EMG amplitude of the hindlimb muscles (see Table 2), especially of the knee flexors (see Fig. 2C). For example, after the NE application, the amplitude of LSt showed an increase of 584%±22 from the intact values, compared to 211%±18 measured before injecting the drug. LGM, which was almost non detectable pre-drug (except in falling episodes) was increased to 252%±35 of intact values after NE application. There was also a stabilizing effect on the fore- and hindlimb coupling pattern reflected by better organized foot fall patterns for all 4

limbs. However, it should be noted that there is still a mismatch, although smaller, between the number of steps performed by the hindlimbs and the forelimbs (the walking frequencies in the fore- and the hindlimb are; 1.3Hz and 1.1Hz respectively), which again results in a shift of the coupling phase between the two girdles (Fig. 2C).

The effects of NE application early after the spinal lesion on cat EB8 locomotion, were similar to those observed in cat EB7 and resulted in an improvement of the regularity, the ability to walk for a longer period of time at a given speed and a better adaptation to speed changes. This is nicely reflected in the step cycle duration of the fore- and the hindlimbs calculated from the EMG activity of the LTriL and LVL, respectively. Pre-drug, the cycle duration at 0.1m/s did not differ significantly in the fore- and the hindlimbs; however they were highly variable (variance ratio test, $p<0.01$); 1239±223 and 1554±635 ms (average±SD), respectively. Post NE, the cycle duration (0.4m/s) in the fore- and the hindlimbs showed a better match and a much reduced variability; 852±63 and 854±69 ms, respectively. Changes were observed in the average angular ranges of the movement of all joints. However, they were less pronounced compared to those observed in cat EB7. After NE application, the average range of hip angular excursion showed an increase from 21 degrees pre-drug to 30 degrees, and in the knee an increase of 8 degrees was observed. In the ankle and MTP joints, the range of movement increased only slightly (3 degrees). No major pre- and post-

drug changes were observed in the normalized amplitude of the hindlimb EMG (see Table 2), and their durations showed variable tendencies. Prolongation relative to the pre-drug situation was observed in the LSt duration while LSrt and the RGM decreased significantly ($p<0.01$).

----------insert Table 2 near here----------

Application of NE during the plateau-period:

The injection of NE in cat EB7 was further repeated during the plateau-period. A representative example for such an experiment is given in Fig. 3 and summarized in Table 3 (this experiment was not done in cat EB8).

Pre-drug (Fig. 3B), the cat could follow a treadmill speed of 0.3m/s. However, compared to the intact state (Fig. 3A), its walking pattern was irregular as reflected in the abrupt changes in the joint angular traces and the variability in the EMGs burst duration and amplitude. The coupling between the fore- and the hindlimbs was variable as well, as illustrated by the dashed lines connecting the foot falls of the homolateral limbs. As with application of NE early after the lesion (Fig. 2), NE improved locomotion also when given during the plateau-period. However, there were some differences between NE effects in both periods. In the two situations the stepping of the hindlimbs became much more regular as noted in the foot

fall diagrams and in smoother angular excursion traces (Fig. 3C). However, during the plateau-period, NE effects on angular excursion were more limited and an increase is observed only in the angular excursion range of the knee, from 12 degrees pre-drug to 34 degrees post NE. As with NE injection early after the lesion, here as well, there was a general increase in the EMG amplitude (see Table 3), especially noticeable in the extensors, compared to a more pronounced increase in knee flexors activity early after the lesion. The increase in EMG activity is accompanied by some decrease in the bursts duration compared to the pre-drug situation. However, these changes were less pronounced compared to NE effects early after the lesion. The fore-and hindlimbs coupling improved considerably in both situations. However, NE injection during the plateau-period stabilized both the coupling pattern and the phase drifts between the homolateral fore- and the hindlimbs to a pattern which resembles the intact one (Fig. 3A), as illustrated by the vertical lines in the foot falls diagrams (Fig. 3C).

----------insert Table 3 near here----------

Clonidine:

In light of the improvement in the walking seen after injection of NE, we have further tested the α_2 -noradrenergic agonist, Clonidine, which is implicated in initiation and modulation of locomotion in the completely

transected spinal cats (For review see Rossignol, 1996). However, the effects of i.t injection of Clonidine early after these partial spinal lesions were quite different from those observed in the complete spinal cat and were also different from the effects of NE in the partial spinal cat at that stage (see Fig. 2). Not only there was no improvement in the walking, but on the contrary, Clonidine appeared to deteriorate the locomotion capabilities of the cats. However, since the pre-drug pattern was highly deficient (see Fig. 2B) it was difficult to assess the changes qualitatively. In the plateau-period, the adverse effect of Clonidine was very obvious in both cats. Then, even one bolus of Clonidine (0.8-1.9mM/100μl) was sufficient to cause a major reduction in the weight support of the hindlimbs, increase wobbliness, stumbling or falling, which limited the cats to a few steps at a time at very low treadmill speeds (0.2m/s). An example of the effects of Clonidine on the walking of cat EB8 is illustrated in Fig. 4. Before the injection of Clonidine (Fig. 4A), the cat could walk rather nicely at a treadmill speed of 0.4m/s. Thirty minutes after injection of Clonidine (1.9mM/100μl) the gait became very wobbly and after a few highly irregular steps, the cat stumbled (Fig. 4B). The time it took the cat to regain an upright position is indicated by the dotted line and the empty space in the stick figure diagram. During that section, it was not possible to digitize the position of the reflective markers. Notice that even when it regained an upright position it had major problems supporting the hindquarters and maintaining walking. This is also reflected in the EMG

pattern (Fig. 4B) which shows an increased variability (see as well histograms in Fig. 6 C and D) . The effects of Clonidine on the locomotion of cat EB7 are illustrated in Fig. 5B. Fourty-five minutes after injection of clonidine (1.9mM/100μl) the cat can hardly support its hindquarters as illustrated by the stick figure diagram and can hardly step as seen as well from the disorganized angular traces and the EMG pattern. The amplitude of the EMGs decreased relative to the pre-drug situation, a change which is especially noticeable in LSt and in the left and right extensors, GM (see as well related histograms in Fig. 6C). These detrimental effects were long-lasting, and a return to the pre-drug walking capacities was seen only the day after. The effect of Clonidine could be mostly reversed by an injection of Yohimbine (α_2 -noradrenergic antagonist) as illustrated in Fig. 5C for cat EB7. Sixty minutes post injection of one bolus of 20.5mM/100μl Yohimbine, given 45 minutes post injection of Clonidine (1.9mM/100μl), the weight support of the hindquarters improved, the regularity of the stepping increased and the cat achieved better walking speeds, of up to 0.3m/s. The quantitative effects on the burst durations and on EMG amplitudes are summarized in histograms of Fig. 6 (E-F and G-H for cat EB7 and EB8 respectively).

Methoxamine:

Since there was a major locomotor improvement after injection of NE, but none with the α_2 -noradrenergic agonist, Clonidine, the effects of an α_1 -noradrenergic agonist, Methoxamine, were tested. It should be noted that Methoxamine was applied only during the plateau-period. The results of such experiments are illustrated in Figs. 7 and 8 and the EMG values are summarized in Table 4.

Methoxamine highly improved the locomotion of cat EB7. An hour and 30 minutes after application of 4mM/100µl Methoxamine, the stepping was forceful and the foot stumped on the treadmill. The steps were more saccadic, regular and performed with a better lateral stability. The regularity of the steps is clearly noted when comparing the consecutive stick figure diagrams pre- (Fig. 7B) and post Methoxamine (Fig. 7C). The related angular excursion traces show that the movements of the joints are smoother and reproducible from cycle to cycle, because all the abrupt changes characteristic of the pre-drug walking disappeared. The average range of movement at the hip, ankle and MTP joints decreased after the application of Methoxamine (from 20 degrees down to 14, 33 to 22 and from 57 to 44 degrees, respectively) while a slighter decrease was observed in the range of knee movement (from 27 to 23 degrees). The activity of the hindlimb muscles markedly changed and a general increase in the normalized amplitude comparing to the pre-drug situation was

noted (see especially RGM). In addition, a significant increase ($p<0.01$) in the duration of LSt was observed (see Table 4).

After the administration of Methoxamine the cat did not improve its speed performance. However, there was a major improvement in the ability of the cat to maintain locomotion at the same speed range for longer periods of time. Pre-drug, the cat could make about 6 consecutive steps, while after application of Methoxamine it did 20 steps.

The coupling between the homolateral fore-and hindlimb was changed as well after the drug application, as illustrated in Fig. 8. The graphs of the phase of burst onset of LTriL and LVL (relative to RSt) show that, pre-drug, the coupling phase between the homolateral fore- and hindlimb varied between 0.4-0.8, which corresponded to a diagonal type of coupling, illustrated in the related average foot fall diagram. Post Methoxamine, the phase of coupling closely resembled to the intact range (Fig. 8A) and it fluctuated around the 0.25. The related foot fall diagrams show that the coupling pattern used by the cat had changed. However, in contrast to the intact coupling pattern, the forelimbs were leading. The general improvement in the locomotion after Methoxamine was long lasting and could be observed for 4-5 hours.

Methoxamine improved the locomotion of cat EB8 as well (see Table 4). As with cat EB7 the regularity of the hindlimbs stepping improved as indicated by a decreased variability of the hindlimb step cycle duration (variance ratio test, $p<0.01$). There was a stabilization of

the coupling between the homolateral fore-and hindlimbs, around a phase value of 0.2, as generally observed in the intact situation and a decrease in the average range of angular displacement, which affected the hip, knee and ankle joints (from 39 down to 33 degrees, from 38 to 26 and from 30 to 19 degrees, respectively). However, the EMGs decreased somewhat in contrast to that observed in cat EB7 (see Table 4).

----------insert Table 4 near here----------

Serotoninergic drugs:

Several serotoninergic drugs were tested :, the neurotransmitter itself, 5HT, the $5HT_{1,2,3}$ agonist, Quipazine, the 5HT precursor, 5HTP, and a $5HT_{1A}$ agonist ,DPAT. All these drugs, except the antagonist DPAT, improved locomotion in a similar manner. They differed mainly in their time of action; 5HT and 5HTP did not last long (< 2 hours) whereas the effects of Quipazine could be observed for up to 5 hours after application. DPAT caused a detrimental effect on locomotion of EB7 by inducing, as soon as 20 minutes post application, a severe foot drag of both hindlimbs causing the cat to stumble very often. This effect, however faded away after one hour.

A representative example for the effects of 5HTP application on the overall kinematics of locomotion is given for cat EB8 in Fig. 9. The effects on the EMGs are summarized in Table 5. 5HTP improved the lateral

stability and the regularity of the walking. The stick figure diagram (Fig. 9C) illustrates a prolongation of the cycle duration (see Table 5). However the average values were not significantly different (see Table 5). The effects of 5HTP did not last long and more pronounced effects were observed after application of the 5HT agonist, Quipazine.

A representative example for the effects of Quipazine for cat EB7 is given in Fig. 10 and 11 and summarized in Table 5. Pre-drug, the cat's walking was irregular as illustrated in the stick figure diagram (Fig. 10B) showing 5 consecutive steps, each of a different duration. In addition, the angular movement of the joints was variable and included abrupt, sudden changes, noted in the related angular excursion traces. The raw EMGs reflect this disorganized walking as indicated by sudden increases in the EMG activity such as the one at the end of the first LGM burst. The EMG activity of the different muscles not only varied in amplitude and duration but also in their time course (see especially right and left GM).

After injection of Quipazine (1.2mM/100μl) the walking of the cat was highly improved. It showed better lateral stability and it could take long regular steps at a constant speed of 0.3m/s. Pre-drug, the cycle duration was 986±273 ms while after Quipazine it was 1200±109 ms ($p<0.05$) which was, however, still shorter than the intact step cycle duration at 0.3m/s (1372±62 ms). The regularity and the stability of the stepping is also noted in the angular excursion traces, which in addition show a major increase in the average range of angular excursion of the

hip, knee and ankle pre- and post-drug; from 17 to 27 degrees, 21 to 29 degrees and from 19 to 28 degrees , respectively. The values of angular excursion, post Quipazine, resemble more the intact values (26 in the hip, 32 in the knee and 36 in the ankle). No change was observed in the average range of angular excursion of the MTP joint which was kept reduced relative to the intact.

The EMG activity reflected the increase in regularity and prolongation of the steps. There was a general tendency for an increase in the burst duration relative to the pre-drug situation (see Table 5), especially noted in the extensor duration which was significantly longer even compared to the intact situation ($p<0.01$).

Quipazine stabilized the interlimb coupling as well (Fig. 11). Fig. 11B shows variable relations in the phase coupling values between the fore- and the hindlimbs, with episodes in which the forelimbs precedes the hindlimbs (see negative values of LTriL). This variability is expressed as well in a continual transition between 3 main types of coupling patterns. These are illustrated in the related foot fall diagrams. The upper one shows one step cycle with homolateral in-phase coupling, the middle one shows an average of 3 steps taken with almost a normal coupling pattern (see average foot fall diagram of the intact situation in Fig. 11A) and the foot fall diagram at the bottom is the average of 3 steps taken with diagonal coupling. Post Quipazine, the cat was walking constantly with an homolateral, in-phase, coupling pattern which is illustrated in the

related foot fall diagram, averaging 13 consecutive steps. However this more robust coupling pattern seemed to be only a result of improved hindlimb stepping regularity (variance ratio test, $p<0.01$) and not a result of tighter coupling relations between the fore- and the hindlimbs, as even post-drug a constant drift in the phase difference between the limbs persisted (see phase plot in Fig. 11C.)

----------insert Table 5 near here----------

Combination of Methoxamine and Quipazine:

As detailed before, both Methoxamine and Quipazine improved locomotion. However, the first drug increased the amplitude of the EMGs, decreased the cycle duration and the joint angular excursion, while the later drug prolonged the step cycle duration. Both drugs were given at the same time in one bolus. The results of such an experiment are illustrated in Fig. 12 and 13. In Fig. 12, only the pre- and post-drug situations are compared because, walking at speed of 0.35m/s was not tested. in the intact situation.

Pre-drug, the stepping of the cat was rather nice as illustrated by the consecutive stick figure diagram, the related angular displacement traces and the raw EMGs in Fig. 12A. However the cat could not perform more than 10 consecutive steps at that speed (0.35m/s).

Sixty-five minutes post application of one bolus of the combined solution, which contained only half the doses(2mM Methoxamine+ 1.2mM Quipazine/100μl) used when each one of the drugs was tested separately, an elegant and maintained walking was observed, with much improved lateral stability . The stepping was characterized by somewhat shorter steps (966±32 ms pre-drug to 896±102 ms post-drug), which was accompanied by a slight reduction in the average range of the angular displacement. The EMG duration and amplitude showed various changes as summarized in Fig. 14 (see histograms A and B).

The improvement in the maintenance of the walking is illustrated in Fig. 13 by plotting the step cycle duration of the whole consecutive stepping sequence performed during one recording session. Pre-drug, the cat is walking up to 0.35m/s. However it had major difficulty maintaining this speed for more than 10 cycles, and after several steps in which it touched the back of the treadmill, the speed was decreased to 0.3m/s.. Notice as well that the walking was irregular as reflected by step to step fluctuations in the cycle duration. After application of the drug combination, the speed range the cat could follow was up to 0.4m/s, which it could maintain regularly as indicated by the reduced variability of the step cycle duration, which resembles the intact situation. Notice however that in the intact situation, walking at 0.3m/s was somewhat too slow for this cat and this was expressed in higher step cycle variability.

Cat EB8 also showed an improvement in the walking after the application of the combined solution which was accompanied by an increase in the step cycle duration calculated from the LVL; 829± 82 ms and 910±83 ms pre- and post-drug, respectively ($p<0.05$). Changes were observed in the average range of angular excursion. The hip, ankle and MTP movement range decreased (29 to 23, 29 to 21 and 48 to 43 degrees pre and post-drugs, respectively). Significant increases was found in the amplitudes of all the flexors (see Histograms C and D in Fig. 14) e the extensor amplitudes did not change, but their duration was significantly increased ($p<0.01$) compared to the pre-drug situation.

Discussion:

In this work, it was shown that different noradrenergic and serotoninergic drugs can improve or deteriorate the locomotion of cats with partial but large ventral and ventro-lateral lesions to the spinal cord. In the discussion, the extent of the spinal lesion of different descending pathways with the implications for the descending noradrenergic and serotoninergic tracts will first be described and then, the results of intrathecal drug application in the partially spinal cat will be discussed and compared to their known effects on the locomotion of the complete spinal cat. Different possible mechanisms by which the drugs may exert their effects will be suggested, as well their clinical implication.

The extent of the spinal lesions in relation to descending noradrenergic and serotoninergic pathways:

The extent of the lesions to noradrenergic and serotoninergic pathways after ventral and ventro-lateral spinal lesions at the thoracic level, sparing to different degrees the dorsolateral funiculi, could only be evaluated indirectly. An estimation of the effect of the spinal lesion to these pathways could be given by comparing the results of the examination of the spinal site of lesion and of WGA-HRP labelled cells counts in the brain stem nuclei and motor cortex to what is known from

the literature about the noradrenergic and serotoninergic pathways in the spinal cord.

Our results show that cat EB7 was submitted to the most extensive spinal lesion (T13) as clearly noted from the physical damage at the spinal site of lesion. The low numbers of HRP labelled cells in the PRF and especially in the LVN confirm extensive damage to the pontine reticulospinal pathway and to the lateral vestibulospinal pathway, which have axons descending in adjacent regions in the ventral spinal quadrants (Brodal, 1969, Kuypers, 1981). The major decrease in the HRP labelled cells in the MRF confirms the severe damage to medullary reticulospinal pathways which descends in the lateral funiculi (Brodal, 1969, Kuypers, 1981, Petras, 1967, Kuypers and Maisky, 1975, 1977). The integrity of the dorsolateral funiculi was evaluated by cell counts in the RN and motor cortex. On the right RN, 58% HRP labelled cells were found, compared to only 4% on the contralateral side, implying that the left DLF contained relatively a larger number of intact axons. This is supported by the two fold increase of the number of HRP labeled cells in the right motor cortex, a phenomenon which might correspond to plastic changes such as sprouting (for discussion see Brustein and Rossignol, 1998). In summary then, according to the inspection of the site of spinal lesion and the number of HRP labelled cells in the brainstem, the lesion of cat EB7 encompasses the ventral and most of the ventro-lateral

quadrants on both sides, sparing axons mostly in the dorsal columns and in the left DLF.

Cat EB8 was subjected to a severe spinal lesion; however, as noted from the HRP labelled cell counts and from the relatively quick locomotor recovery, it was less pronounced compared to cat EB7. HRP cell counts in the LVN show that the vestibulospinal tract is mostly damaged. However, cell counts in the PRF show asymmetric damage to adjacent regions in the ventral funiculi. Severe damage was observed to the ventro-lateral funiculi as the medullary reticulospinal pathways on both sides were largely damaged. The number of labelled axons in both red nuclei indicates relatively numerous intact axons on both DLF (55%-66%). However the number of HRP-labelled cells in the right motor cortex indicate that the left corticospinal tract was rather damaged (28%), while the cell counts in the contralateral motor cortex showed an increase to 140% in the HRP labelled cells. In summary, these findings show that in cat EB8 the lesion to the ventral quadrants was extensive but somewhat asymmetrical and that the lateral funiculi were damaged considerably on both sides. The DLF on the right contained larger numbers of intact axons compared to the left one.

Retrograde histofluorescence and HRP studies show that noradrenergic pathways originating in nucleus subcoeruleus, Kolliker-Fuse and cell bodies in A5, send fibers through both the ventro-lateral and the dorsolateral funiculi, while nucleus locus coeruleus, which is the

main source of spinal noradrenergic fibers, projects primarily through the ventro-lateral funiculus. The noradrenergic fibers innervate the lumbar spinal cord bilaterally, although the ipsilateral contribution dominates (Stevens et al. 1985, Kuypers and Maisky, 1977, Kuypers, 1981, Marshall, 1983). The serotoninergic axons originating in the raphe nuclei such as raphe pallidus and obscurus descend mainly in the ventral and ventro-lateral funiculi while axons from raphe magnus descends primarily through the DLF (Martin et al. 1978, Kuypers, 1981).

In reviewing these findings, it could be suggested that most of the noradrenergic and the serotoninergic axons passing in the ventral and ventro-lateral quadrants of cat EB7 were probably damaged while the ones passing in the left DLF were mostly preserved. In cat EB8, however, the existence of serotoninergic axons in the left ventral quadrant cannot be excluded. However, extensive damage was probably done to serotoninergic and noradrenergic fibers passing in the lateral funiculi.

Despite the extensive spinal lesions observed in both cats to the important descending pathways such as the reticulospinal, vestibulospinal pathways and which probably encompassing as well the noradrenergic and serotoninergic pathways, both cats recuperated voluntary quadrupedal locomotion. However, both cats suffered from poor lateral stability, irregular stepping and problems in maintaining constant interlimb coupling. The extent of these locomotor deficits is correlated with the extent of the spinal lesion. The less lesioned cat , EB8, recovered more

quickly and exhibited better locomotor performance long-term post lesion. These observations may explain why generally larger doses of the tested drugs were given in order to see effects in cat EB8, long-term post lesion (see Table 1). However, the effects of all the applied drugs show similarities in both cats, in a way that drugs such as Clonidine had a detrimental effect in both cats and drugs that caused a pronounced improvement in the walking of cat EB7, may have caused more subtle effects on the walking of cat EB8, but they were never detrimental.

Injection of noradrenergic drugs:

Summary of the effect of noradrenergic drugs injection in the partial spinal cat:

Injection of the neurotransmitter itself, NE, early after the lesion, was highly effective in cat EB7 and in cat EB8. Both cats showed major improvement in speed performance and in stepping regularity accompanied by better joint angular movement and a better homolateral fore- and hindlimb coupling. These effects were more pronounced in cat EB7, compared to cat EB8, probably due to the differences in the extent of the spinal lesion. In both cats, , however, NE improve neither the weight support nor the lateral stability of the hindlimb to the point that they could walk quadrupedally on their own. The improvements in stepping regularity and in fore- and hindlimb coupling were also observed after application of NE long-term post lesion, during the plateau-period (tested in cat EB7 only) . At that stage the effects on angular displacement were

more limited and a more pronounced increase was observed in extensor activity, accompanied by a general decrease in the burst duration. The latter effects resembled more the observed after application of α_1 -agonist, Methoxamine. The pronounced increase in extensor amplitude may explain the improvement in weight support and in lateral stability. However, in contrast to NE at that stage, Methoxamine also caused a general decrease in the joint angular excursion, which may explain why despite the increased regularity and better weight support there was no improvement in the speed performance. The regularity of the hindlimb stepping, better stability and interlimb coupling can, however, explain better walking maintenance at the same speeds. It is interesting to note that cat EB8 showed a better regularity and a decrease in angular displacement after Methoxamine, as did cat EB7, but without major effects on the amplitude of activity of the recorded muscles.

In contrast to the locomotor improvement seen after NE and the α_1 -agonist, Methoxamine, the α_2-agonist ,Clonidine caused deterioration of the walking in both cats. There was a major decrease in weight support and in lateral stability of the hindquarters which consequently resulted in irregularity of the stepping, a decrease in the speed that the cat could maintain.

Taken together, the deterioration of locomotion after Clonidine and the pronounced improvement after Methoxamine may explain why NE has less pronounced effects compared to Methoxamine alone, as NE

exerts a combined effect on locomotion through activation of both the α_2 and $\alpha 1$ noradrenergic receptors.

Comparing the effects of noradrenergic drugs in cats with complete and partial spinal lesions:

Acute spinal cats: The involvement of the noradrenergic system in locomotion of the acute paralyzed complete spinal cat was demonstrated by using the noradrenergic precursor L-DOPA (Jankowska et al. 1967a ,1967b). In the presence of L-DOPA, stimulation of flexor reflex afferents (FRA) released a long latency and long duration alternating discharge in flexors and extensors nerves, as well as in interneurons, suggesting that L-DOPA could affect neurons implicated in the generation of spinal locomotor rhythmicity by releasing Noradrenaline from terminals of descending noradrenergic pathways (Anden et al. 1966a). When given after Nialamide, a monoamine oxidase inhibitor, L-DOPA was shown to evoke a detailed alternating pattern recorded in muscles nerves of fictive acute spinal cat with similarities to the normal pattern (Grillner and Zangger, 1979). It was shown as well that, i.v. injection of L-DOPA or of the α_2 noradrenergic agonist, Clonidine, in the acute spinal cat, improved weight support and allowed locomotion of the hindlimbs on a treadmill (Forssberg and Grillner, 1973). The direct involvement of NE was demonstrated by administration of NE itself through an intrathecal cannula which, like L-DOPA, evoked long latency reflexes following

stimulation of high threshold afferents. Intrathecal application of NE to the spinal cord also inducined and maintained locomotion (Kiehn et al. 1992).

Chronic spinal cats: In the early chronic spinal cats, Clonidine injected i.p. or i.t. was found to initiate hindlimb locomotion (Barbeau and Rossignol, 1991, Barbeau et al. 1993, Chau et al. 1998b). NE itself was reported to initiate locomotion but had different effects on the stepping characteristics compared to Clonidine. Methoxamine as well could induce locomotion at that stage but the locomotion was not as sustained as with Clonidine (Chau et al. 1998a). The partial spinal cat, in contrast to the complete spinal cat could initiate hindlimb stepping voluntarily even at early stage post lesion. However quadrupedal locomotion could not be expressed because of major problems in supporting the weight of the hindquarters and because of poor lateral stability. Such an observation was reported as well after depletion of both NE and 5HT from the brain stem and spinal cord in otherwise intact cats, which showed severe ataxia (Steeves et al. 1980). Any attempt by these cats to walk resulted in loosing balance and falling. These cats could, however, show stepping movements when laying on their sides. Their ability to initiate locomotion was further supported by the finding that MLR-evoked locomotion was not abolished after such treatment (Steeves et al. 1980). NE was not sufficient to improve the postural deficits observed in the partial spinal cats early after the lesion, probably because its effects on extensors was less pronounced compared to its effects on flexors. Such a differential

effect on muscle activity accompanied by a knee sag was reported as well after Clonidine application in the complete spinal cats (Chau et al. 1998b). However NE application early after the lesion improved the rhythmicity and the regularity of the walking, much like that observed in the complete spinal cat. Long-term post lesion, during the plateau-period, NE as well as Methoxamine markedly increased, the extensor activity. Such improvement in muscle tonus was also observed after Methoxamine in the late complete spinal cats (Chau et al. 1998b). However this was not accompanied by changes in the angular displacement or in the step cycle duration, in contrast to the effect of Methoxamine in the partially spinal cats, which reduced the angular displacement and bursts duration.

Proposed mechanisms for the drugs action:

The improvement in locomotion of the partial spinal cat after NE and Methoxamine injections can be attributed to global changes in spinal neuron excitability (Grillner, 1981). According to this suggestion, the slight hyperpolarization caused by NE, which results in a decrease in membrane conductance, will cause a general potentiation of 25 to 50% of other non-NE synaptic input. Possible participation of noradrenergic drugs in mechanisms of gain amplification was suggested as well by Hounsgaard et al (1988) and by Conway et al. (1988). L-DOPA and Clonidine were found to induce plateau potentials in spinal MN of acute complete spinal cats (Conway et al. 1988); as does Methoxamine in the

decerebrate cat preparation (Lee and Heckman, 1996, Lee and Heckman, 1997). The appearance of plateau potentials during locomotion, initiated by L-DOPA in the fictive spinal cat (Schomburg and Steffens, 1996), was proposed as a mechanism which will reduce the need for sustained synaptic drive from the rhythm generator during locomotion, by maintaining excitability at a constant level, so that only short lasting excitation or terminating inhibition is needed to start or stop the activity in the MNs. This may explain as well the major improvement in regularity of the walking after Methoxamine and NE in both cats, even without major changes in EMG amplitudes as observed in cat EB8. NE and Methoxamine probably provided a constant background excitability on which the signals from the rhythm generator could be expressed reliably and consistently.

In contrast to NE and Methoxamine, application of Clonidine in the partially spinal cat had a detrimental effect on the walking. This is in some contrast to the observations in late complete spinal cats (Barbeau et al. 1993, Chau et al. 1998), in which small doses (1μg/100μl) of Clonidine given i.t, modulated the stabilized spinal hindlimb walking, to increase the step length , joint angular displacement and the amplitude and duration of flexors. However larger dose (10μg/100μl i.t) of Clonidine in the late spinal cat, decreased the amplitude of the extensors, reduced the weight support of the hindlimbs and induced a pronounced foot drag much like in the partial spinal cat. For the partially spinal cat these effects were

much more severe since the cats could hardly continue walking quadrupedally. The detrimental effect of Clonidine on the walking of partial spinal cats, its beneficial effects in the early spinal cats and, its dose dependent effects in late complete spinal cats could be explained by a difference in the α_2 receptor population remaining in each condition. After a complete spinal transection, there is a removal of the central presynaptic α_2 receptors, and the effects of the drugs are mainly due to activation of the remaining α_2 postsynaptic receptors (Langer, 1977). In the partially spinal cats, some presynaptic α_2 receptors must be present on remaining descending adrenergic terminals, mainly in the dorsal horn where they are mostly concentrated (Giroux et al. 1995). Thus, in the partial spinal cat, application of Clonidine will affect both pre- and postsynaptic receptors. Activation of α_2 presynaptic receptors results in a decrease in NE release from noradrenergic terminals (Marshall, 1983). Not only is a decrease in the transmitter liberation from of central NE terminals is expected but also a significant decrease in the transmission from primary sensory afferents which were found to contain up to 20% of the spinal cord presynaptic α_2 receptors (Howe at al 1987). In addition, it is possible that the number of α_2 presynaptic receptors might be even larger after the partial lesion, a result of sprouting of primary afferents as observed in the cat dorsal root ganglion after hemisection (Helgren and Goldberger, 1993). Such plastic changes could be observed in the corticospinal pathways of these cats, as already mentioned before (Jiang

and Drew, 1996, Brustein and Rossignol, 1998, Brustein et al. 1997). L-DOPA, the noradrenergic precursor, is known to depress activity from primary afferents but not of group I (Anden et al. 1966b). The local application of NE and NE agonists was found as well to depress transmission in interneuronal pathways from group II afferents (Jordan et al. 1977, Bras et al. 1989, Bras et al. 1990) an effect which was observed in populations of neurons at L4-5, where the tip of the intrathecal cannula is located in both cats. It is interesting to note that the depressive effect was primarily exerted by α_2 agonists such as Tizanidine. The depressive effect of descending systems on interneuronal pathways which mediate the reflex action of group II afferents on MN was further demonstrated by stimulation of the locus coeruleus and subcoeruleus of decerebrated, otherwise intact cat (Jankowska et al. 1993), suggesting differential gating effects of peripheral afferents by the noradrenergic system. Such differential effects of peripheral input was proposed (Bras et al. 1989) to transform neurons receiving both group II and group I input, to neurons processing information related to the dynamic aspects of the movement. It was further reported that L-DOPA reduced spasticity in paraplegic patients (Eriksson et al. 1996). The above mechanisms may not be beneficial for the partial spinal cat, as a reduction in the transmission from the periphery may interfere with one of the compensatory mechanisms the cat may develop post lesion to maintain weight support (Brustein and Rossignol, 1998, Grillner, 1972, Guertin et al. 1995) and on

which it relies as well for locomotor feedback after large damage to descending and ascending pathways.

Another important aspect is the effect of NE and Methoxamine on the coupling between the fore-and the hindlimbs. In the complete spinal cat, fore- and hindlimb coupling does not obviously recover, while the partial spinal cat recovers voluntary quadrupedal locomotion. However, it is highly irregular, as illustrated by variations in the coupling phase between the homolateral fore- and the hindlimbs. In the most lesioned cat such as cat EB7, it was accompanied by different walking frequencies of the two girdles as well. Methoxamine and NE both stabilized these locomotor aspects. As discussed earlier, NE and Methoxamine may contribute to improve the depolarizing background activity on which the signals from the rhythm generator can be expressed consistently. Stabilization of the hindlimb walking pattern will result in a generally more organized quadrupedal coupling,. It is possible, as well, that the observed improvement in the hindlimb locomotion results in a more consistent information flow between the two girdles through the remaining ascending and descending spinal pathways to better integrate their activity. A modulatory action (facilitatory and inhibitory) of NE was observed on ascending information from muscle and skin afferents, information which is important to the control and planning of movement (Jankowska et al. 1997). In the case of the partial spinal cat an inhibitory effect may reduce excess and variable information ascending to higher centers, resulting in

a more constant feedback through the remaining descending pathways. On the other hand, facilitation observed in ascending systems such as the dorsal spinocerebellar tract is suggested to increase the number of transferred nerve impulses and also to increase synchronization of these signals by reducing the range of their latencies to half. In both cases, facilitation or depression, the total outcome of NE action may contribute to stabilization and improvement of the locomotion as seen in the partial spinal cat.

Injection of serotoninergic drugs:

Summary of the results of serotoninergic drugs in the partial spinal cat:

Serotonin (5HT), the neurotransmitter itself, its precursor, 5HTP and Quipazine a $5HT_{1,2,3}$ agonist, improved lateral stability and weight support of the hindlimbs, which was reflected in a more regular walking and a more stable interlimb coupling pattern. Here again the locomotor improvement was more pronounced in the more deficient cat EB7. In addition the serotoninergic drugs seemed to prolong the step cycle duration, in both cats. This was accompanied by an increase in flexor and a slighter increase in the extensor burst duration in cat EB8 and a significant increase in extensor burst duration of cat EB7, as well as slight decrease in the normalized EMG amplitude. These effects were different, especially in cat EB7, from the ones observed after application

of NE or Methoxamine which decreased the step cycle duration, the joint angular displacement and increased EMG amplitudes of flexors and extensors.

The $5HT_{1A}$ agonist, DPAT, had a detrimental effect on the locomotion of cat EB7 as a result of a severe foot drag in both hindlimbs, which caused the cat to stumble often. However, compared to Clonidine, this effect was short-lasting and was not accompanied by an apparent decrease of the weight support of the hindlimbs or an increased wobbliness.

The effects of Serotoninergic drugs after partial spinal lesion in comparison to the complete spinal cat:

In contrast to NE and Clonidine, the 5HT precursor, 5HTP, was not found to be effective in evoking locomotor rhythm neither in the low spinal decerebrate cat, (Grillner and Shik, 1973), nor in the complete chronic spinal cat during the first week after spinalization, while Clonidine or L-DOPA caused a dramatic change in kinematics and EMG pattern (Barbeau and Rossignol, 1990, Barbeau and Rossignol, 1991, Barbeau et al. 1993). However, in both preparations, there was a marked increase in the tonic activity in all muscles (Grillner and Shik, 1973)) or at least in the flexor activity when walking on the treadmill (Barbeau and Rossignol, 1991). A marked increase in the amplitude of extensor and flexor muscles as well as in their duration was observed in the chronic late spinal cat after i.p. injection of 5HT precursor, 5-HTP and of Quipazine and 5-

MeODMT, which are 5HT agonists (Barbeau and Rossignol, 1990, Barbeau and Rossignol, 1991). In light of these results, the serotoninergic drugs seemed to be appropriate candidates to improve the pronounced postural deficits of the partial spinal cat, such as poor lateral stability and weight support of the hindlimbs, causing a wobbly irregular walking with repeated stumbling. These deficits are expected from the damage to descending pathways such as the vestibulospinal tract which exert strong excitatory effects on extensor MNs (Orlovsky, 1972) as well as from lesions to ventral and descending pathways that are implicated in postural control (Mori, 1987).

The origin of these pathways with excitatory postural effects is at the rostral position of the nucleus raphe magnus, so it is highly probable that serotoninergic raphe spinal neurons are implicated in the postural effects seen after stimulating this area, such as increased activity in the soleus muscle, an increase in the hindlimb force. These effects were accompanied by long lasting depolarization of extensor α MNs (Mori, 1987). The involvement of serotonin in the modulation of the membrane properties of MNs in the decerebrate cat was demonstrated also by Hounsgaard et al. (1988). It was shown that the bistable properties mainly of extensor MNs, which disappeared after acute spinalization, could be restored after I.V. application of large doses of 5HTP. The effect of 5HTP on the bistable properties of the MNs have many similarities to the effect of L-DOPA (Conway et al. 1988). However they differ in their targets and

strength of action. 5HTP strongly affected extensor MNs, while L-DOPA effect both extensors and flexors but to a lesser extent. This may explain why we observed a tendency to increases, in step cycle and in burst durations after 5HT, especially in extensors (See cat EB7), while after application of NE, early after the lesion, a general increase in the amplitude of all muscles but especially in flexors was noted, accompanied by shortening of the burst duration. During the plateau-period, however, the effects of NE and Methoxamine on extensors were somewhat more pronounced than on flexors. This may be explained by the fact that early after the lesion the activity in the extensors is highly decreased because of a major reduction in strong excitatory input from descending pathways. At that stage, a very strong excitatory input is needed to increase their weak activity. However, long-term post lesion, some adaptive processes have taken place (see Brustein and Rossignol, 1998, for discussion), and weight support of the hindlimbs improved, i.e. the baseline activity of these muscles is much better and less input is needed to increase their weak action. This possible explanation have support from observations that the level of the bias current applied to extensor MNs in the decerebrate cat, had an important effect on expression of the bistable properties (Hounsgaard et al. 1988).

The involvement of serotoninergic drugs in restoring tonic background activity in extensors was further demonstrated by Miller et al. (1996). $5HT_2$ agonist , DOI, restored the tonic background activity in GM

and soleus and facilitated the GM stretch reflex which was abolished after acute spinalization of the decerebrate cats. Such a tonic excitation is suggested already by Mori (1987) to be the main action of serotoninergic system.

However, 5HT, like NE, was found to have a specific modulatory effect on transmission from muscle afferent (Bras et al. 1990, Bras et al. 1989, Jankowska et al. 1993, 1997). 5HT was found to depress transmission from group II afferents, but not of group I afferents. However, it effectiveness was different then seen after NE. NE had more pronounced effects in depressing potentials in the ventral horn, and its action appeared sooner and was faster relative to 5HT (Bras et al. 1989). It was further suggested that this depression may involve different membrane receptors at different locations. α_2 adrenoreceptors were found to be effective primarily in the intermediate zone and in the ventral horn while 5HT $_{1A}$ was effective in the dorsal horn (Bras et al. 1990). Such influences were observed as well after stimulation of the raphe nucleus (Jankowska et al. 1993). 5HT was also found to affect the ascending transmission from group II afferents (Jankowska et al. 1997). These modulatory effects may explain why in cat EB8, which had better weight support, 5HT drugs improved the regularity of the locomotion without a major effect on EMG activity.

It is interesting to mention here that the effectiveness of $5HT_{1A}$ agonist, DPAT, in depressing input from group II and group I (Bras et al.

1990) was limited to the dorsal horn, in contrast to the other tested drugs. Miller et al. (1996) also showed no effect on the excitability of tonic background activity in extensors or on the stretch reflex of the GM after spinalization (in contrast to the $5HT_2$ agonist). These results may explain the appearance of foot drag and the decrease in the locomotor performance in cat EB7 after application of DPAT.

Both Quipazine and Methoxamine given separately had beneficial effects on locomotion. Their combination was most efficient in improving the locomotion of the cats and only half the dose of each drug used individually was sufficient to give a pronounced effect. The combination of both drugs induced the most regular and maintained locomotion, with elegant and smooth movements. This overall impression is obtained by observation of video recordings and is not necessarily expressed as vividly in the stick figure diagrams, because of the limitation of this illustration to one plane of movement.

Taken together it seems that the serotoninergic and the noradrenergic systems, by changing the neuronal excitability, can modulate the pattern of locomotion. In the absence of or after reduction of these modulatory descending inputs, signals for generation of the walking rhythm can not be consistently expressed resulting in irregular walking. A lesion to these descending pathways effect as well the coupling between

the fore- and the hindlimbs. It seems that serotoninergic and noradrenergic neurotransmitters probably contribute to continual stabilization of the fore- and hindlimb coupling by modulating the transmission of ascending information from the muscle and skin afferents.

Our results have important clinical implications, showing that the effects of some drugs such as Clonidine depend on whether or not the spinal lesion is complete. In the complete spinal cat Clonidine may exert beneficial effects; however in the partial spinal cats, its effects may interfere with compensatory mechanisms the cat developed after the lesion. This may indicate that different classes of drugs may be used in patients with different types of spinal cord injuries. In addition, a most important finding is that these drugs effects can be integrated into the residual voluntary locomotor control to improve locomotion and its postural aspects. It is then probable that such changes in neuronal excitability could be used beneficially by patients who still have residual capabilities to induce the rhythm of walking but are unable to sustain it.

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Tables and Tables footnotes:

Table 1: Drugs Injected

The drugs used in the different experiments are listed according to their role and commercial source. The range of doses tested in each cat is given together with the days post spinal lesion on which the experiments were carried out.

Table 1: Drugs Injected

Drug name	Role	Source	Dose (mM/100µl) Injection days (post lesion)	
			Cat EB7	Cat EB8
Noradrenaline (NE)	Neurotransmitter	RBI	**0.8- 4.7** (9,14,17,34,49, 104,107,147,182)	**1.6- 6.3** (6, 8,21,23, 70, 135)
Clonidine	α_2- noradrenergic agonist	SIGMA	**0.6- 5.6** (16, 33, 128, 169,190, 205, 210)	**1.9- 5.6** (14,65,76,84,86, 91,98)
Methoxamine	α_1- noradrenergic agonist	RBI	**2- 4** (196,198, 103,239)	**4- 6** (104, 107)
Yohimbine	α_2- noradrenergic antagonist	RBI	**12.8- 20.5** (205, 210, 212)	**12.8- 20.5** (84,86, 91, 98, 100)
Serotonin (5HT)	Neurotransmitter	RBI	**2.4- 7.1** (42, 44,120, 126, 175, 177)	**1.2- 2.3** (16, 35, 63)
Quipazine	5HT agonist	RBI	**0.6- 2.2** (224, 227, 231, 234)	**1.2-3.4** (112, 119, 127)
DPAT	$5HT_{1A}$ agonist	RBI	**1.5** (266, 268)	**1.5** (154,156)
5HTP	precursor	SIGMA	**1.1- 2.3** (280, 282, 287, 289)	**2.3- 4.5** (168, 170, 175)

Table 2: Amplitude and duration of EMGs after NE application (early after the spinal lesion)

The normalized and averaged amplitude of EMG bursts pre- and post **NE** injection **early after the spinal lesion**, are given for each one of the cats, as percent of the intact values ± the coefficient of variation (cv) and are listed for selected muscles in the fore- and the hindlimbs, together with their burst duration in real time (ms±S.D.). ND= not detectable during the experiment although the signal was present in other circumstances. The statistical significance of all values was tested using a Student t-test, comparing representative values taken from intact, pre- and post-drug trails (*=$p<0.05$, **=$p<0.01$).

Table 2 continue:

		Intact (0.4m/s)		**Pre-drug** (0.1m/s)		**Post-drug** (0.4m/s) 160min after NE 1.6mM/100µl	
		6 days post lesion					
EB8	LSt	100±10 (n=12)	178±48	89±17 (n=2)	76±11*	76±31** (n=7)	103±15**
	RSt	100±9 (n=10)	144±34	83±25 (n=4)	154±51	73±18** (n=7)	124±16
	LSrt	100±12 (n=12)	383±50	129±15** (n=4)	644±267**	133±10** (n=7)	369±22
	LGM	100±14 (n=12)	646±81	ND	ND	57±4** (n=7)	575±77
	RGM	100±9 (n=12)	510±83	75±14** (n=4)	1355±171**	82±25* (n=7)	417±79*
	LTriL	100±10 (n=12)	741±77	70±3** (n=3)	1056±307**	90±13* (n=7)	616±55**
	RTriL	100±7 (n=12)	612±62	108±12 (n=4)	1228±216**	103±7 (n=7)	507±54**
	hindlimb step cycle		1081±170 (n=12)		1554±635* (n=3)		852±63** (n=7)
	forelimb step cycle		1081±193 (n=12)		1239±223 (n=4)		854±69** (n=7)

Table 2: Amplitude and duration of EMGs after NE application (early after the spinal lesion)

		Intact (0.4m/s)		**Pre-drug** (0.2m/s)		**Post-drug** (0.4m/s) 20min after 3 doses of **NE** 1.6mM/100µl	
				9 days post lesion			
	Muscle	Normalized amplitude (%±cv)	duration (ms±SD)	Normalized amplitude (%±cv)	duration (ms±SD)	Normalized amplitude (%±cv)	duration (ms±SD)
EB7	LSt	100±11 (n=17)	193±19	211±18** (n=8)	226±111	548±22** (n=12)	154±46**
	RSt	100±16 (n=16)	215±29	227±36** (n=7)	441±228**	302±31** (n=11)	184±52
	LSrt	100±10 (n=17)	303±40	137±41** (n=7)	466±106**	209±24** (n=11)	380±58
	LGM	100±9 (n=16)	678±64	ND	ND	239±35** (n=11)	521±83**
	RGM	100±14 (n=17)	581±81	245±47** (n=7)	565±228	113±25 (n=7)	401±85**
	LTriL	100±9 (n=16)	801±47	47±21** (n=6)	851±142 (n=6)	41±20** (n=12)	569±110**
	RTriL	100±6 (n=17)	752±77	111±14* (n=8)	873±175*	132±11** (n=12)	570±110**
	hindlimb step cycle		1099±57 (n=15)		2084±816** (n=4)		934±125** (n=11)
	forelimb step cycle		1098±61 (n=15)		1003±189 (n=7)		770±65** (n=12)

Table 3: Amplitude and duration of EMGs after NE application (plateau-period) The normalized and averaged EMG amplitude pre- and post **NE** injection **during the plateau-period** (same format as Table 2)

Table 4: Amplitude and duration of EMGs after Methoxamine application (plateau-period) The normalized and averaged EMGs amplitude pre- and post injection of **Methoxamine** during the plateau-period, are presented for both cats. Same format as Table 2.

Table 3: Amplitude and duration of EMGs after NE application (plateau-period)

		Intact (0.3m/s)		**Pre-drug** (0.3m/s)		**Post-drug** (0.3m/s) 20min after **NE** 3.2mM/100µl	
				104 days post lesion			
	Muscle	Normalized amplitude (%±cv)	duration (ms±SD)	Normalized amplitude (%±cv)	duration (ms±SD)	Normalized amplitude (%±cv)	duration (ms±SD)
EB7	LSt	100±10 (n=7)	233±43	125± 30 (n=18)	175±78	194±23** (n=18)	130±48**
	LSrt	100±7 (n=7)	384±58	117± 13** (n=18)	327±95	123±16** (n=18)	300±51**
	RSrt	100±10 (n=6)	453±58	86± 15* (n=16)	250±53**	161±18** (n=18)	240±34**
	LGM	100±8 (n=6)	670±113	390± 6** (n=17)	685±135	576±13** (n=18)	614±61**
	RGM	100±17 (n=7)	563±80	105± 43 (n=17)	538±87	324±25** (n=18)	470±62**
	LTriL	100±17 (n=6)	1051±83	28±9** (n=18)	659±119**	32±11 (n=17)**	600±75**
	RTriL	100±8 (n=7)	1006±65	ND	ND	105±6 (n=18)	551±81**
	hindlimb step cycle		1372±62 (n=6)		1065±152** (n=18)		864±74** (n=18)
	forelimb step cycle		1367±80 (n=6)		931±108** (n=18)		871±113** (n=17)

Table 4: Amplitude and duration of EMGs after Methoxamine application (plateau-period)

		Intact (0.3m/s)		**Pre-drug** (0.3m/s)		**Post-drug** (0.3m/s) 80min after **Methoxamine** 4mM/100µl	
				196 days post lesion			
	Muscle	Normalized amplitude (%±cv)	duration (ms±SD)	Normalized amplitude (%±cv)	duration (ms±SD)	Normalized amplitude (%±cv)	duration (ms±SD)
EB7	LSt	100± 10 (n=7)	233± 43	183± 87 (n=6)	191±84	201±36** (n=17)	341±83**
	LSrt	100± 7 (n=7)	384± 58	130±14** (n=6)	249±48**	156±15** (n=17)	278±42**
	RSrt	100± 10 (n=6)	453± 58	105±17 (n=6)	214±54**	156±20** (n=16)	248±46**
	LGM	100± 8 (n=6)	670± 113	180±14** (n=6)	676±84	231± 9** (n=17)	630±61
	RGM	100± 17 (n=7)	563± 80	130± 42 (n=6)	493±76	325±27** (n=17)	454±65**
	LTriL	100± 17 (n=6)	1051± 83	24± 8** (n=6)	637±78**	26±6** (n=17)	647±82**
	RTriL	100± 8 (n=7)	1006± 65	222±10** (n=6)	656±68**	246±12** (n=17)	558±85**
	hindlimb step cycle		1372±62 (n=6)		938±53** (n=6)		881±56** (n=17)
	forelimb step cycle		1367±80 (n=6)		933±110** (n=6)		856±83** (n=17)

Table 4 continue:

		Intact (0.4m/s)		**Pre-drug** (0.4m/s)		**Post-drug** (0.4m/s) 50min after **Methoxamine** 6mM/100µl	
		104 days post lesion					
EB8	LSrt	100±12 (n=12)	383±50	137± 29** (n=14)	339±67	120±17** (n=17)	379±47
	RSrt	100±10 (n=12)	410± 45	121± 17** (n=14)	340± 80*	98±14 (n=12)	431±52
	RSt	100±9 (n=10)	144±34	151± 25** (n=14)	146±63	124±26* (n=17)	124±63
	LGM	100±14 (n=12)	646±81	59± 28** (n=11)	698±190	55±20** (n=17)	681±75
	RGM	100±9 (n=12)	510±83	178± 14** (n=14)	511±83	135±19** (n=17)	505±60
	LTriL	100±10 (n=12)	741±77	80 ±23** (n=14)	541±71**	77±13** (n=17)	702±82
	RTriL	100±7 (n=12)	612±62	102± 12 (n=14)	671±206	151±61 (n=16)	680±234
	hindlimb step cycle		1081±170 (n=12)		890±227* (n=10)		958±71* (n=17)
	forelimb step cycle		1081±193 (n=12)		799±108** (n=14)		950±92 * (n=17)

Table 5: EMGs amplitude and duration after Quipazine application (plateau-period)

The normalized and averaged EMG amplitude pre- and post injection of **Quipazine** (cat EB7) and **5HTP** (cat EB8) during the plateau-period are presented for both cats. Same format as Table 2.

Table 5: EMGs amplitude and duration after Quipazine application (plateau-period)

		Intact (0.3m/s)		**Pre-drug** (0.3m/s)		**Post-drug** (0.3m/s) 75min after **Quipazine** 1.2mM/100µl	
				227 days post lesion			
	Muscle	Normalized amplitude (%±cv)	duration (ms±SD)	Normalized amplitude (%±cv)	duration (ms±SD)	Normalized amplitude (%±cv)	duration (ms±SD)
EB7	LSt	100±10 (n=7)	233±43	366±71* (n=13)	121±38**	160±28** (n=12)	234±70
	LSrt	100± 7 (n=7)	384±58	206±26** (n=13)	270±109*	132±12** (n=12)	312±63**
	RSrt	100±10 (n=6)	453±58	155±23** (n=12)	206± 40**	152±20** (n=12)	244±54**
	LGM	100±8 (n=6)	670 ±113	235±23** (n=12)	781±190	180±23** (n=11)	877±98**
	RGM	100±17 (n=7)	563± 80	246±47** (n=12)	407±152*	141±23** (n=11)	667±133**
	LTriL	100±17 (n=6)	1051± 83	43±28** (n=13)	610±100**	44±11** (n=12)	810±121**
	RTriL	100±8 (n=7)	1006±65	221±18** (n=13)	564±105**	237±13** (n=12)	738±100**
	hindlimb step cycle		1372±62 (n=6)		987±273** (n=13)		1200±109** (n=11)
	forelimb step cycle		1367±80 (n=6)		817±124** (n=13)		1087±135** (n=12)

Table 5 continue:

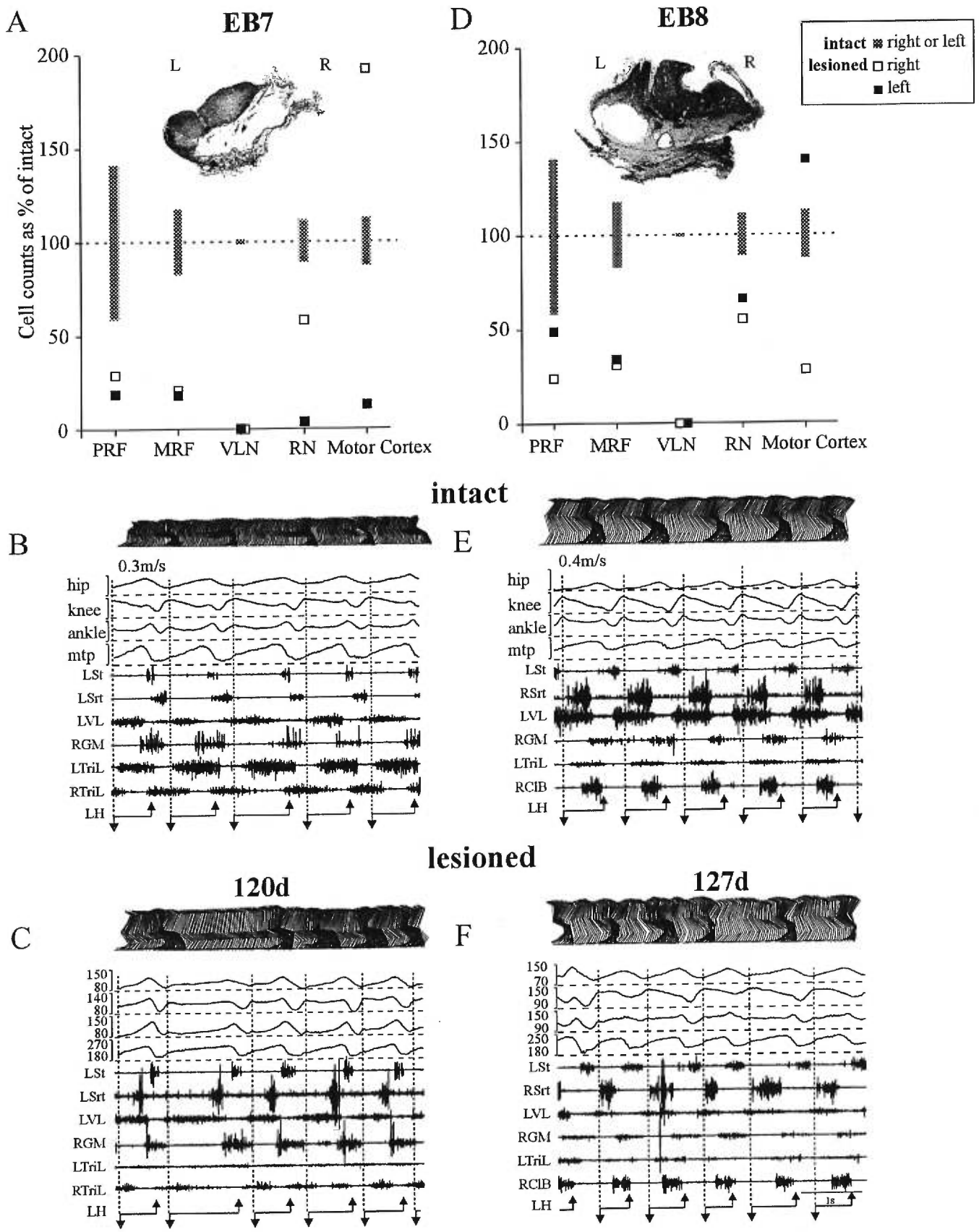
		Intact (0.4m/s)		**Pre-drug** (0.4m/s)		**Post-drug** (0.4m/s) 150min after **5HTP** 2.3mM/100µl	
				168 days post lesion			
EB8	LSrt	100±12 (n=12)	383±50	140±14** (n=19)	275±48**	133±12** (n=5)	296±22**
	RSrt	100±10 (n=12)	410±45	142±14** (n=19)	372±103	118±7* (n=5)	487±61
	RSt	100±9 (n=10)	144±34	177±24** (n=19)	123±19*	118±4 (n=5)	197±51*
	LGM	100±13 (n=12)	646±81	42±11** (n=19)	667±82	ND	ND
	RGM	100±6 (n=7)	510±83	114±14* (n=19)	486±82	115±11 (n=5)	527±56
	LTriL	100±10 (n=12)	741±77	73±12** (n=19)	648±126*	64±6** (n=5)	790±68
	RTriL	100±7 (n=12)	612±62	70±8** (n=19)	545±70*	68±5** (n=5)	629±107
	hindlimb step cycle		1081±170 (n=12)		930±94** (n=17)		990±43 (n=5)
	forelimb step cycle		1081±193 (n=12)		946±100* (n=18)		1031±80 (n=5)

Figure Legends:

Fig. 1: **The extent of the spinal lesion and the locomotor deficits during the plateau-period**: The extent of the spinal lesion and the locomotor deficits long-term post spinal lesion are illustrated in A-C for cat EB7 and in D-F for cat EB8. A and D. A summary of the histological findings for cat EB7 and EB8, respectively. The photomicrograph of a cross section stained with Kluver-Barrera, taken from the spinal site of lesion,, illustrates the extent of the maximal spinal lesion. The graphs illustrate the number of HRP labelled cells found after the spinal lesion in the left and right; Pontine reticular formation (PRF) , Medullary reticular formation (MRF), Lateral vestibular nucleus (LVN), Red nucleus (RN) and motor cortices, expressed as percent of the mean values obtained from 3 intact cats. The shaded vertical bars crossing the 100 level illustrate the coefficient of variation of the average cell counts in the intact situation. B and E, illustrates the locomotion of cats EB7 and EB8 in their intact state, using consecutive stick figure diagrams and angular displacement traces of the left hindlimb together with the raw EMGs of the same 5 step cycles. The vertical dotted lines on the angular traces and raw EMGs indicate the foot contact of the left hindlimb (LH), whereas foot falls are illustrated by the horizontal heavy lines at the bottom. The arrow heads indicate the foot contact (down) and foot lift (up). C and F illustrate, as in B and E, the locomotion of the cats post spinal lesion. To give an indication of the spatial coordinates, in all the stick figure diagrams, the tibia of cat EB7 and cat EB8, are 11 and 10 cm, respectively. See Methods for an explanation of the variations in the vertical dimension.

Fig. 7: **Injection of methoxamine during the plateau-period (cat EB7):**

Representative walking sections taken at the same treadmill speed (0.3m/s) to compare the locomotion of cat EB7 in . **A**, intact situation **B,** pre-drug and **C,** 80 minutes post i.t. injection of 4mM/100µl Methoxamine. For details see Fig. 1. The related bursts amplitude and duration are given in Table 4.


A
EB7
D
EB8
intact ▩ right or left
lesioned □ right
■ left
Cell counts as % of intact
200
150
100
50
0
L
R
PRF
MRF
VLN
RN
Motor Cortex
intact
B
E
0.3m/s
0.4m/s
hip
knee
ankle
mtp
LSt
LSrt
RSrt
LVL
RGM
LTriL
RTriL
RClB
LH
lesioned
120d
127d
C
F
150
80
140
80
150
80
270
180
70
150
90
150
90
250
180
1s

EB7

Intact **Day 196 post-lesion**

A **B** pre-drug **C** post-methoxamine

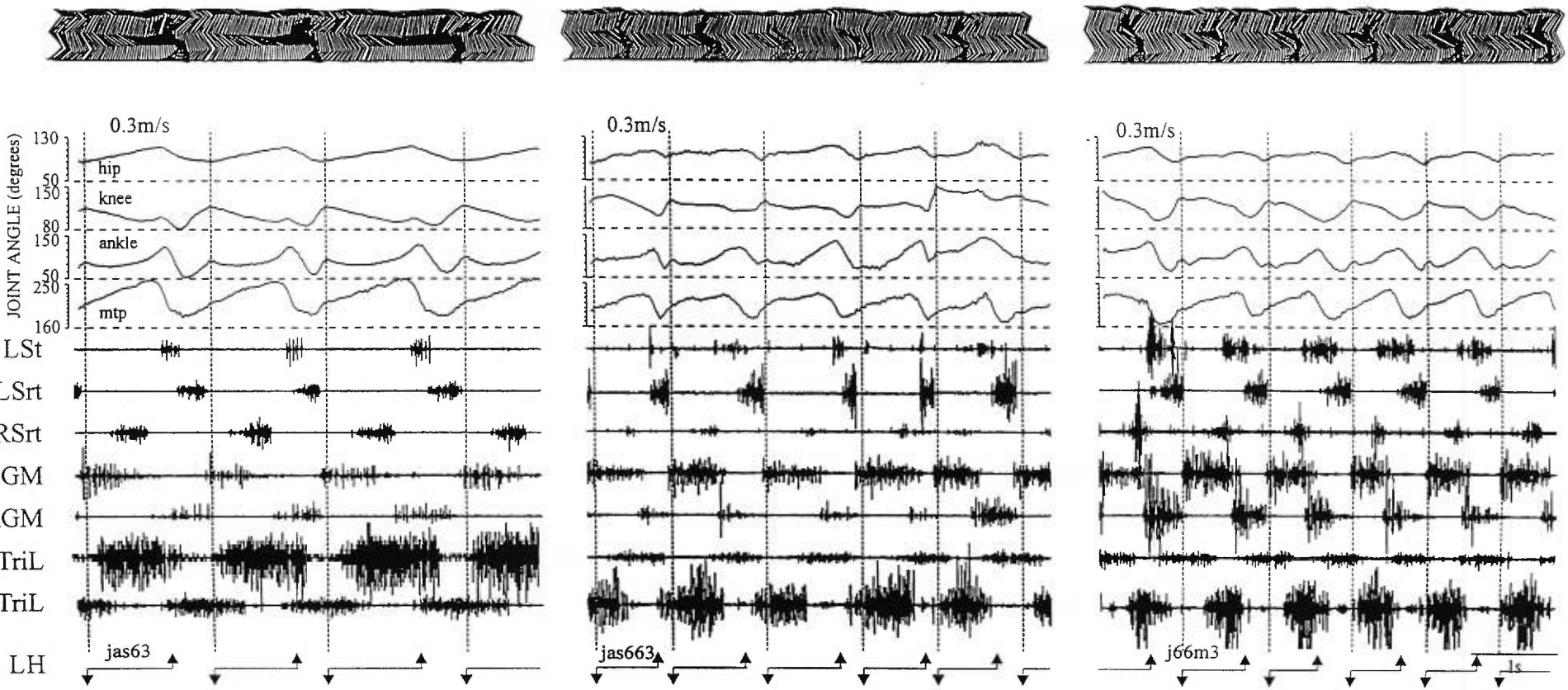


Fig. 9: **Injection of 5HTP (cat EB8):**

Stick figure diagrams of the left hindlimb, taken in **A.** intact situation. **B**, pre-drug and **C**, 150 minutes post i.t injection of 2.3mM/100μl 5HTP. In all the stick figures the tibia =11 cm, respectively. (For explanations about the variations in the vertical dimension see Methods). The corresponding average amplitude of EMGs and burst duration recorded during that walking sequence are summarized in Table 5.

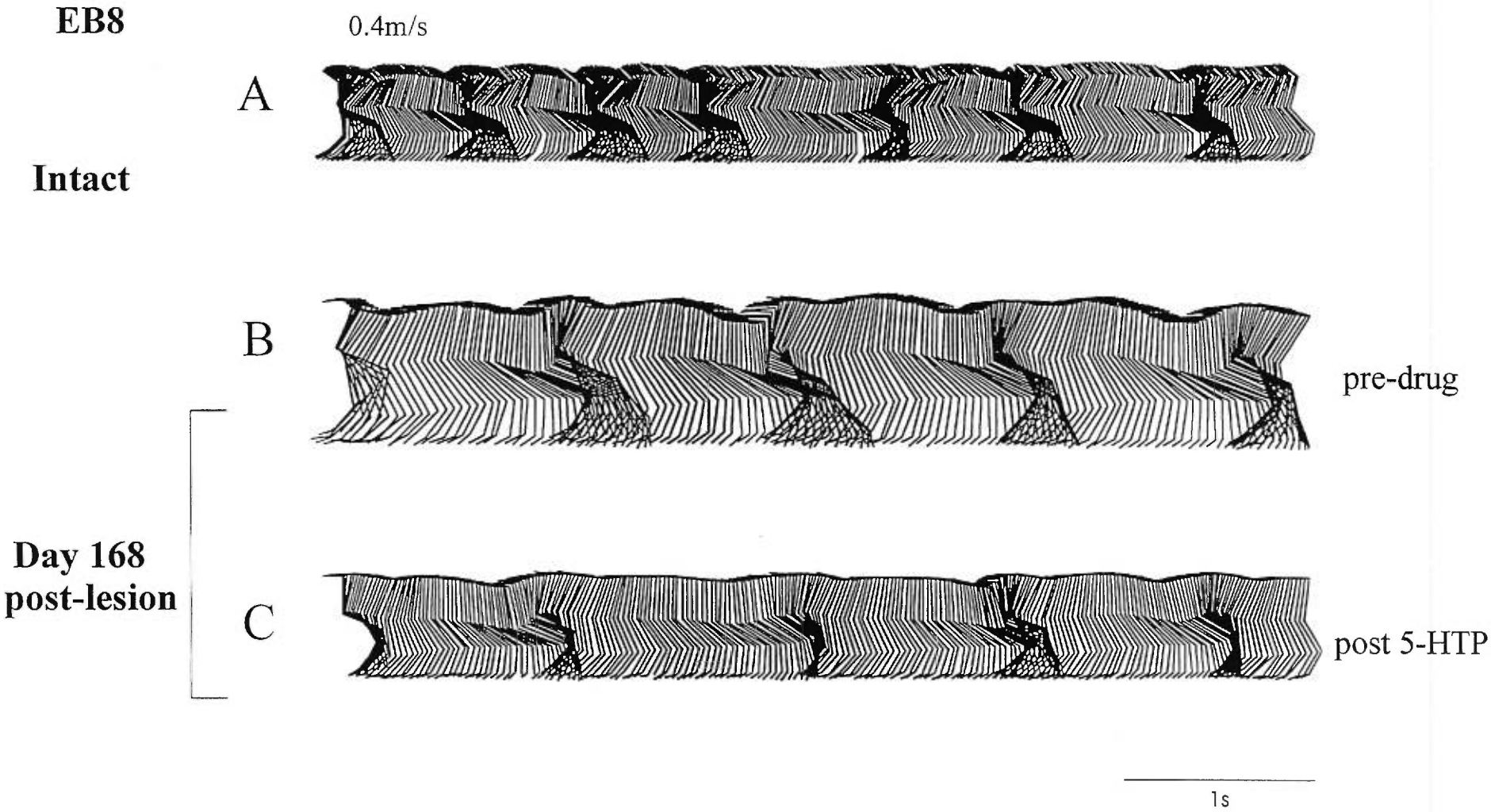

EB8
0.4m/s
A
Intact
B
pre-drug
Day 168
post-lesion
C
post 5-HTP
1s

Fig. 10: **Injection of Quipazine (EB7):**

The consecutive stick figure diagrams, joint angular traces of the left hindlimb and the corresponding EMG activity of selected muscles in the fore- and the hindlimb (for details see Fig. 1) illustrate the walking capacities of cat EB7. **A**. intact situation. **B,** pre- drug and **C**.75 minutes post 1.2mM/100µl Quipazine.

(The average amplitude and duration of EMG bursts are summarized in Table 5)

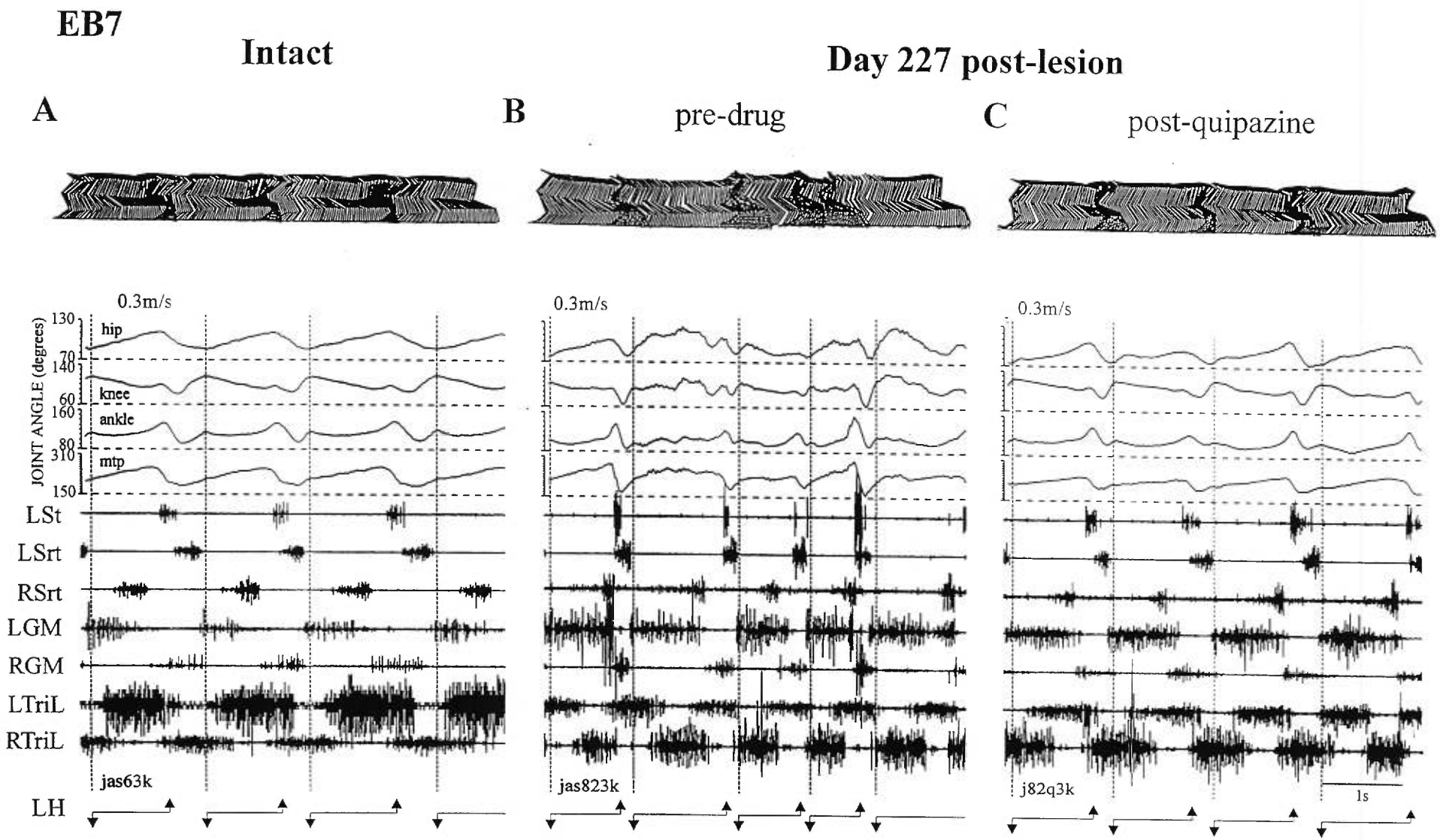

EB7
Intact
Day 227 post-lesion
A
B
pre-drug
C
post-quipazine
0.3m/s
0.3m/s
0.3m/s
JOINT ANGLE (degrees)
130
70
140
60
160
80
310
150
hip
knee
ankle
mtp
LSt
LSrt
RSrt
LGM
RGM
LTriL
RTriL
LH
jas63k
jas823k
j82q3k
1s

Fig. 11: **Interlimb coupling after Quipazine injection (cat EB7):**

The data used to illustrate the coupling relations is taken from the walking sections of Fig. 9 and correspond to **A,** intact situation **B,** pre-drug and **C,** 75 minutes post i.t. injection of 1.2mM/100μl Quipazine using the format of Fig. 8. Notice, however, that in B, 3 average foot falls diagrams are illustrated to show the variability in the coupling pattern used by the cat in the pre-drug situation. L=left, R=right, H=hindlimb, F=forelimb.

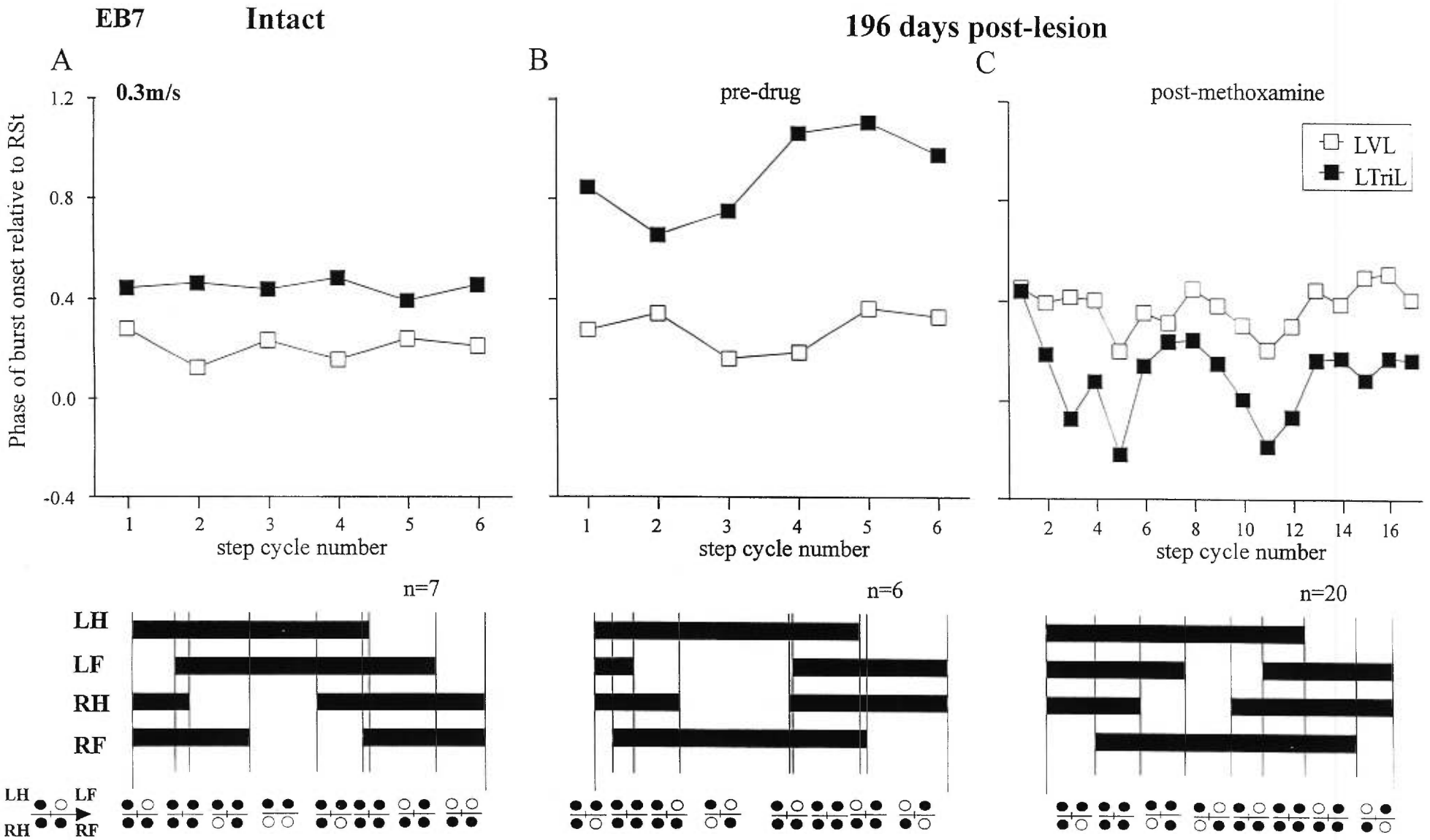

EB7
Intact
196 days post-lesion
A
B
C
0.3m/s
pre-drug
post-methoxamine
LVL
LTriL
Phase of burst onset relative to RSt
1.2
0.8
0.4
0.0
-0.4
1
2
3
4
5
6
step cycle number
2
4
6
8
10
12
14
16
n=7
n=6
n=20
LH
LF
RH
RF

Fig. 6: **Histograms of EMG amplitude and burst duration after clonidine and clonidine followed by Yohimbine (cat EB7 and EB8):**

Pairs of histograms illustrate, on the left, the average and normalized EMG amplitude expressed as percent of the intact ± coefficient of variation (CV) and on the right , the related averaged burst duration ± SD, for each cat. A-D, injection of Clonidine, and E-H, injection of clonidine followed by Yohimbine.

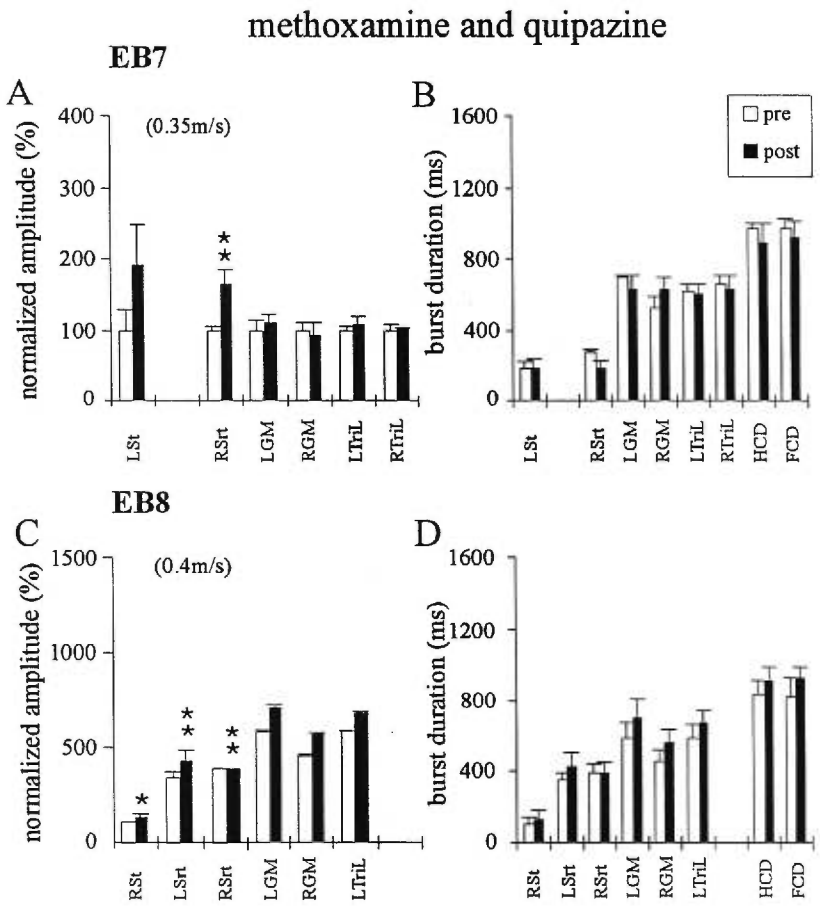
Time post drug injection and their doses: A-B, cat EB7, 30 minutes post 0.8mM/100µl Clonidine at day 51 post lesion. C-D, Cat EB8, 30 minutes post 1.9mM/100µl Clonidine, at day 14 post lesion. E-F, Cat EB7: 45 minutes post 1.9mM/100µl Clonidine and 60 minute post 20.5mM/100µl Yohimbine at day 210 post lesion. G-H, Cat EB8: 30 minutes post 1.9mM/100µl Clonidine and 60 minutes post 20.5mM/100µl Yohimbine, at day 98 post lesion. Pre= pre-drug, Post = post-drug, HCD= hindlimb cycle duration, FCD= forelimb cycle duration, C+Y= injection of Yohimbine after injection of Clonidine. The statistical significance of the changes in EMG amplitude are compared to the pre-drug situation except in the Clonidine experiment where comparison is relative to the intact. (student t-test, *=$p<0.05$, **=$p<0.01$)

Fig. 8: **Interlimb coupling after Methoxamine injection (cat EB7):**

The data used to illustrate the coupling relations are taken from the walking sections illustrated in Fig. 6 and correspond to **A,** intact situation **B,** pre-drug and **C,** 80 minutes post i.t. injection of 4mM/100µl Methoxamine. The graphs illustrate the homolateral fore- and hindlimb coupling using the phase values of burst onset of an extensor muscle in the fore- and the hindlimb, TriL and VL, relative to LSt, respectively, for several consecutive step cycles. The normalized foot falls at the bottom of each graph demonstrate the average coupling pattern the cat used in each walking sequence. The filled horizontal rectangle represent the stance phase of each one of the limbs. The vertical lines divide the average step cycle according to the number of supporting limbs. For each division, the related figurines demonstrate which limb was in contact with (filled circles) or lifted of, the ground. L=left, R=right, H=hindlimb, F=forelimb.

recovery are discussed in the first paper of the thesis while in the second paper, the possible mechanisms for the drugs effects are discussed. I have therefore chosen to extend the discussion in this section to include different mechanisms of plasticity in the locomotor system after spinal cord lesions, a central issue of this thesis and one which is of major importance if one is to develop proper rehabilitation strategies after spinal cord injuries.

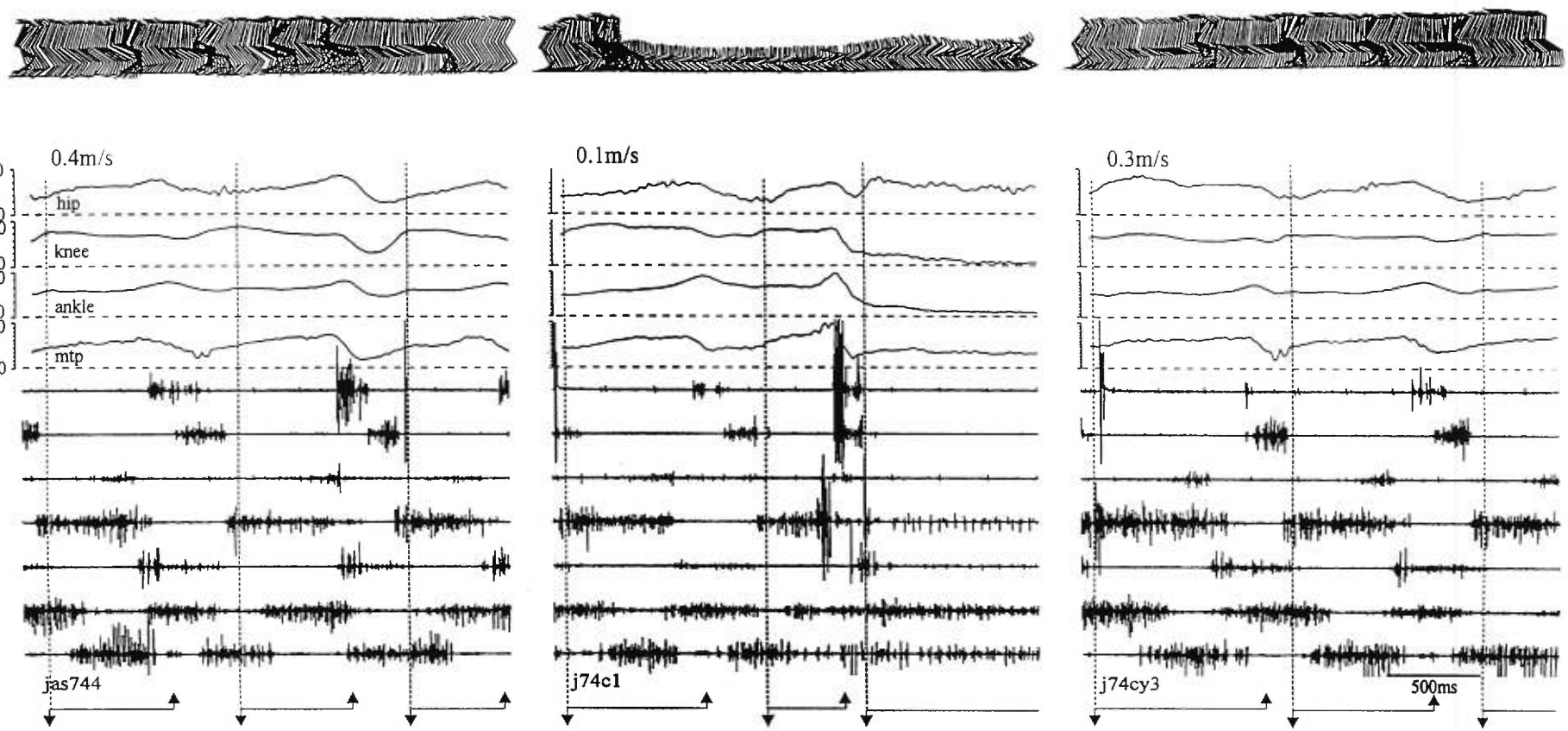
The evidence presented in this study, as well in other studies, indicates that locomotion recovery is possible even after extensive spinal cord lesions to ventral and ventrolateral pathways (Bem et al. 1995, Gorska et al. 1993a, Zmyslowski et al. 1993) such as the reticulo- and the vestibulospinal, which were formerly suggested to be necessary and essential for locomotion (Eidelberg, 1981). Our observation does not lessen the importance of these pathways in normal locomotion. On the contrary, the long period it took most lesioned cats to recover weight support and walking indicates that the contribution of these pathways under normal conditions is of major importance and, in keeping with the initial deficits, they are probably involved in the control of weight support, of lateral stability and in the initiation of locomotion. It is important to emphasize, that in our lesion paradigm, which encompassed more then one pathway, trying to relate a specific function to a specific pathway, should be done with caution. However, it is tempting to speculate, based on our results and those of other studies in the decerebrate, fictive or intact cat preparations, which documented the activity in a specific pathways (see introduction sections corresponding to "implication of descending pathway is regulation and adaptation of locomotion" and in "initiation of locomotion") that the postural deficits may be attributed to the elimination of the lateral vestibulospinal pathways and to severe damage to pathways from the DTF and the VTF areas of the brain stem, all implicated in adjustments of muscle tone (Orlovsky, 1972b, Mori, 1987,

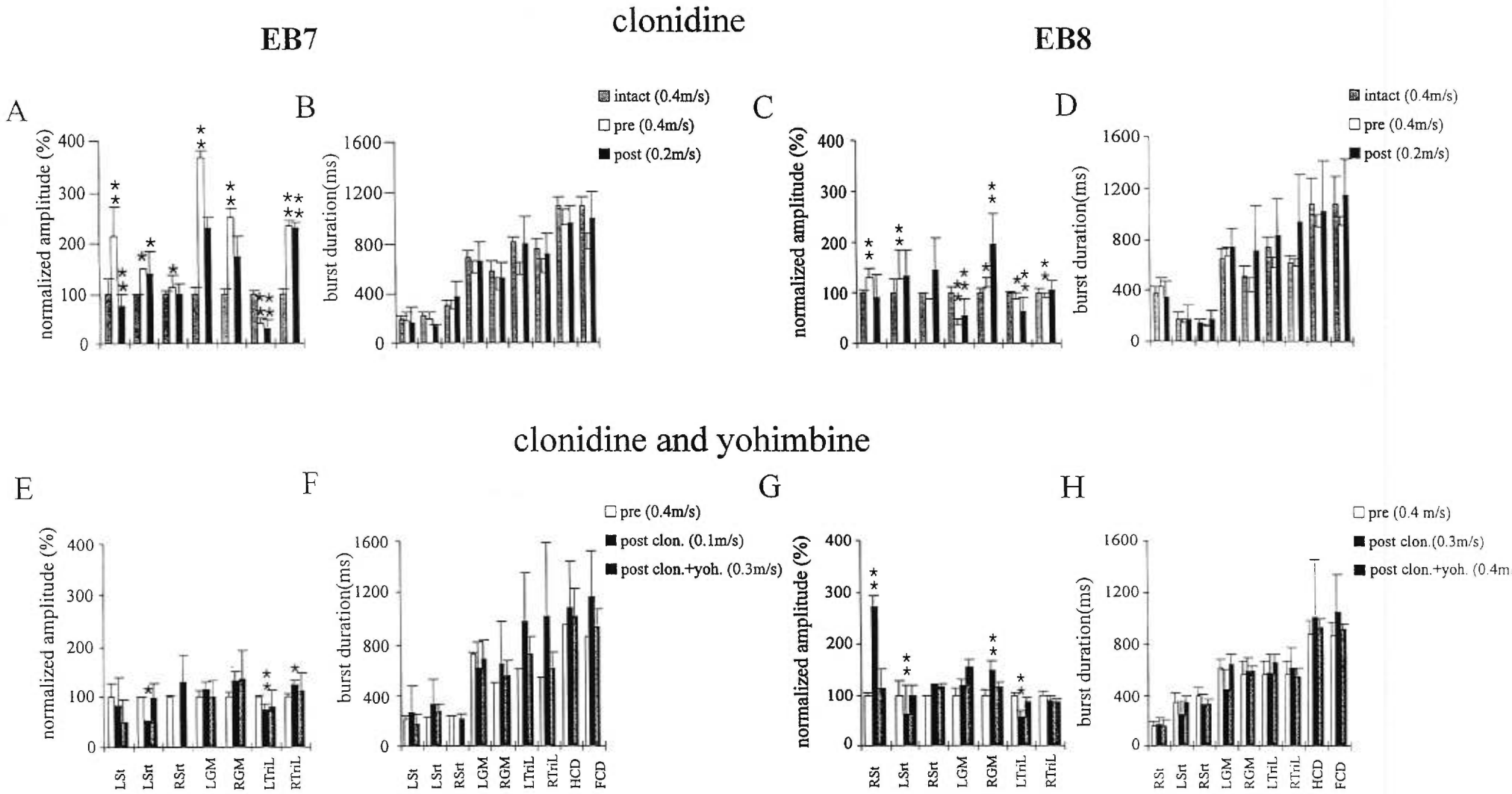

methoxamine and quipazine
EB7
A
(0.35m/s)
normalized amplitude (%)
400
300
200
100
0
LSt
RSrt
LGM
RGM
LTriL
RTriL
B
pre
post
burst duration (ms)
1600
1200
800
400
0
LSt
RSrt
LGM
RGM
LTriL
RTriL
HCD
FCD
EB8
C
(0.4m/s)
normalized amplitude (%)
1500
1000
500
0
RSt
LSrt
RSrt
LGM
RGM
LTriL
D
burst duration (ms)
1600
1200
800
400
0
RSt
LSrt
RSrt
LGM
RGM
LTriL
HCD
FCD

EB7

Day 210 post-lesion

A pre-drug **B** post-clonidine **C** post-clonidine and yohimbine




clonidine
EB7
EB8
A
B
C
D
normalized amplitude (%)
burst duration(ms)
intact (0.4m/s)
pre (0.4m/s)
post (0.2m/s)
clonidine and yohimbine
E
F
G
H
pre (0.4m/s)
post clon. (0.1m/s)
post clon.+yoh. (0.3m/s)
pre (0.4 m/s)
post clon.(0.3m/s)
post clon.+yoh. (0.4m
LSt
LSrt
RSrt
LGM
RGM
LTriL
RTriL
HCD
FCD
RSt

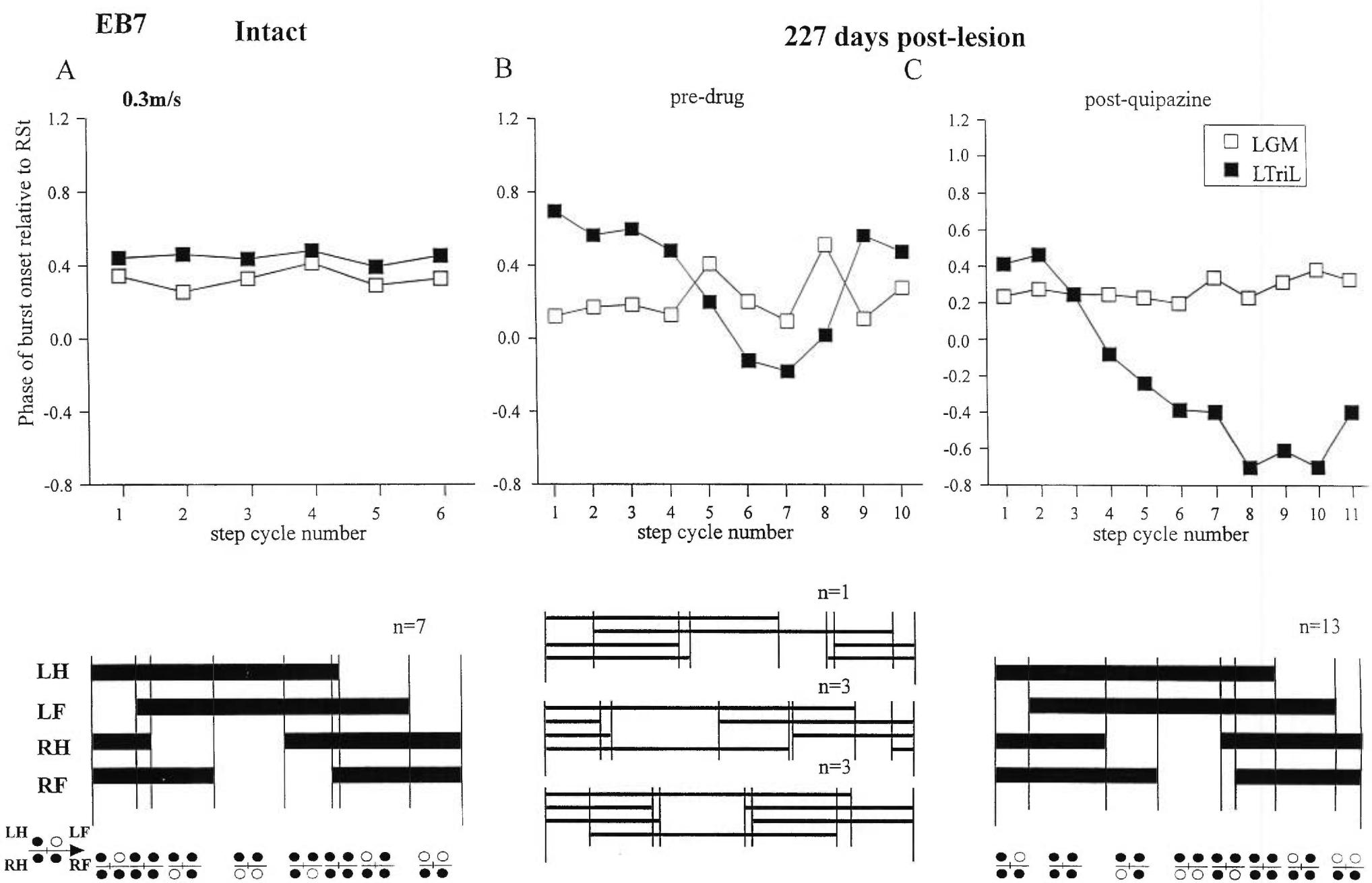

EB7
Intact
227 days post-lesion
A
B
C
0.3m/s
pre-drug
post-quipazine
LGM
LTriL
Phase of burst onset relative to RSt
step cycle number
n=7
n=1
n=3
n=3
n=13
LH
LF
RH
RF

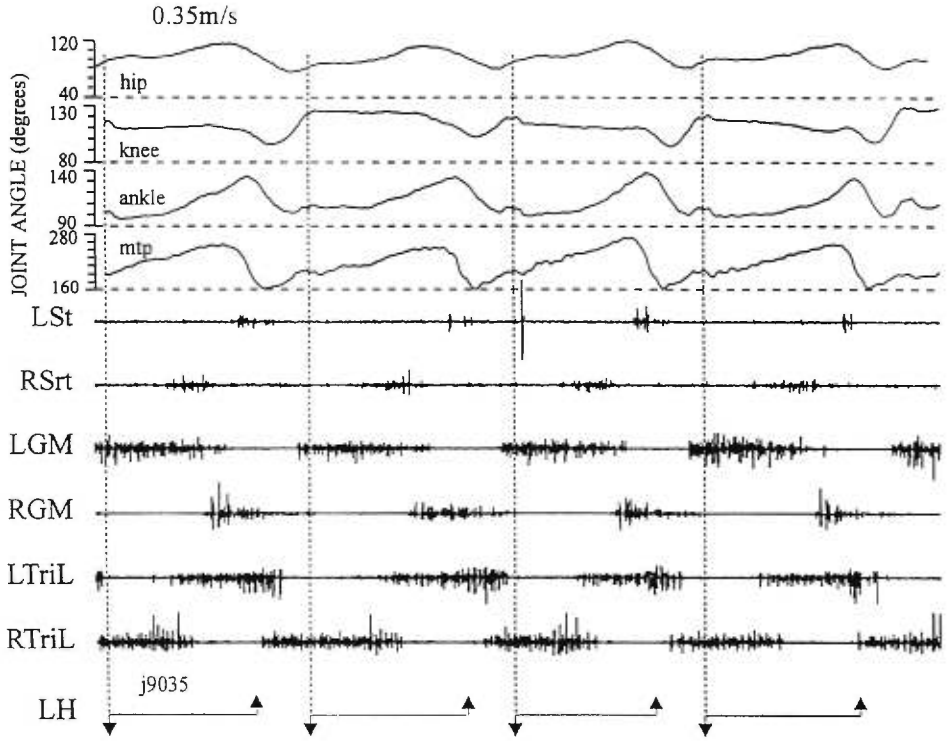
Fig. 12: **Injection of Methoxamine and Quipazine (cat EB7):**

The consecutive stick figure diagram, the related joint angular displacement traces and raw EMGs illustrate walking sequences at 0.35m/s. **A,** pre- and **B,** 75 minutes post i.t injection of Methoxamine and Quipazine combination (2mM and 1.2mM/100µl, respectively), at day 245 post lesion. For details see Fig. 1.

EB7

Day 245 post-lesion

A pre-drug



B post-methoxamine and quipazine

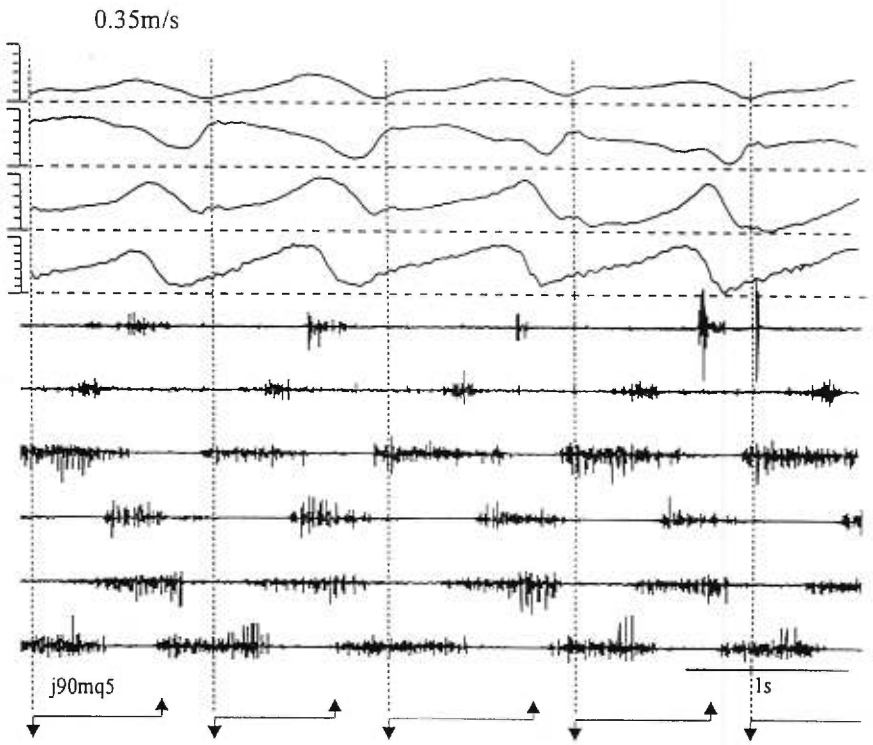


Fig. 13: **Duration of consecutive step cycles after injection of Methoxamine and Quipazine:**

The cycle duration (ms) of consecutive steps taken during one experimental session at different treadmill speeds (indicated by the arrows) are illustrated for cat EB7. **A,** intact situation, **B**, pre-drugs and **C**, 75 minutes post combined injection of Methoxamine and Quipazine (2mM and 1.2mM/100μl, respectively).

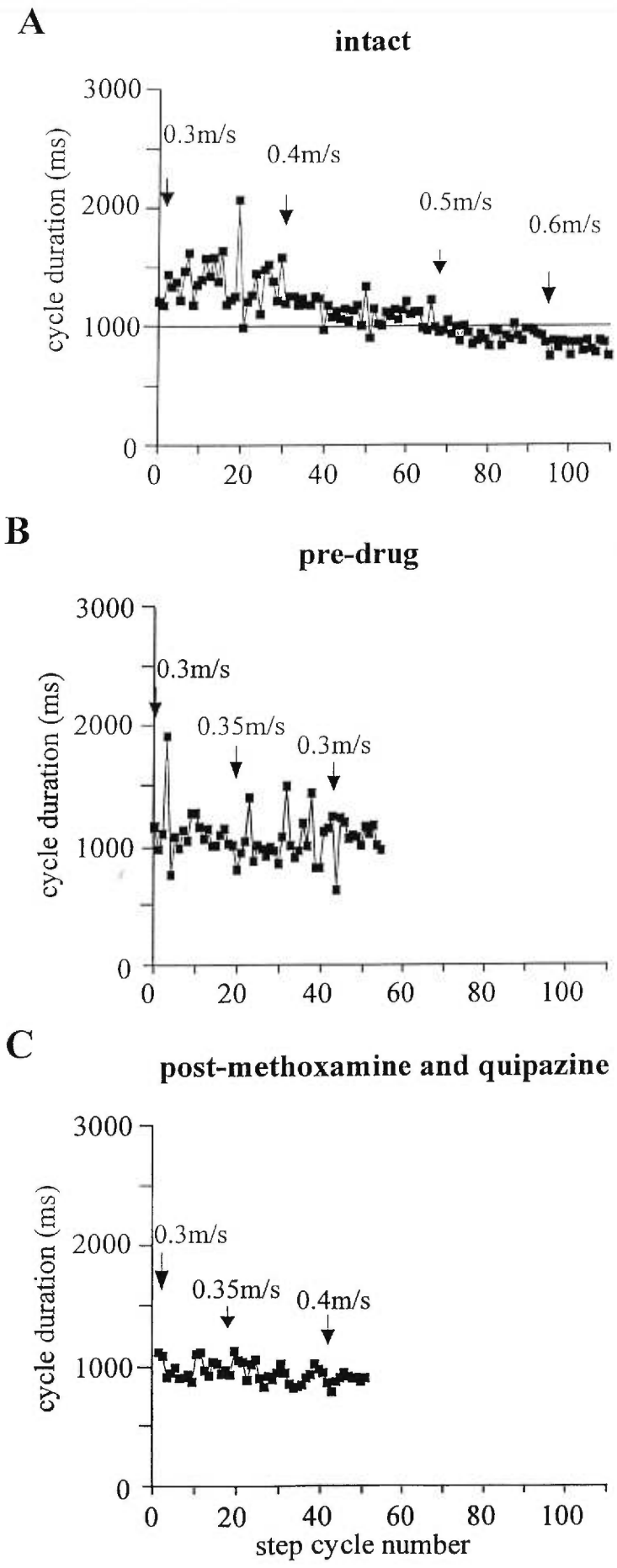

A
intact
3000
2000
1000
0
cycle duration (ms)
0.3m/s
0.4m/s
0.5m/s
0.6m/s
0
20
40
60
80
100
B
pre-drug
3000
2000
1000
0
cycle duration (ms)
0.3m/s
0.35m/s
0.3m/s
0
20
40
60
80
100
C
post-methoxamine and quipazine
3000
2000
1000
0
cycle duration (ms)
0.3m/s
0.35m/s
0.4m/s
0
20
40
60
80
100
step cycle number

Fig. 14: **Histograms of amplitude and duration of bursts (cat EB7 and EB8) after a combined injection of Methoxamine and Quipazine:**

The pairs of histograms illustrate the average and normalized EMG amplitude and the related burst duration. A-B, Cat EB7, 65 minutes post (2mM+1.2mM)/100µl Methoxamine and Quipazine , respectively, at 245 days post lesion. C-D, Cat EB8, 75 minutes post (4mM+2.4mM)/100µl Methoxamine and Quipazine, respectively, at day 133 post lesion. for details see Fig. 6.

GENERAL DISCUSSION

The results presented in this thesis show that cats with extensive spinal lesions (at the thoracic level) including the ventral and ventro-lateral pathways and to a different extent the dorsolateral pathways, can recover quadrupedal voluntary locomotion overground and on the treadmill. Initially, none of the cats could support the hindquarters or walk with their hindlimbs. They then gradually regained weight support of the hindlimbs and the ability to walk quadrupedally without any external support or stimulation. However, their walking was irregular, with poor lateral stability, inconsistent interlimb coupling and often stumbling which limited the number of consecutive steps that they could take, as well as the range of speeds they could follow. The more extensive was the lesion, the longer was the time course for recovery and the more limited was their locomotor performance. However, the regularity and endurance of the walking could be improved by certain types of drugs, especially by the $\alpha 1$ -adrenergic agonist, Methoxamine, and the serotoninergic agonist Quipazine. Combination of both these drugs gave the best results. On the other hand, other drugs such as the α_2 -adrenergic agonist, Clonidine, and DPAT, the 5-$HT1_A$ agonist, had a detrimental effect on the walking pattern. The specific mechanisms that may underlie the observed

1989). The initial lack of locomotion with the hindlimbs may be attributed to damage in reticulospinal pathways which are suggested to convey the information from the MLR, as shown in the decerebrate acute cat (Steeves and Jordan, 1980, Noga et al. 1991). Yet, lesions in chronic animals restricted to a specific pathway, for example to the lateral vestibular nucleus in the cat (Yu and Eidelberg, 1981) or to the medial system in the monkey (Lawrence and Kuypers 1968a,b) resulted in less pronounced initial postural and locomotor deficits. This may suggest that the deficits observed with our most lesioned cats are a result of a combined lesions to the systems implicated in initiation of locomotion and in the control of locomotor related postural adjustments, impeding proper integration between both mechanisms, on which full expression of locomotion depends (Sherrington, 1910, Mori et al. 1978, Noga et al. 1991, Mori et al. 1992).

However, even the most lesioned cats in our study finally recovered quadrupedal locomotion suggesting that the nervous system can adapt and compensate up to a certain extent for severe damage to these important locomotor implicated, reticulo- and vestibulospinal pathways.

The interpretation of the recovery process after subtotal spinal lesions is problematic. On the one hand the recovery can be attributed to remaining axons in pathways that normally mediate the locomotor function

or, on the other hand, it can be attributed to functional " take-over" by other, non damaged, pathways.

In our lesion paradigm, a residual population of medullary reticulospinal axons will always remain in the DLF, intermingled with the rubrospinal pathways (Petras, 1967, see also Fig.4 in the introduction), as seen even after the most extensive lesions (see cat EB7). In this context, Sabel (1997), has suggested an hypothesis to explain recovery of function even with a very low number of remaining axons. According to this hypothesis of "the minimal residual structure" or "within system recovery", a certain minimal number of axons within a structure is required for recovery following CNS injury. In his example, 10% of remaining axons in the optic nerve (following a crush) are sufficient to sustain vision. These remaining axons are suggested to undergo genetic, metabolic and electrophysiological reorganization which results in amplification of their action. As already mentioned the "within structure recovery", requires a minimal number of remaining axons, each structure with its own "threshold". Significant behavioral deficits will be expressed only when the size of the lesion is close to that "threshold". Our results show that cats with larger number of remaining axons in the reticulospinal pathways recover remarkably and the long term deficits are minimal. In contrast, the most extensively lesioned cats, in which less then 20-30% of the reticulospinal axons remained, express pronounced initial and long term deficits, suggesting, that in these cats the "threshold" for the

Fig. 2: **NE injection, early after the spinal lesion (cat EB7):**

The treadmill locomotion of cat EB7 is illustrated using representative consecutive stick figure diagrams and angular displacement traces of the left hindlimb, the related raw EMGs and foot fall diagrams. **A**, Intact situation . **B**, pre-drug. **C**, 20 minutes post i.t. injection of 3 consecutive doses of 1.6mM/100µl NE . The dashed vertical lines on the angular traces and raw EMGs correspond to left foot contact. The horizontal lines in the foot fall diagrams illustrate the period the limb is in contact with the treadmill (stance) while the empty spaces correspond to the swing period. The dashed lines connecting the foot falls illustrate, when possible, the coupling relations between homolateral fore- and hindlimb. L=left, R= right, H=hindlimb, F=forelimb. (the values of the step cycle duration, normalized EMG amplitude and bursts duration are summarized in Table 2)

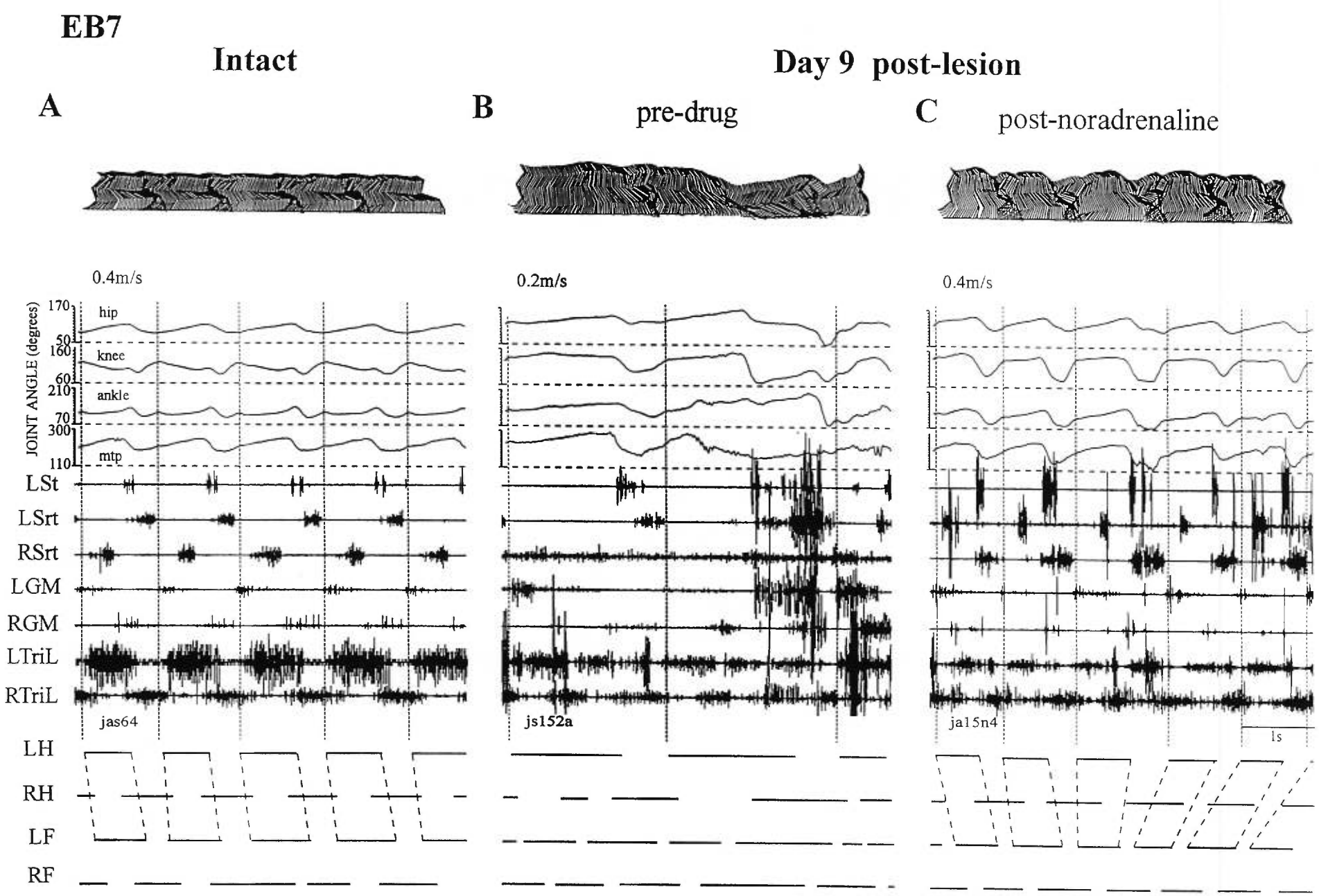

EB7
Intact
Day 9 post-lesion
A
B
pre-drug
C
post-noradrenaline
0.4m/s
0.2m/s
0.4m/s
JOINT ANGLE (degrees)
170
50
160
60
210
70
300
110
hip
knee
ankle
mtp
LSt
LSrt
RSrt
LGM
RGM
LTriL
RTriL
jas64
js152a
ja15n4
1s
LH
RH
LF
RF

Fig. 3: **NE injection during the plateau-period (cat EB7):**

Representative walking sections taken at the same treadmill speed (0.3m/s) to compare the locomotion of cat EB7 in **A**, intact situation, **B**, pre-drug and **C**, 20 minutes post i.t. injection of 3.2mM/100µl NE. For details see Fig. 2. The related average bursts amplitude and duration are given in Table 3.

EB7

Intact

Day 104 post-lesion

A

B pre-drug

C post-noradrenaline

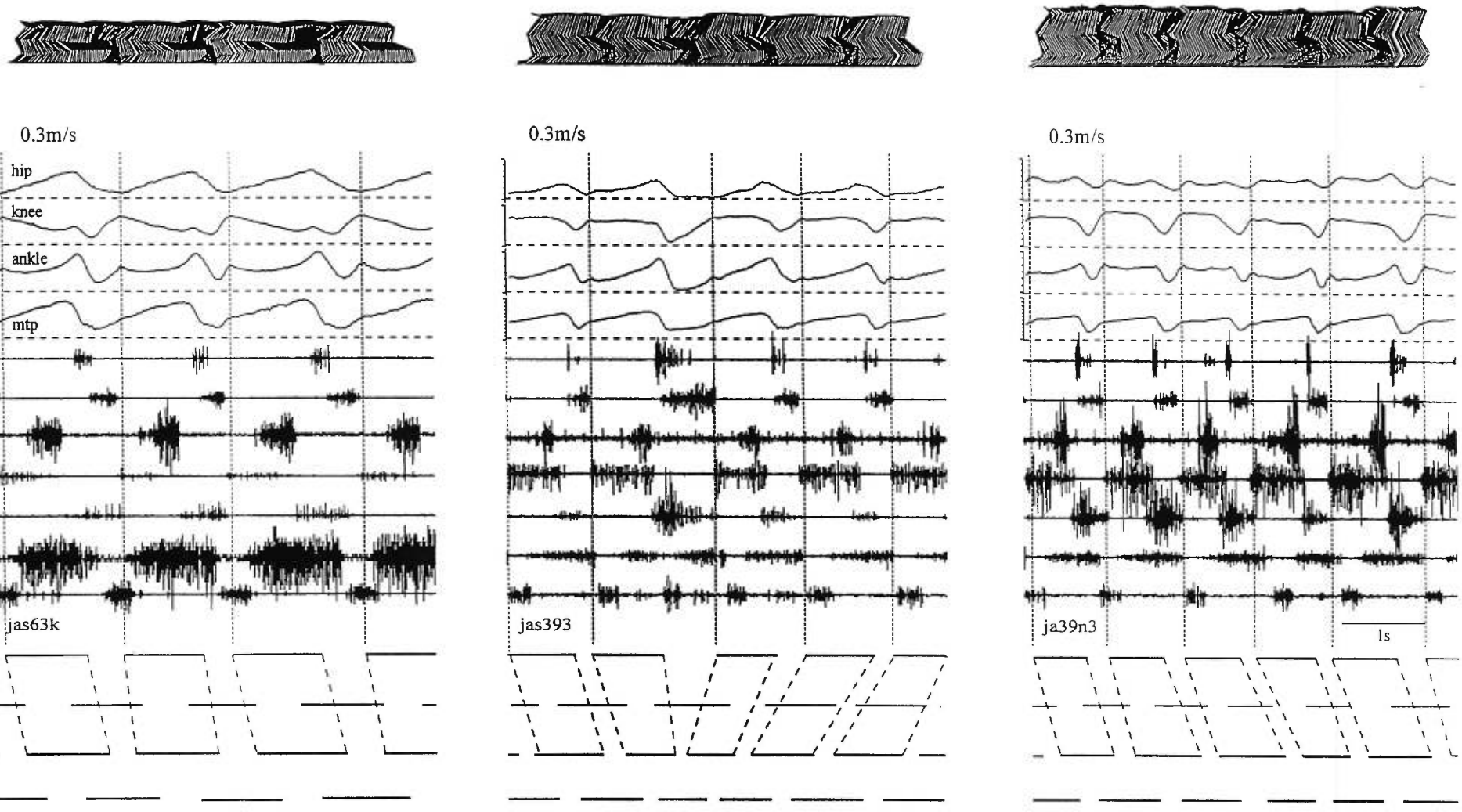


Fig. 4: **Clonidine injection after recovering quadrupedal walking (cat EB8):**

Representative walking sections taken 14 days post lesion. **A** pre-drug and **B**, 30 minutes post i.t injection of 1.9mM/100µl Clonidine. The consecutive stick figure diagrams, angular displacement and raw EMGs in each one of the situations illustrates the stepping sequence of about 8 seconds (for details see Fig. 1). Pre-drug the cat stepped consecutively at 0.4m/s, while post Clonidine the locomotor pattern is highly deteriorated. The missing steps represented by the dotted lines in the stick figure diagram in B and the missing joint angular traces correspond to the time it took the cat to recover an upright position and continue walking, after it fell during that sequence. It was impossible to reconstruct the limb movement as the position of the reflective markers was hidden from the camera.

EB8

Day 14 post-lesion

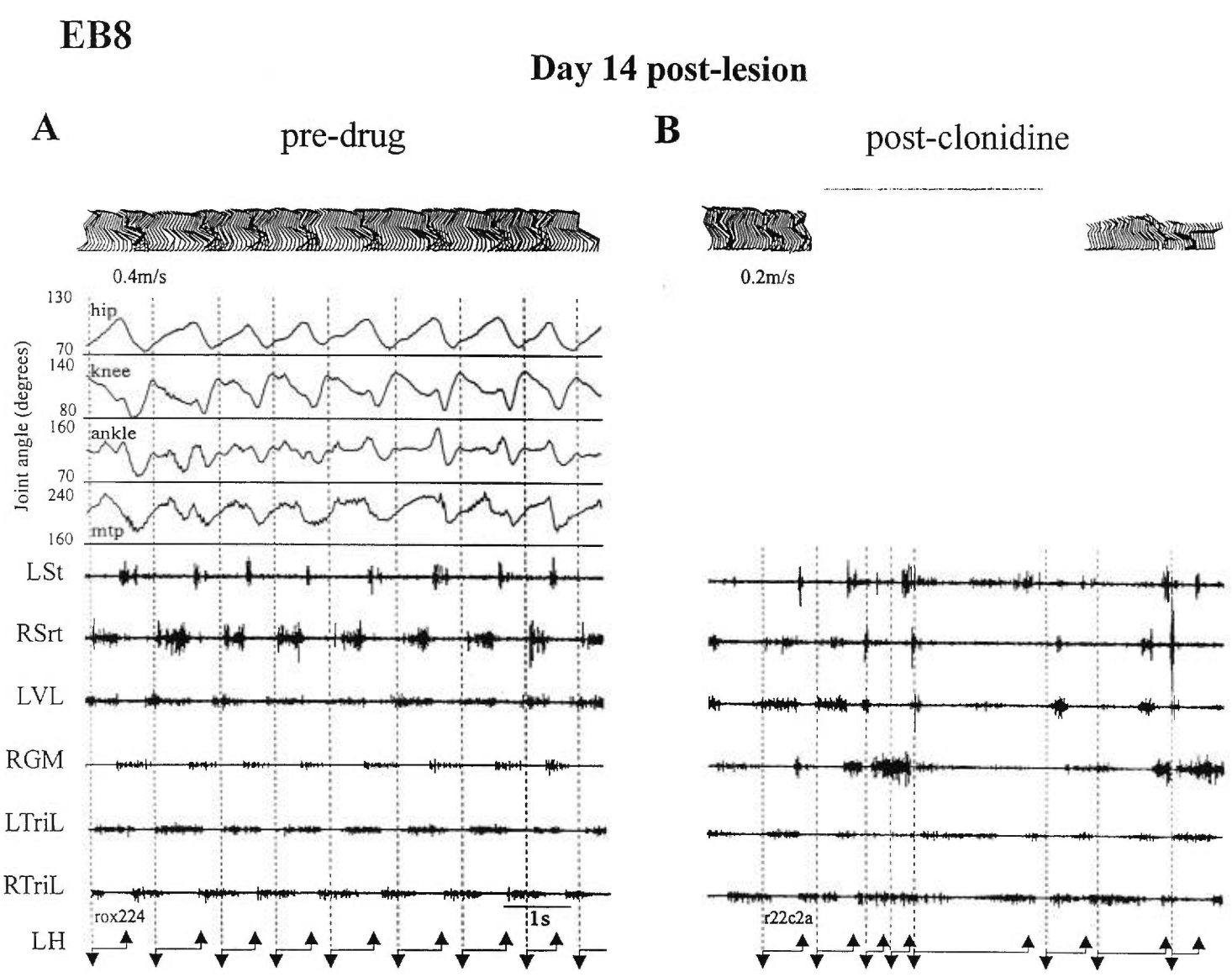


Fig. 5: **Injection of Clonidine followed by injection of Yohimbine (cat EB7):**

Treadmill stepping sequences taken 210 days post lesion. **A**, pre-drug and **B** and **C** post-drugs. The consecutive stick figure of the left hindlimb, the angular displacement and the raw EMGs in B illustrate that, 45 minutes post i.t injection of 1.9mM/100µl clonidine, the cat sagged and could not maintain the weight of the hindquarters nor walk.

However, as illustrated in C; 60 minutes after i.t. injection of 20.5mM/100µl Yohimbine, given 45 minutes after Clonidine, the walking of the cat improved considerably as noted from regular stick figure diagrams, joint angular displacement traces and raw EMG taken at treadmill speed of 0.3m/s.

reticulospinal system was attained according to Sabel's hypothesis. Therefore, in the case of the less lesioned cats (EB1-EB4) the recovery might be due to amplification in the function of remaining axons, but in the case of the most lesioned cats such as cat EB7 in which the "threshold" is attained, and the recovery cannot be exclusively attributed to the remaining reticulospinal pathways, we suggest that other remaining pathways are implicated in the recovery.

A functional "take-over" by other descending pathways was suggested by (Alstermark et al. 1987a), after serial lesions to descending pathways implicated in food-taking movements in the cat. Normally, food-taking movements are mediated by the corticospinal and the rubrospinal pathways. Lesions to these two pathways, at the cervical level, abolish the movements which, however, slowly recover within a month. The recovery is suggested to be due to "take-over" by a third pathway, the reticulospinal pathway. Food-taking movements are not abolished if part of the rubrospinal pathway is preserved. Further, the ability to retrieve food persists even if the remaining rubrospinal pathway is removed a month later. The recovery is, however, abolished after a third section to the ventrolateral quadrants. The authors suggest that during the time between the two first lesions, the reticulospinal pathway has been gradually implicated in the food taking task, which underlies the quick recovery and results in less important deficits, even in the absence of the principal pathways after the second lesion. The plastic changes involved

in this process are not detailed in that study; however the authors suggest an increase in the efficacy of the corticobulbar pathways.

Plastic changes in cortical pathways, such as outgrowth of pyramidal cell dendrites in layer V, were observed in the motor cortex of the rat. These changes occurred in the intact motor cortex following damage to the motor cortex on the other side (Kozlowski et al. 1996). The dendritic outgrowth was found to depend on usage, as immobilization of the limb innervated by the intact cortex, abolished the phenomenon and was correlated with severe and chronic behavioral deficits. Outgrowth of commissural fibers from the contralateral descending pathways in the spinal cord is suggested to be the basis of motor recovery in hemisected rats (Hariss at al. 1994); however the involved descending pathways were not identified. The growth of crossing fibers in the spinal cord was shown to be the source of gradual motor recovery after hemisection in the monkey (Aoki et al. 1988). In these studies it was shown that stimulation of the motor cortex contralateral to the intact cord led to simultaneous appearance of EMGs in both hindlimbs. This phenomenon is abolished after additional lesion to the remaining corticospinal pathways. This functional recovery was related, as already mentioned, to the outgrowth of crossing fibers, shown using anterograde HRP labelling. In addition, an increase in the density of corticospinal terminals is observed on the intact side. In our studies we have found a remarkable increase in the number of HRP labelled neurons in the cortex contralateral to the intact

DLF of the most lesioned cats. The increase in the number of cells was accompanied by changes in the distribution of the labelled cells (Brustein et al. 1997) to areas which are not normally labelled after HRP injections in the lumbar level, suggesting outgrowth of axons from more rostral regions or from regions that usually innervate the back muscles. We therefore suggest that the gradual recovery observed in our most lesioned cats may in part be attributed to plastic changes in the corticospinal pathways.

From what is known in the literature about the involvement of the corticospinal pathways in locomotion of the intact cat, such a "take-over" by the corticospinal pathway is not of a complete surprise, as it can control the timing, the duration and the amplitude of the EMGs and also be implicated in the initiating the accompanying postural adjustments (Drew et al. 1996b, see also introduction section on "the involvement of descending pathways in regulating and adapting locomotion"). It should be emphasized, however, that the observed recovery is limited. The most lesioned cats show permanent locomotor deficits such as absence of EMG amplitude modulation when walking on a tilted treadmill or deficits in maintaining constant interlimb coupling, indicating that the suggested "take-over" by the corticospinal pathways is incomplete and also points again to the importance of the ventral and ventrolateral pathways to locomotion of the intact cat.

The proposition of corticospinal "take-over" should however be further studied. It would be interesting to try and reversibly inactivate the motor cortex in cats with partial lesions. If indeed the cat relies on this structure to regain walking after the lesion, inactivating the motor cortex, which normally has little effect on unobstructed treadmill locomotion, should have major effects. More histological analysis is also needed to identify the origin of the increase in the number of labelled cells in the motor cortex of these cats. One possibility is an outgrowth of commissural fibers crossing in the spinal level as demonstrated in other studies (Aoki et al. 1988). Another possibility which was not discussed till now, is an increase in the convergence of inputs on propriospinal neurons at the cervical level. These inputs could be from cortical origin (Alstermark et al. 1987b, Alstermark et al. 1991) and also from vestibular, rubral and reticular sources. For example, an increase in the efficacy of the corticoreticulospinal inputs on cervical propriospinal neurons was suggested to explain recovery of food taking movements after partial spinal lesions (Alstermark et al. 1987a). Such functional and anatomical changes may also be at the basis of our observation in cats with larger lesions, in which the forelimbs seem to take a greater propulsive role than usual as revealed by a consistent increase of the activity of the triceps.

Another aspect that may underlie the postural and locomotor recovery of our cats indicate that the partial lesioned cat may also depend on peripheral transmission and segmental reflexes to maintain

weight support. Such a dependency is already suggested to explain the recovery of weight support in complete spinal cats (Grillner, 1972, Pratt et al. 1994). Therefore, interfering in transmission from the periphery may abolish important inputs on which the partial spinal cats relay on to maintain posture and for local feedback. We have demonstrated that after quadrupedal locomotion is recovered, i.t. injection of Clonidine, the α_2-agonist, markedly decreases weight support of the hindlimbs and increase the wobbliness of the cats, together causing a marked limitation of walking. These adverse effects of clonidine on locomotion in the partial spinal cat are probably mediated by activation of presynaptic α_2 receptors, which cause a decrease in transmitter release. Such receptors can be found on peripheral afferents (Howe et al. 1987), in addition to remaining descending terminals. A depressive effect on spinal reflexes on muscle tonus was also observed in human patients after L-DOPA administration (Eriksson et al. 1996).

It also possible that the partial spinal cat shows increased sensitivity to Clonidine (compared to the intact spinal cat, Giroux et al. 1996), because of an increase in the population of presynaptic α_2 receptors on peripheral afferents, due to sprouting (Goldberger and Murray, 1978, 1988, Helgren and Goldberger, 1993). Sprouting in dorsal root ganglions was associated with the recovery of segmental reflexes (Helgren and Goldberger, 1993) and may contribute to amplification and assuring transmission from the periphery.

Till now it was suggested that remaining reticulospinal pathways are probably sufficient to sustain locomotion in some of the cats, but that in the more lesioned cats the recovery process is more complex and may be attributed to changes in descending corticospinal pathways, as well in increased reliance on segmental reflexes and peripheral inputs. A combination of local spinal adjustment and supraspinal implication in the adaptation and recovery process was also suggested to take place after unilateral peripheral lesions, such as neurectomy. In this work (Carrier et al. 1997), appended in the thesis, we have shown quick adaptation of locomotion to neurectomy of part of the common peroneal nerve (which cause denervation of the ankle flexors, Tibialis anterior and extensor digitorum longus), in the otherwise intact cat. The cat shows only minor deficits and, most importantly, the cat can sustain symmetrical walking. A detailed analysis shows an increase in the activity of the hip and knee flexors in the affected limb. However, after complete spinalization, these cats (in contrast to the normal spinal cat, which recovers symmetrical hindlimb locomotion), reveal an asymmetric and disorganized walking pattern of the hindlimbs, with a pronounced hyperflexion on the neurectomized side. Since this non functional pattern contains some aspects of the compensatory response seen before spinalization, and cats that were neurectomized after recovering stable spinal hindlimb locomotion, maintain symmetrical hindlimb stepping as the normal spinal cat, it was suggested that the adaptation to the neurectomy in the intact

cat included modifications at the spinal level and in descending supraspinal pathways. Such combined adjustments were also suggested by (Wolpaw, 1997) for operant conditioning of the spinal stretch reflex in monkeys, rats and in humans. Wolpaw's (1997) and our results after peripheral lesions, suggest changes in multiple sites, even after relatively minor interference or changes in the locomotor demand. Wolpaw (1997) suggests that one of the reasons for these complex interactions is to allow the incorporation of the new motor strategies emerging as a response to the lesion or to a new motor task, while preserving at the same time, other remaining functions, a process which probably underlies normal learning. Learning a motor task by complete spinal cats (Hodgson et al. 1994) was found to be task dependent (see also Viala et al. 1986 for training of spinal rabbits). A cat that was trained to walk on a treadmill could not perform well a standing task and vice versa. Cats that were subjected to alternating training program, improved their capacities in one task but did not retain their former capacities.

The importance of training is further emphasized in the studies of Chau et al. (1998a). Training combined with drug application, soon after complete spinalization, resulted in earlier expression of the hindlimb locomotion in complete spinal cats. Our results of drug application in partial spinal cats are also encouraging, showing that the action of certain drugs can be incorporated into the residual voluntary locomotor pattern and improve it. However, the drug studies also show that the effects, as in

the case of clonidine, depends on whether the lesion is complete and that the applied drug therapy with human patients should probably be specifically designed in relation to the type of the spinal lesion.

In summary, the studies in this thesis taken in perspective with former studies (Eidelberg, 1981, Afelt, 1974, Bem et al. 1995, Jiang and Drew, 1996, Nathan, 1994) show that integrity of even minimal parts of various tracts, whether located in the ventral or the dorsal quadrants of the spinal cord are probably sufficient to sustain locomotor function.

From the discussion up to now it is also evident that the nervous system implicated in locomotion can adapt to peripheral lesions and up to a certain extent also to complete spinal lesions. These results are important when implementing rehabilitation programs after lesions to the locomotor systems. According to our studies, it is therefore of importance to maintain and protect remaining axons after a spinal injury (through neuroprotection and better management) and enhance their residual locomotor functions to allow "within system plasticity" through application of adequate training programs to enhance "take-over" by other locomotor pathways. Pharmacotherapy is also suggested to be an important tool by which residual function can be improve, also allowing for better training. The different capacities of the partial spinal cat compared to the complete spinal, to adjust and retain a new motor tasks may indicate the important role of descending pathways in the integration of new acquired locomotor strategy into existing locomotor pattern after injuries.

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APPENDIX A

ɔcomotion of the Hindlimbs After Neurectomy of Ankle Flexors in tact and Spinal Cats: Model for the Study of Locomotor Plasticity

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rier, Lynda, Edna Brustein, and Serge Rossignol. Locomo- of the hindlimbs after neurectomy of ankle flexors in intact spinal cats: a model for the study of locomotor plasticity. *J. rophysiol.* 77: 1979–1993, 1997. To study the potential plastic- of locomotor networks in the spinal cord, an important issue locomotor rehabilitation after spinal injuries, we have investi- d the locomotor performance of cats before and after a unilat- denervation of the ankle flexors tibialis anterior (TA) and nsor digitorum longus (EDL) both in cats with intact spinal l and after spinalization. The effects of the inactivation of the e flexors were studied in three cats with intact spinal cord ng periods of 4–7 wk. Cats adapted their locomotor perfor- ce very rapidly within a few days so that the locomotor behav- ppeared to be unchanged practically. However, kinematic anal- of video records often revealed small but consistent increase nee and/or hip flexion. These changes were accompanied by e increase in the amplitude of knee and hip flexor muscle ity. Cats maintained a regular and symmetrical walking pattern the treadmill for several minutes. Two of these cats then spinalized at T13 and studied for ~1 mo afterward. Whereas nally cats regain a regular and symmetrical locomotor pattern spinalization, these cats had a disorganized and asymmetrical motor pattern with a predominance of knee flexion and absence lantar foot contact of the denervated limb. Another cat first spinalized and allowed to recuperate a regular symmetrical motor performance. Then it also was submitted to the same ateral ankle flexor inactivation and studied for ~50 days. The maintained a well-organized symmetrical gait although there almost no ankle flexion on the denervated side. There was no gerated knee hyperflexion and gait asymmetry as seen in the previous cats spinalized only after they had adapted to the rvation of ankle flexors. It is concluded that, after muscle rvation, locomotor adaptation is achieved through changes oc- ing at different levels. Because cats spinalized after adaptation ıe neurectomy had an asymmetrical locomotor pattern domi- d by hyperflexion, it is suggested that the spinal circuitry has ı modified during the adaptive process, presumably through action of corrective supraspinal inputs. Indeed spinal cats do ıormally display such abnormal hyperflexions, and neither did ɔne cat denervated after spinalization. On the other hand, be- e the modified locomotor pattern in the spinal state is not tional and contains only some aspects of the compensatory onse seen before spinalization, it is suggested that the complete tional adaptation observed in intact cats after peripheral nerve ıns may depend on changes occurring at the spinal and the aspinal levels.

RODUCTION

ıne of the major hopes in the field of locomotor rehabili- ɔn after spinal cord injury is that the combined approach harmacology, locomotor training, and functional electrical stimulation could lead to a gradual improvement of the residual locomotor capabilities (Barbeau and Fung 1994; Barbeau and Rossignol 1994; Dietz et al. 1994; Rossignol and Barbeau 1993, 1995; Wernig and Muller 1992; Wernig et al. 1995). The successful outcome of such rehabilitation strategy assumes that the locomotor control mechanisms have some degree of plasticity and that the improvement of the residual locomotor function can be retained. Various observations may argue for or against such locomotor plasticity.

It currently is believed, on the basis of a large body of experimental evidence from several animal species, that the basic locomotor pattern is generated largely at the spinal cord level and is regulated through afferent feedback and descending commands, as summarized in various reviews (Barbeau and Rossignol 1994; Delcomyn 1980; Grillner 1981; Pearson 1993; Rossignol 1996; Rossignol and Dubuc 1994). In that framework, one could ask how animals adapt their locomotion to new conditions such as damages to peripheral nerves, muscles, or joints and where do these adaptive changes take place? Although there is some evidence of anatomic plasticity (sprouting) in descending pathways and afferent pathways after spinal lesions or dorsal root sections (Goldberger and Murray 1982) that may underlie functional changes (Goldberger and Murray 1978), it is not clear, however, if the spinal locomotor generating circuits are modifiable functionally. Some of the evidence from cats (Forssberg and Svartengren 1983; Gordon et al. 1986) and rats (Sperry 1940–1942) indicates that, after transposing antagonist muscles of the ankle, the functions of the transposed muscles are retained, suggesting that there is little plasticity in the spinal locomotor mechanisms. For instance, the transposed ankle extensor muscle continues to discharge not in accordance with its new biomechanical function (ankle flexion) but according to its former function (ankle extension) and connectivity (hardwired networks).

On the other hand, there is the view that there can be some functional plasticity within certain simple spinal reflexes that may even persist after spinalization. Wolpaw has shown, with operant conditioning, that monkeys can be trained to upregulate or downregulate the monosynaptic H-Reflex and that this "learned" asymmetry may persist after spinalization (Wolpaw 1994; Wolpaw and Lee 1989; Wolpaw and O'Keefe 1984; Wolpaw et al. 1983a,b). Work on the flexion reflex in the cat also suggests some degree of plasticity even in spinal animals (Durkovic 1975, 1983; Durkovic and Damianopoulos 1986; Misulis and Durkovic 1982, 1984), as previously suggested (Shurrager and Culler 1940; Dykman and Shurrager 1956).

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Other types of evidence of spinal plasticity also have been tained from the study of locomotion in spinal animals. ter spinalization, kittens (Forssberg et al. 1980a,b) as well adult cats (Barbeau and Rossignol 1987, 1994; Belanger al. 1996), can walk with the hindlimbs on a treadmill, d the quality of such locomotion is a function of regular eight-bearing training (see also Edgerton et al. 1983; Smith al. 1982). Early locomotor training using clonidine, an pha-2 noradrenergic receptor agonist that can induce locotion in the first hours after a spinalization (Forssberg and illner 1973) also appears to accelerate the recuperation of comotion (Barbeau and Rossignol 1994; Barbeau et al. 93; Rossignol and Barbeau 1993). Finally, it has been stulated that there is specificity in the training of spinal ts such that cats trained to stand or to walk will have a tter standing or walking performance, respectively (Hodgn et al. 1994). This combined work then suggests some gree of plasticity within the spinal cord and more specifilly in the locomotor control mechanisms.

The muscle transposition paradigm described above may present an extreme case of the demand on the CNS for nctional locomotor plasticity, which requires the rewiring neural circuits to adapt the function of transposed muscles. her strategies may be more economical and efficient. As matter of fact, work on tendon transposition or axotomy specific hindlimb nerves (Gordon et al. 1980) often refer "tricks" (Sperry 1942) developed by the animal to comnsate for their neuromuscular deficit. For instance, to obvie the deficit in one joint due to muscle transposition of at joint, the animal can increase the excursion of other nts. Where are these tricks generated in the tripartite conl scheme for locomotion mentioned above? Indeed, when organism adapts its locomotor behavior to permanent anges in its limbs or joints, are the adaptive changes incorrated eventually within the spinal circuitry so that deending voluntary commands trigger spinal circuits, which eady have been modified to take into account the state of e peripheral apparatus? Another alternative is that these aptive changes are achieved by modifying descending the ntrol commands themselves to the spinal circuits so that ch step is modified appropriately to offset the deficit? In e first alternative, most changes would occur in the spinal rd, and in the second alternative, most changes would cur in the descending commands.

To study this question, we have investigated the locomotor havior in cats before and after a unilateral denervation of o large ankle flexors of one limb, tibialis anterior (TA) d extensor digitorum longus (EDL). After a period of aptation of treadmill walking with this new neuro-muscu- state, two of the cats were spinalized at T13, and their comotor performance studied for several days. In another t, the same neurectomy was performed after spinalization a time when the cat already had regained its locomotor pabilities. The data suggest that, in normal cats, adaptation such neuromuscular impairments involves changes both spinal and supraspinal levels. The results of this study have en presented in preliminary form (Carrier et al. 1992).

ETHODS

eneral experimental protocol

All the surgical procedures described below were reviewed and proved by the University Ethics Committee. Four adult cats, purchased through a local supplier approved by the university, were trained progressively during a period of 2–4 wk to walk on a treadmill, in a Plexiglas enclosure, at constant speeds ranging from 0.2 to 0.8 m/s so that they became accustomed to the laboratory environment and maintained a nearly constant position on the treadmill. Electromyographic (EMG) electrodes then were implanted and several control recording sessions of EMGs and kinematics were taken for several days to establish reliable baseline control values for each cat (43 for *cat 1*, 27 for *cat 2*, 33 for *cat 3*, and 12 for *cat 4*). Thereafter, the ankle flexor muscles TA and EDL were denervated and cats that were studied again for several days (51 for *cat 1*, 27 for *cat 2*, and 40 for *cat 3*). Two of the cats (*cat 1* and *cat 3*) were spinalized, and their locomotor performance studied for 24 and 32 days, respectively. In *cat 4*, the order was reversed; the cat was spinalized 12 days after implantation, the neurectomy was performed 22 days after the spinalization, and the cat was killed 52 days later. *Cat 2* was used only to investigate the effect of neurectomy and was not spinalized. The number of recording sessions in between these events also varied from 3 to 18 depending on cats. Only certain recordings on specific days were used for complete analysis and display.

Training procedures after spinalization

From 3 to 4 days after spinalization, training of the spinal cat was performed one or twice daily (a recording session could replace a training session). Cats were trained to walk with their forelimbs standing on a platform placed a few centimeters above the belt and a Plexiglas separator placed so that the hindlimbs would not impede each other as can happen when cats have an exaggerated adduction after spinalization. In the early days after spinalization the weight of the hindquarters was supported partially by holding the tail, and locomotion was facilitated by manual perineal stimulation. In the two cats in which neurectomy was performed before spinalization, clonidine 150–200 μg/kg was given intraperitoneally on four occasions to initiate walking movements (Barbeau et al. 1987) mainly in the first week postspinalization so that the expression of the locomotor pattern could be studied during that period without further plastic changes that could be brought about by the subsequent intensive training.

Surgical procedures

EMG IMPLANTATION. The general methods have been described earlier (Belanger et al. 1996). After fasting overnight, cats were premedicated with Acepromazine (Atravet, 0.1 mg/kg sc) and atropine (0.05 mg/kg sc) and then given a dose of pentobarbital (Somnotol 35 mg/kg iv). Penicilline G (40,000 U/kg im) also was given at the time of surgery. Up to 14 muscles were implanted chronically. Teflon-insulated stainless steel wires (AS633, Cooner Wire, Chatsworth, CA) were soldered to two 15-pin connectors (TRW Electronic Components Groups, Elk Grove Village, IL) cemented to the skull of the animal and the other end of the wires was led subcutaneously to selected muscles. After removing the insulation for ~3 mm of the wires inserted in the muscle belly itself, each wire was attached individually by a silk thread at its entry in the muscle, and both wires were tied together at their exit from the muscle belly. The following muscles were implanted on both sides (i = ipsilateral to the camera, usually the left of the animal; co = contralateral): flexor muscles, IP, iliopsoas; Srt, sartorius anterior; St, semitendinosus and extensor muscles; Glu, gluteus; VL, vastus lateralis; GL, gastrocnemius lateralis; and GM, gastrocnemius medialis. Before denervation or spinalization, several recording sessions ensured that the EMG signals were stable and consistent so that the changes seen after such procedures were not due to changes in recording conditions. After surgery, cats were placed in their individual cage with an infrared light to maintain temperature overnight. Buprenorphine hydrochloride (Temgesic,

05–0.01 mg/kg im) was given for 24–48 h every 6–8 h. Amoxline (Amoxil 22 mg/kg) was given twice a day orally for a 10-' period.

JRECTOMY. The neurectomy was performed also under gen- l anesthesia as described above. The common peroneal nerve the left of the animal was dissected just below the lateral aspect he knee so that muscular branches going to the anterior part of leg and innervating TA and EDL could be identified. Two ıtures were placed, and the nerve was cut in between. The ximal stump was enclosed in a polymer cap to prevent regrowth. : superficial peroneal nerve was preserved. In a terminal experi- ıt in *cat 1*, we decerebrated the animal and stimulated the ımon peroneal nerve above the lesion with strengths of ≤300 , and, when the limb was pendant, the prominent response was nee flexion sometimes accompanied by what appeared to be a y small ankle flexion of a few degrees only. When the limb ; flexed and the knee joint kept in one position, there was no ı of ankle flexion after stimulation. At autopsy, the nerves were ;ected to confirm the intended lesion.

NALIZATION. The spinalization was performed at T13 also er barbiturate anesthesia and with postoperative analgesia. The ıal cord was sectioned completely with ophthalmic scissors after ning the dura, and the wound was filled to the sides and to the :om of the vertebral canal with pieces of oxidized regenerated ulose (Surgicel). Cats were kept in separate cages and were led manually twice a day (Belanger et al. 1996). They were ıned regularly and weighed and examined by a veterinarian at :t once a week. Necessary blood samples and antibiograms were formed through our University Health Services.

:ording and analyses

3. The EMG signals were amplified differentially using a d-pass filter of 100 Hz to 3 kHz and recorded on a 14-channel S tape recorder (Vetter model 4000A with a typical rise time alf-amplitude of 200 μs at the speed of recording). The EMGs e played back on an electrostatic polygraph (Gould 1000) and :cted sequences were digitized at 1 kHz using a PDP 11/34 ıputer. An interactive custom made EMG analysis program was l to extract cycle and EMG burst values for statistical analyses display. Furthermore, the EMG bursts could be averaged and ıalized over many cycles using either one of the EMGs or a :matic event such as paw contact as a trigger.

EMATICS. To analyze the kinematics of locomotion, six light- :cting disks (made from 3 M reflecting tape) were stuck on tip of the iliac crest, the great trochanter, the knee's lateral dyle, the lateral malleolus, the fifth metatarsophalangeal joint, the tip of the third toe. The cat walked freely on the treadmill, ıg connected to the equipment only through the flexible cables n the head plugs to the preamplifier input box. Locomotion was ırded using a Digital 5100 shutter camera and a Panasonic AG 0 video tape recorder. With an exposure time varying between and 2 ms, it was possible to have a sharp image of the reflective kers for every field, thus a time resolution of ±1 field (16.7 . Using a two-dimensional peak performance system, the six ıts were digitized for every field. From the x and y coordinates ach point, the kinematics of locomotion could be reconstructed displayed as stick diagrams, trajectories of the points, or joint ular excursions of the hip, knee, ankle, metatarsophalangeal TP) joints. In all angular displays, flexion is downward except the MTP joint where dorsiflexion of the toes corresponds to an 'ard deflection of the trace. Other events, such as paw contact lift off, were identified and used to align normalized joint ırsions or EMG signals. Finally, the EMG signals and the video ges were synchronized using a SMPTE time code (time code erator Skotel TCG-80N and time code reader TCR-80N) re- led simultaneously on the analogue tape containing the EMG data as well as on one of the voice channels of the video tape and on the image itself.

RESULTS

Kinematics and EMG changes after neurectomy

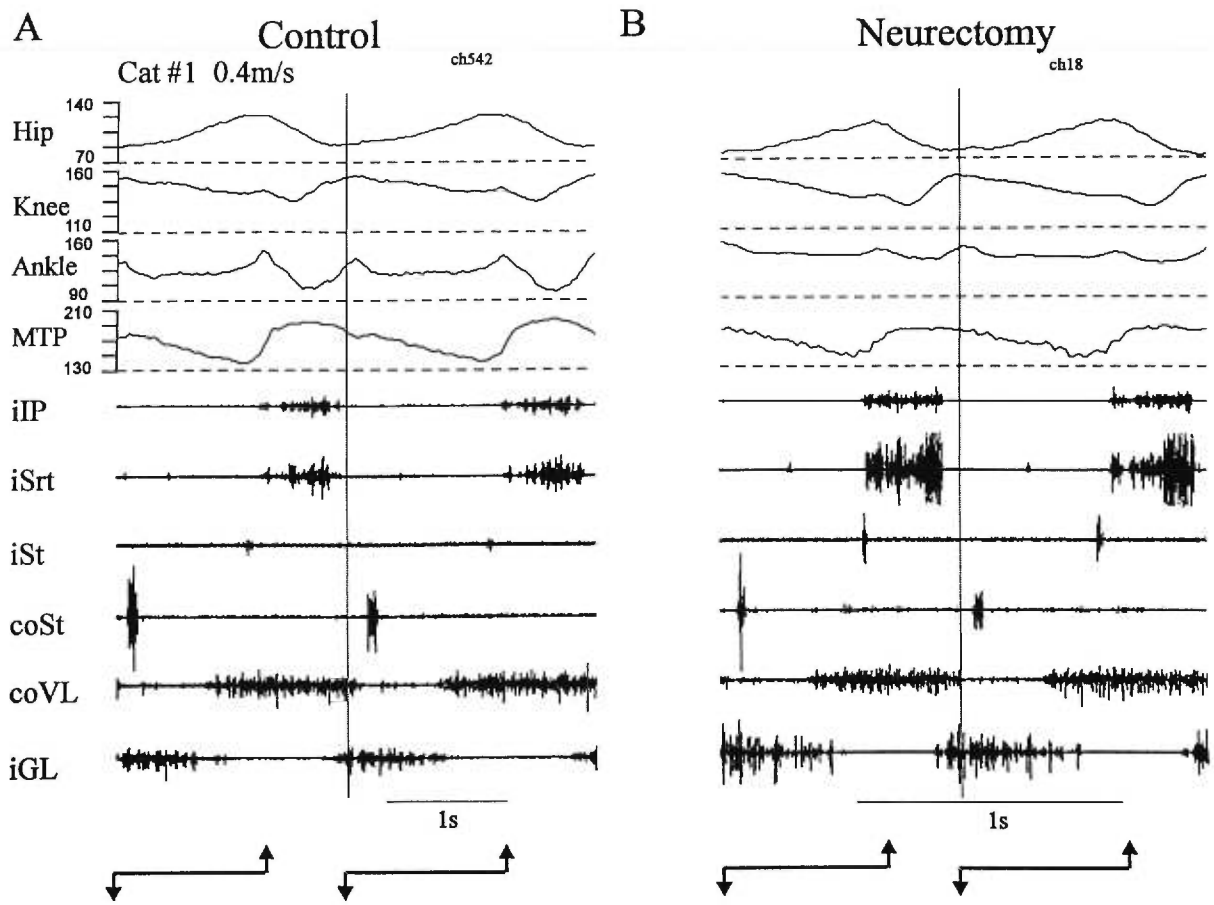
All cats were tested on the treadmill 2–3 days after the neurectomy. We were struck by the fact that all cats showed very little locomotor deficits at slow walking speeds of 0.3–0.5 m/s. They had more difficulty walking at moderate speeds of 0.7–0.8 m/s and tired more rapidly. Careful visual observation suggested a decreased ankle flexion but a more conspicuous hip abduction during swing. This hip abduction disappeared after the first week in all but one cat, in which it persisted at a low level.

Figure 1 shows two step cycles at 0.4 m/s taken during the control period (Fig. 1*A*) and 25 days after the TA-EDL neurectomy (Fig. 1*B*). In Fig. 1*A*, the synchronized kinematics and EMGs illustrate a normal locomotor pattern. Just before lift-off, the knee flexor St discharges as a very brief burst, which, together with foot muscles (not illustrated), would clear the paw from the ground and start the swing phase. The hip, knee, and ankle flex while the toes dorsiflex during the first half of swing, a phase referred to as the flexion phase (F) in the step cycle defined by Philippson (1905). During this flexion period, the hip flexors, IP and Srt, start their discharge, which continues into the next phase, the first extension phase (E1), during which the knee and ankle extend to contact ground for the next stance phase. Extensor muscles, such as iGL, are activated just before foot contact, which starts the second extension phase (E2), and are active for most of the stance phase.

After the neurectomy of TA and EDL, the kinematics changes of the various joints (except for the ankle) were subtle (Fig. 1*B*) and hardly could be detected by eye only. As expected, there was a marked reduction, but not a complete abolition, of ankle excursion (from 30 to 13°, see Table 2) and a reduced dorsiflexion of the MTP joint (from 66 to 43°, see Table 2). The knee and hip showed an increase of peak-to-peak excursion of only a few degrees. These small kinematic changes, however, were accompanied by an increase in the amplitude of the St EMG, as well as a marked increase in the amplitude and duration of the hip flexor Srt. The delay seen normally between the onset of ST and the onset of IP or Srt was reduced after neurectomy (compare Fig. 1, *A* and *B*; see also Fig. 4), and the onset of the hip flexor discharge was more abrupt.

The cycle duration and structure was similar for the same belt speed (0.4 m/s) before and after neurectomy when the animal had recovered fully as seen in Fig. 1. Table 1 shows that the cycle duration pre- and postneurectomy was similar for the same belt speed in *cat 1*. In *cat 2*, the overall cycle remained also the same although there was a small (but significant) increase in the swing phase duration. In *cat 3*, the cycle duration was decreased significantly from 996 to 851 ms due to a shorter stance. This decrease of cycle duration, although initially seen at 0.6 m/s, recovered after 4 wk so that pre- and postneurectomy step cycles were the same.

Figure 2, *A* and *C*, illustrates one step cycle before and after neurectomy in a stick figure format for the same cat. Again the changes are very subtle if any. There is a significant reduction in ankle and MTP excursion as well as a



3. 1. Kinematics and electromyographic (EMG) activity of locomotion at 0.4 m/s before and after neurectomy of lis anterior (TA) and extensor digitorum longus (EDL) in *cat 1*. In *A*, 2 consecutive cycles from control period are trated. For hip, knee, and ankle, downward deflection of traces indicate flexion whereas for metatarsophalangeal (MTP), ard deflection indicates plantar flexion. Note that for knee, apparent flexion occurring during stance is due to a gradual ıage of joint marker due to skin movements. ↓, paw contacts, ↑, paw lifts, ——, stance period. Vertical lines are aligned downgoing arrows. In *B*, similar displays taken from 2 cycles 25 days postneurectomy. Gains of EMG amplifiers were : before and after neurectomy.

ıewhat greater flexion of the knee at the end of the swing se after neurectomy. The overall shift of the hip toward :e flexion is due to a more ventral placement of the light ective disk at the iliac crest. In the angular traces (Fig.), the total peak-to-peak hip excursion is the same as in . 2*B*, but a study of the first derivative of such traces icated a small increase in hip flexion velocity after neu- omy. In this example, the angular traces of the knee joint ore and after neurectomy can be superposed, although knee is somewhat more flexed at the onset of E1. Table ıows that for the hip and knee, the peak-to-peak excur- ıs (dif) are very similar before and after neurectomy. hough the ankle and MTP joints excursions are reduced, cat managed to clear the tip of the foot from the belt as n in the trajectory of the foot point in Fig. 1*C*. Thus by y small step-by-step adjustments that tend to get blurred he averaging process, the animal managed to clear the t from the ground. In *cat 2*, the changes observed were ıewhat more pronounced. For example, both before and :r neurectomy, the hip reached a maximum extension of 20° but reached a peak flexion of 85° after neurectomy ıpared with 95° before. Similarly, the overall peak-to- k excursion of the knee was 33° compared with 27° before neurectomy. In *cat 3*, the total peak-to peak excursion of the hip was increased by 4.5° and the knee by 5–6°. These changes were consistent and were augmented with increasing speed of the treadmill.

In Fig. 2, *A* and *C*, the trajectory of the foot marker below the stick figures also shows a somewhat greater vertical component after neurectomy. The vertical position of the toe point or the marker placed on the MTP joint was measured during the swing phase for the three cats, and it was found that the foot was raised up to 5–7 mm more during swing than in the control period. In summary, then, it appears that through the combined effects of small increases in the flexion of hip and/or the knee, the animals could raise the paw a few millimeters higher during the swing to compensate for the reduced ankle flexion.

Figure 3 shows the averaged EMG of >110 cycles taken in different control recording sessions and a similar number of cycles postneurectomy at a speed of 0.7 m/s and mainly serves to illustrate the overall envelope of the EMG bursts. There was a marked increase in amplitude of all flexor bursts on the ipsilateral side whereas the changes in contralateral flexors were minimal (coIP, coSrt) or absent (coSt). It should be noted that St had a small second burst of activity

BLE 1. *Changes in overall cycle, swing, and stance ation*

Condition	*Cat 1*	*Cat 2*	*Cat 3*	*Cat 4*
trol				
	23	18	72	9
ycle	1,009 ± 83	1,004 ± 74	996 ± 107	1,010 ± 70
wing	313 ± 32	257 ± 18	316 ± 42	302 ± 38
ance	696 ± 63	747 ± 73	680 ± 90	708 ± 72
neurectomy				
	25	25	10	NA
ycle	953 ± 85	1,068 ± 79	851* ± 55	
wing	286 ± 44	305* ± 35	324 ± 40	
ance	667 ± 62	763 ± 61	527* ± 32	
spinal				
	18	NA	6	11
ycle	621 ± 43		775 ± 172	726* ± 21
wing	309 ± 43		226* ± 54	201* ± 18
ance	312* ± 42		547 ± 151	526* ± 23
spinal				
stneurectomy				
	NA	NA	NA	6
ycle				672* ± 16
wing				243* ± 18
ance				428* ± 18

alues of means ± SD are in ms. Changes are presented for all conditions l cats walking at 0.4 m/s. The statistical significance ($*P < 0.01$) of ges in a given condition is established in relation to the immediately eeding condition.

before foot contact that was clear at 0.7 m/s after neurec- y. Changes in the timing were also noticeable. Whereas he control state, the IP and Srt clearly discharged later ı St, the delay was reduced after neurectomy and even ersed at higher speeds (see also Fig. 4). It is also clear activity of the ipsilateral extensor muscles was increased ıewhat, especially the ankle extensors (iGM and iGL) the hip extensor (iGlu).

he interlimb coordination remained very good after neu- omy as demonstrated by the onset of the coSt, relative St, which remained at ~50% in Fig. 3 (see also Fig. 8 a slower speed). This further suggests that the animal did limp or else underuse its lesioned limb during walking. reover, the animal could adapt its gait to overcome an tacle [a cylinder placed on the treadmill belt (Drew 8)]. In the intact state, the hindlimb was brought progres- ly forward in a smooth trajectory to step over the obsta- After neurectomy the animal successfully negotiated the tacle by making a larger flexion at the hip and knee joints. ıough this voluntary gait adaptation was not studied sys- atically, the results clearly indicated that compensatory hanisms were adequate to adapt the step characteristics xternal demands.

ı summary, then, after a unilateral TA and EDL neurec- y, cats adapted very well their locomotor pattern through inimal increase in knee and/or hip flexion resulting from ncrease in the amplitude of discharge of the knee and hip ors. The cats maintained a well-coordinated locomotor ern, and, by casual observation only, it was difficult to ct any locomotor deficit.

ınges after spinalization

he changes in the locomotor pattern seen after spinaliza- were dramatic. Indeed, from previous experience (Barbeau and Rossignol 1987; Belanger et al. 1996), cats usually have a well-established regular and symmetrical locomotor pattern of the hindlimbs 3 wk after spinalization (see also Fig. 9, *D–F*). In *cat 1* (Fig. 5), 19 days after spinalization, on the lesioned side, the hip remained more or less at 120–130° while the knee performed large flexion movements (52 vs. 34° after neurectomy; see Table 2) powerful enough also to swing the ankle (46 compared with 13° postneurectomy; see Table 2). The MTP joint remained ventroflexed during the whole cycle, and the foot did not make any plantar contact. The activity in IP and Srt was sustained throughout these prolonged swing phases whereas the activity in St was more discreet but much more prolonged than usual and certainly much longer than on the contralateral side. The ankle extensor GL started its discharge quite early before foot contact and was not maintained after foot contact, which was made on the foot dorsum. The locomotor pattern of the contralateral limb was not as well organized as normal presumably because of the very abnormal signals it received from the denervated limb. This resulted in a very asymmetrical locomotor pattern best seen in Fig. 5, which shows abnormally long swing phases (even longer than the stance phases) on the denervated side, and the reverse in the normal leg, which has to maintain weight for a greater proportion of time, the ipsilateral limb being incapable of supporting any weight. This asymmetry even increased as time progressed after spinalization (interlimb coupling going from $\phi 0.4$ to $\phi 0.8$). In *cat 3,* which also was submitted to the same sequence of neurectomy followed by spinalization, the results were very similar with a predominance of knee flexion (see Table 2). It is also worth noting that the disorganization of the movement is reflected in the very large SD, especially in *cat 3*.

This abnormal locomotor pattern and the asymmetry is well illustrated by the stick figures and averages of angular excursions shown in Fig. 5. During stance, the dorsum of the foot of the lesioned limb contacted the belt and the MTP joint plantarflexed. During swing, the more or less synchronous flexion at all joints results in an oval-shaped foot trajectory. On the intact side, the steps cycle were somewhat more normal as can be seen from the stick diagrams and foot trajectory (Fig. 5*C*). We will not attempt to describe these pathological locomotor patterns in much further details for each cat except to say that the walking pattern was, at times, even more abnormal with the lesioned limb performing two small incomplete steps for one step on the contralateral side.

The examples shown above were taken from the period where the cats had stable albeit abnormal locomotor performance. However, these anomalies could be seen earlier after spinalization. The first recording sessions were made 3–5 days after the spinalization. From previous experience, 5 days after spinalization cats have only small symmetrical rhythmic movements of the hindlimbs usually with the hip in extension (Belanger et al. 1996). In the cats presented here, the movements were disorganized and asymmetrical with frequent hyperflexions of the knee and hip on the lesioned side, and strong perineal stimulation was required to evoke movements. Only a few steps could be obtained at 0.2 m/s. To facilitate the induction of the locomotor pattern as early as possible after spinalization, clonidine (200 μg/kg ip) was injected (Barbeau et al. 1987); the results are

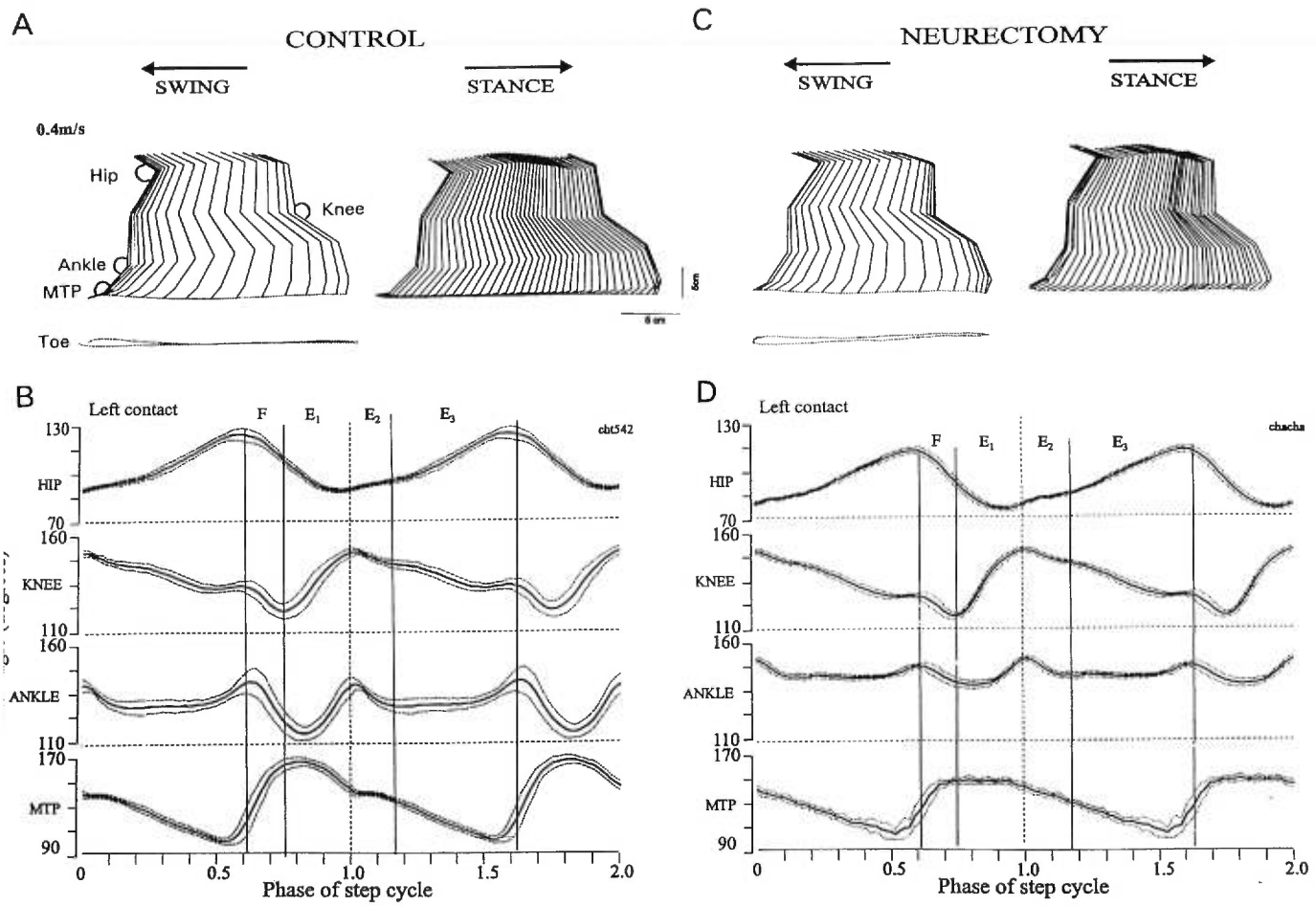
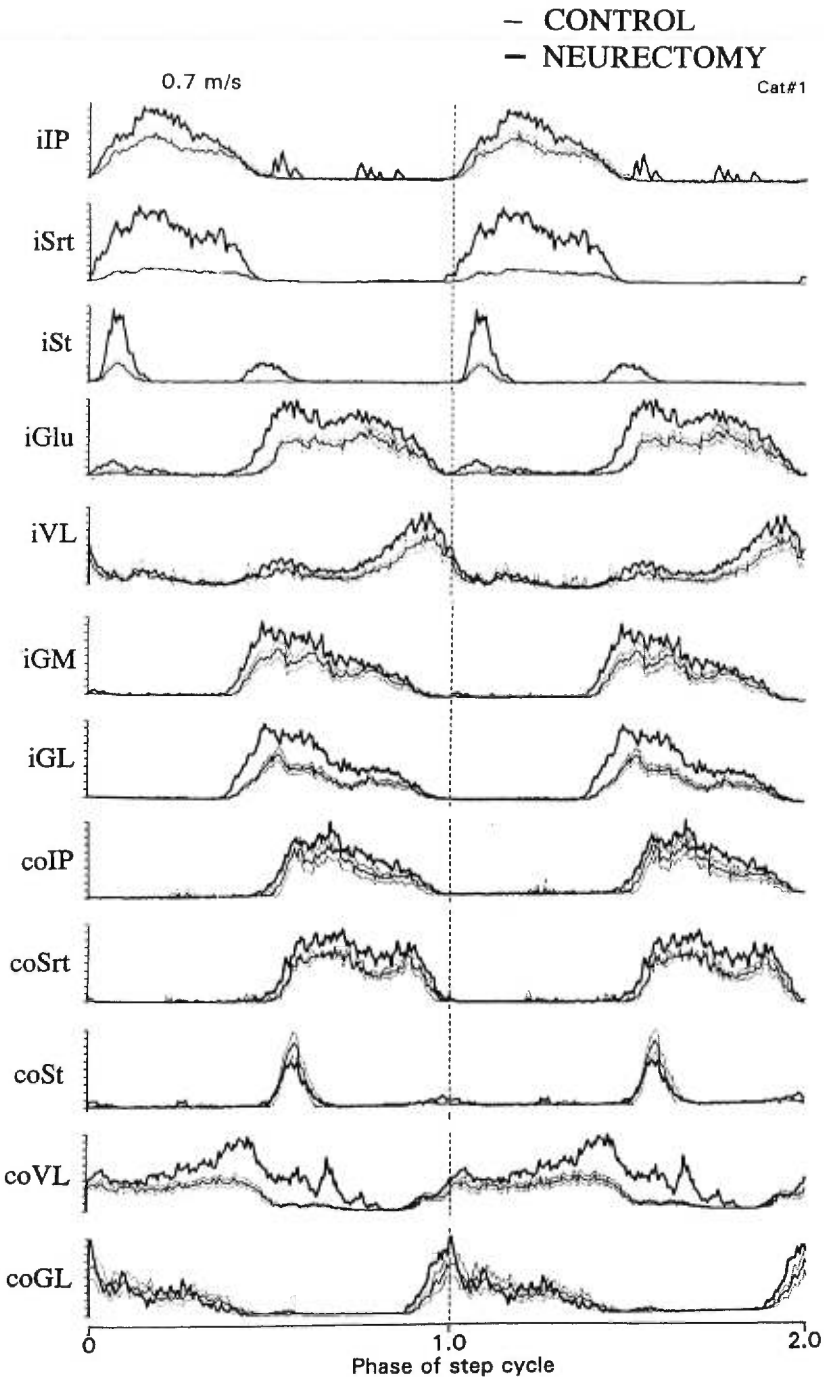


FIG. 2. Kinematic of same control and neurectomy recording sessions as in Fig. 1. *A* and *C*: stick figures of 1 step cycle ı treadmill together with trajectory of toe point for swing and stance. In this display, each figurine of hindlimbs is displaced / an amount equal to linear displacement of paw between video fields so that horizontal calibration is larger than vertical ıe. *B* and *D*: averaged and standard deviation of angles calculated in 7 cycles before and 10 cycles after neurectomy are 'nchronized to left paw contact. Same step cycle is presented twice for more clarity. Philippson's subdivisions of the step /cle (F-E_1-E_2-E_3) are presented on *top*.

ABLE 2. *Angular maxima (Max), minima (Min) and differences (Dif) between Max and Min for the four joints in all cats for all ɔnditions*

	Number of Cycles	Hip			Knee			Ankle			MTP		
		MAX	MIN	DIF	MAX	MIN	DIF	MAX	MIN	DIF	MAX	MIN	DIF
at 1													
Control	7	124 ± 3.7	88 ± 1.2	36	152 ± 1.6	120 ± 3.2	32	144 ± 1.9	114 ± 3.9	30	167 ± 4.8	101 ± 3.1	66
Neurectomy	10	109 ± 2	75 ± 1.5	34	150 ± 1.5	116 ± 1.6	34	151 ± 1.8	138 ± 2	13	149 ± 3	106 ± 3	43
Spinal	18	133 ± 2.3	118 ± 1.5	15	149 ± 1.7	97 ± 3.4	52	200 ± 2.1	154 ± 5.2	46	244 ± 4.3	201 ± 2.4	43
at 2													
Control	8	121 ± 1.9	95 ± 1	26	137 ± 0.8	110 ± 1.4	27	145 ± 4.3	114 ± 1.1	31	177 ± 2.2	145 ± 2.2	32
Neurectomy	8	120 ± 3	85 ± 1.1	35	140 ± 1.8	107 ± 3.2	33	158 ± 2.6	135 ± 1.2	23	152 ± 1.3	113 ± 6	39
at 3													
Control	7	112 ± 3.1	88 ± 1.3	24	130 ± 1	104 ± 2.4	26	150 ± 3.8	121 ± 2.1	29	144 ± 7.8	93 ± 2.4	51
Neurectomy	9	107 ± 3.5	79 ± 2.2	28	138 ± 1.7	107 ± 6.6	31	150 ± 3.2	137 ± 3	13	155 ± 4	105 ± 4.3	50
Spinal	6	104 ± 6.7	79 ± 8.6	25	118 ± 4.9	83 ± 6.4	35	181 ± 1.2	165 ± 6.3	16	237 ± 6.9	187 ± 3.9	50
at 4													
Control	6	123 ± 4	102 ± 2.6	21	139 ± 3.1	108 ± 5.2	31	137 ± 5.1	114 ± 6	23	157 ± 12.8	117 ± 4.1	40
Spinal	11	120 ± 1.4	98 ± 0.7	22	132 ± 2.2	101 ± 1.2	31	137 ± 1.2	122 ± 1.7	15	157 ± 3.6	118 ± 2.1	39
Neurectomy	6	110 ± 3.4	92 ± 3.1	18	137 ± 1.7	106 ± 2.7	31	182 ± 1.1	154 ± 4.4	28	272 ± 4.3	171 ± 4.2	101

Values are means ± SD in ms. All values were obtained from the reconstructed kinematics of several cycles recorded on video tape at 0.4 m/s. MTP, metatarsophalangeal.



3. 3. Averaged EMG recordings before and after neurectomy at 0.7 m/s. Averages are taken from 3 control sessions ans ± 1 SD of 37–113 cycles depending on EMGs) and 3 postneurectomy sessions (days 7, 26, and 46 postneurectomy). ages are triggered on ipsilateral flexor semitendinosus muscle (iSt) and are normalized with respect to time and are ated for 2 cycles.

sented in Fig. 6. Whereas the nonlesioned side performed er normal movements (not illustrated), the lesioned side strated here had very abnormal movements. As can be n in the stick figures, the knee performed brisk hyperflex- movements more or less synchronous with hip flexions hat the limb was not brought forward and the paw landed he dorsum of the toes and remained in that position for rest of the stance, much as it was seen later without gs as described above. The average EMG traces compares the activity in the control period (before neurectomy) and after neurectomy and spinalization. Very large almost synchronous bursts of activity are seen in all flexor muscles. Note that the activity in coSt starts at about $\phi 0.5$ and peaks at $\phi 0.7$, somewhat later than before spinalization, indicating that some symmetry was preserved at this early postspinal stage. This example thus illustrates that the abnormal movements described in previous figures, taken at day 19, already were present 6 days after spinalization, well before the cat

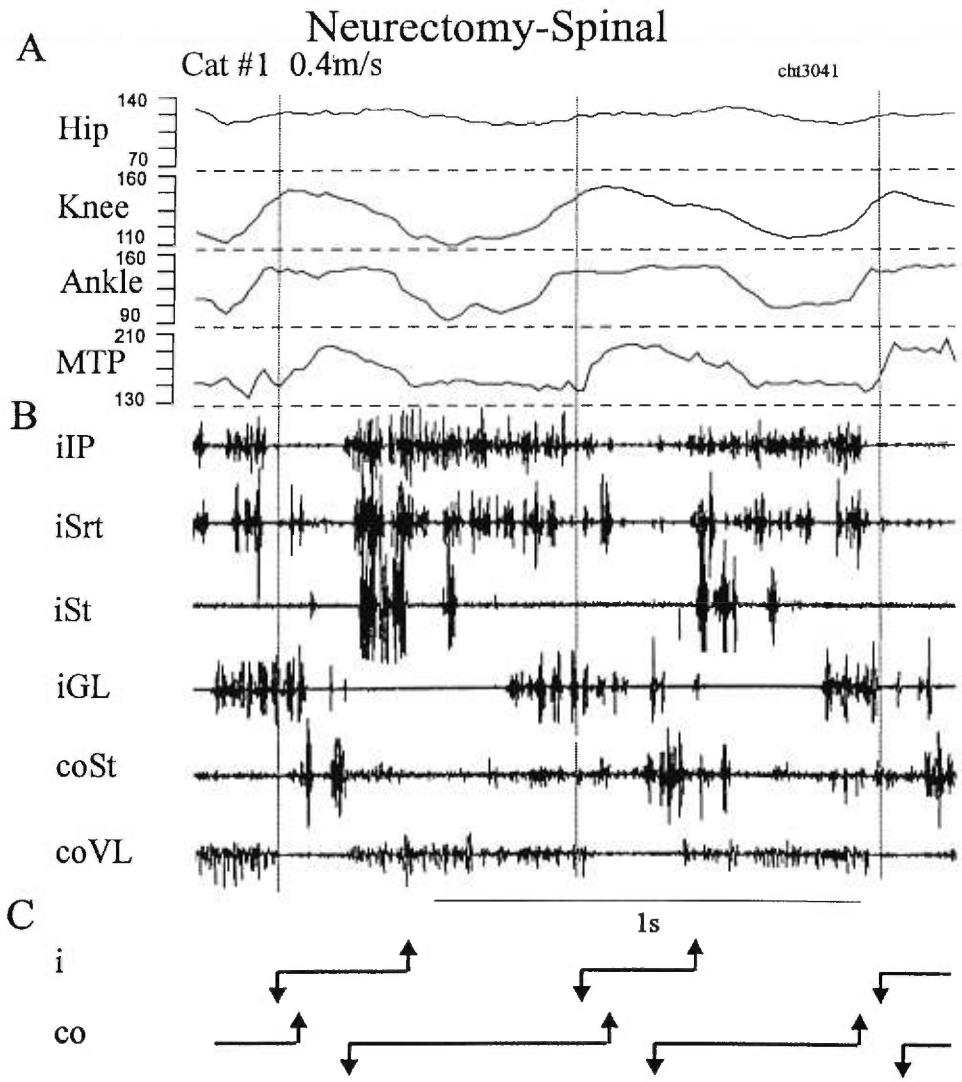
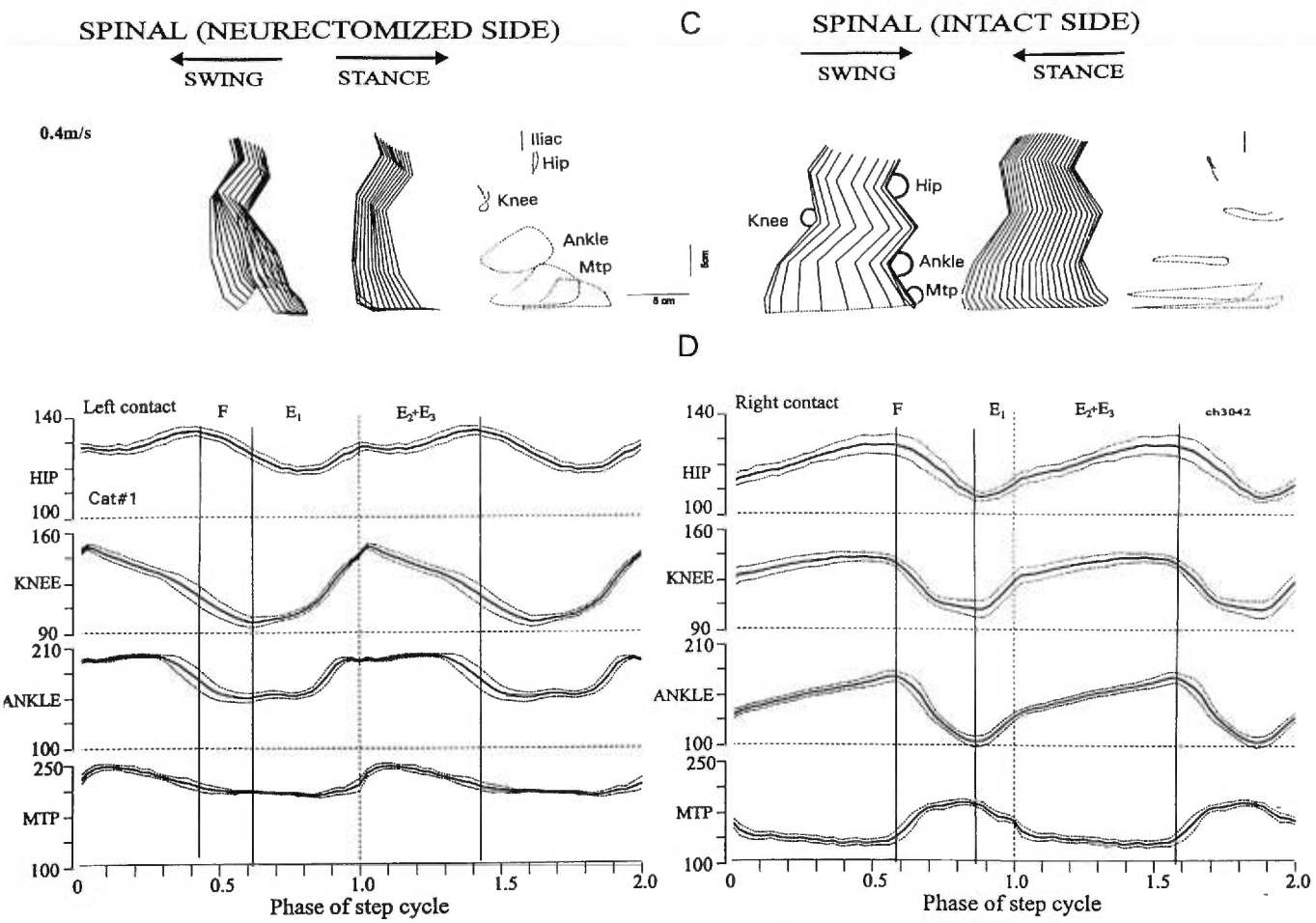


FIG. 4. Two consecutive cycles taken 19 days after spinalization of a neurectomized cat. Presentation is the same as in g. 1 except for duty cycle shown here for 2 hindlimbs. Note marked asymmetry between 2 sides.

ıd any significant locomotor training on the treadmill. We ıve repeated this experiment three times in *cat 3* and essen- ally obtained the same results. We thus could conclude that ıe abnormal pattern seen at a later stage was not due to an ɔnormal training because of the neurectomy but rather that ıe abnormal pattern was there very soon after the spinaliza- ɔn, suggesting that clonidine merely expressed an abnormal ıcomotor pattern already extant in the spinal cord.

The evolution of the EMG patterns as recorded with the ıme gains over the three conditions, i.e., control, postneu- ːctomy, and spinal-postneurectomy at the same speed (0.4 ı/s) is illustrated in Fig. 7. Activity of the ipsilateral flexor ıuscles was increased substantially after neurectomy hereas the contralateral St remains similar in both ampli- ıde and timing. Activity of the extensor muscles also was ıcreased, and there was more overlap between the extensor ːtivity of both legs. After spinalization, there is not only a ıarked asymmetry (see displacement of coSt burst relative ı iSt), but the flexor muscles showed a greater increase in ıplitude and duration, and there was a substantial cocon- action between the hip flexor muscles and ankle extensor muscles. The combined timing and amplitude changes for the three conditions are illustrated more quantitatively in Fig. 8 for *cat 1* at a speed of 0.4 m/s, a speed at which all conditions could be compared.

To evaluate the effect of the ankle flexor neurectomy in a spinal cat, the neurectomy was performed in *cat 4* after it had been spinalized and had recovered hindlimb locomotion. Figure 9*A* shows one normal step in the control period with stick diagrams, trajectories, angular displacements, and EMGs. After spinalization, the swing was very smooth and the stance is shorter compared with normal, an common observation in spinal cats (Belanger et al. 1996). After neurectomy, the locomotor movements were symmetrical, regular, and smooth albeit with only very small flexion of the ankle and absence of plantar foot contact. The large knee hyperflexions seen in the spinal cats that had undergone neurectomy before spinalization were absent in this cat neurectomized after the spinalization. This suggested that the anomalies seen in the former two cats reflected spinal changes that had occurred during the period of adaptation before the spinalization.



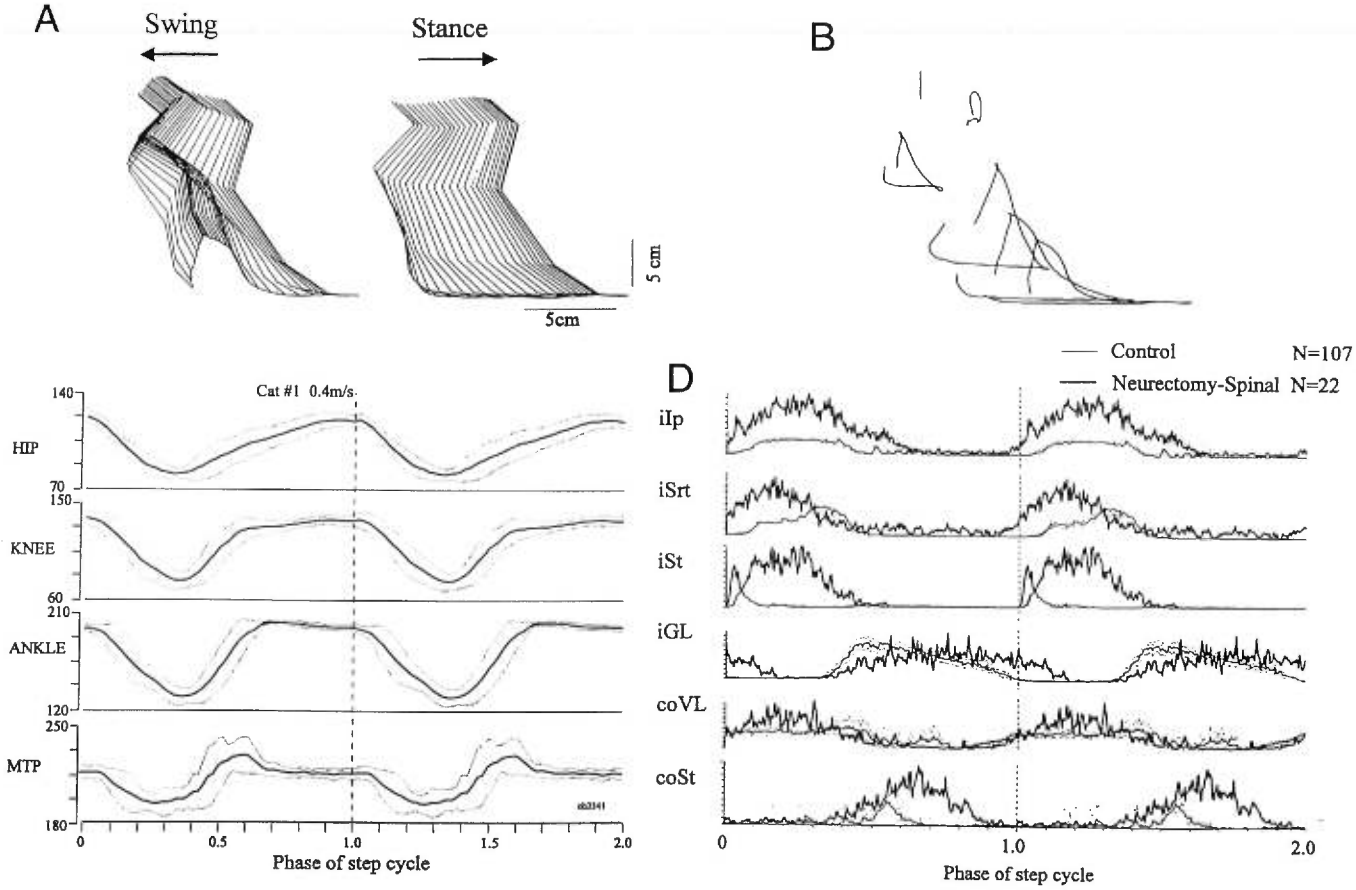
IG. 5. Kinematic after spinalization of a neurectomized cat; same presentation as in Fig. 2. *A* and *C*: stick figures; *B* l *D*, $n = 9$ and 13, respectively.

SCUSSION

nsequences of neurectomy

The results of these experiments show that, in normal nditions, cats adapted their locomotor pattern very well er the inactivation, by neurectomy, of two major ankle xors TA and EDL. This adaptation involved small in- ases in knee and/or hip flexion just enough to clear the w well above ground during the swing phase. The kine- tic compensation was subtle and difficult to perceive by ual observation only. The EMG analyses showed that ere was an increase in the knee flexor St and hip flexors and Srt. Therefore, the compensation is achieved mainly changes occurring at more proximal joints, as described o by others. It indeed has been reported previously, al- ough not analyzed in any detail, that after section of the ole common peroneal nerve, cats had a pronounced foot op and compensated through a greater hip flexion (Gordon al. 1980). In other circumstances, compensation also uld take place in synergist muscles. For instance, in rab- s, progressive section of the tendons of ankle plantarflex- s on one side led to a prolongation of the stance, hyper- xion during swing, and landing on the heel (Stewart 37). When one of the muscles was biomechanically dis- nnected after tenotomy, other synergist muscles compen- ted so that several muscles have to be removed or tenotom- ized before any biomechanical changes could be seen in the locomotor pattern. Similar compensatory mechanisms have been described after the section of nerves to specific ankle extensors (Gordon et al. 1980; Wetzel et al. 1973; Whelan et al. 1995). Although TA and EDL are considered as the main flexors of the ankle, peroneus longus, which was not denervated in our experiments, is also an ankle flexor (Lawrence et al. 1993; Nichols 1994) with a significant moment arm in the plane of flexion when the ankle joint is flexed already such as during swing (Young et al. 1993). This might account for the fact that we still could measure ankle flexion during swing although some of this flexion also might be due to inertial forces, especially when the hip flexion movements were very brisk. This residual flexion was, however, insufficient to compensate for the inactivation of TA and EDL over the time span of the experiments, and therefore compensation from the more proximal flexors, as described above, was needed.

The section of a motor nerve also has sensory consequences. Denervation of TA and EDL eliminates potentially important sensory information from these muscles. Recent data show that stimulation of afferents from TA, possibly from group I and group II afferents, during the flexion phase of fictive locomotion, can reset the cycle to extension (Perreault et al. 1995). It was postulated that these effects could be due to group II spindle secondaries. It then should be



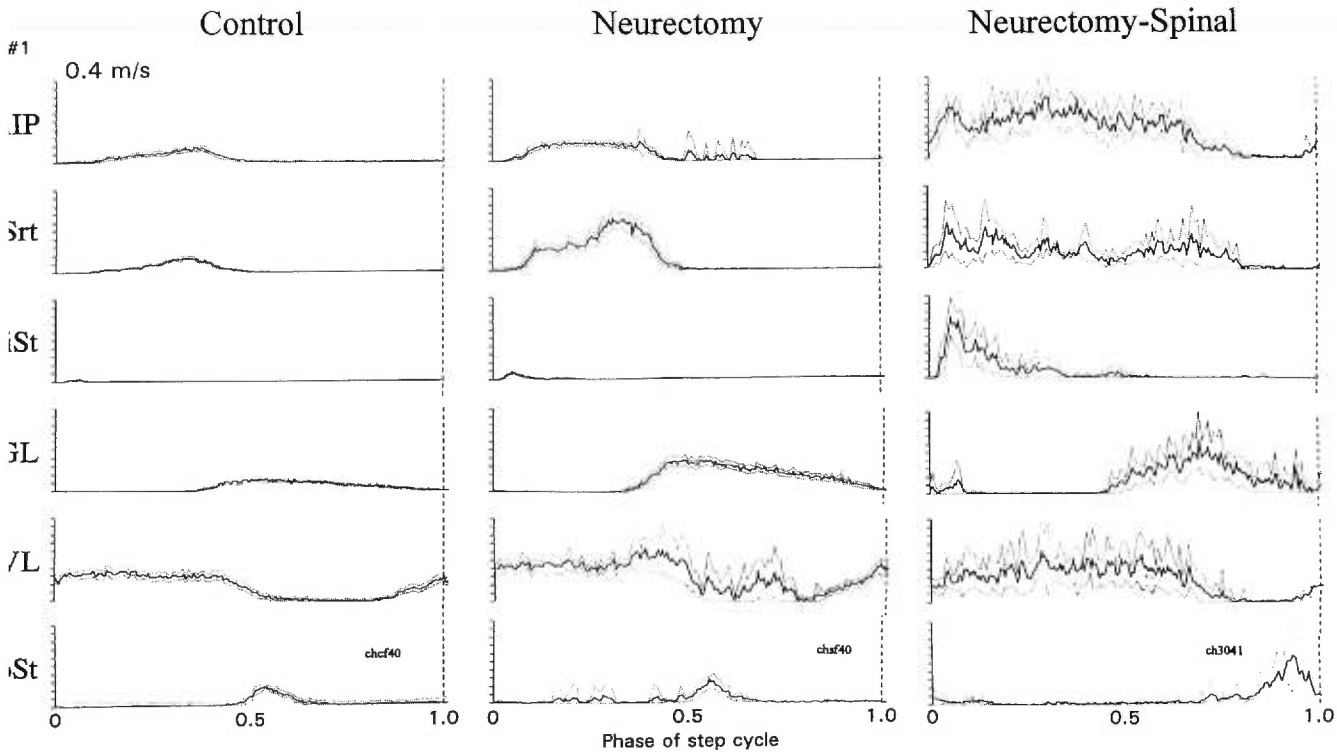
IG. 6. Kinematic and EMG recordings pre- and postneurectomy in *cat 1*, 6 days after spinalization and with 200 μg/ ip of clonidine. *A*: stick figure of swing indicates large flexion of knee and hip. *B*: trajectories of marker points. *C*: ematic analysis of 8 cycles is triggered on forward movement of paw although paw has not lifted yet because of foot g. *D*: EMGs of control (prespinalization) and postspinalization postneurectomy are superimposed and triggered on onset St, which is close to but not synonymous to onset of swing as seen in Fig. 1*A*.

pected that, normally, stimulation of these afferents during nce where flexor muscles are stretched would provide ne positive feedback to extensors and prolong the stance ase (indeed described for Srt stimulation but not for TA l PBSt). In absence of these afferents, this mechanism ıld participate in the decrease of the step cycle mainly e to a shortening of the stance phase seen in one of the s at least in the first few weeks postneurectomy.

nctional plasticity

The above results show that cats adapted their locomotor tern very well after the denervation of ankle flexors ough compensatory movements of other joints. How are se compensatory changes achieved? There are several es of evidence suggesting that changes in the state of the ıro-muscular apparatus will trigger anatomic and physio- ical changes in various structures. For instance, transposi- n of nerves from muscle containing different populations muscle unit types can lead to changes of the distribution unit type in the muscles such that slow motor units can converted to fast units (for review, see Buller and Pope 77). After nerve transposition, new monosynaptic connec- ns are made between the axons originating from a gastroc- nius or plantaris nerve with peroneus longus motoneurons ccles et al. 1960, 1962). The distribution of afferents and efferents to transposed muscles in the various lumbo-sacral roots is also changed (Gordon et al. 1986).

Despite these changes in the reflex circuitry, a number of experiments suggest that there is little functional plasticity in locomotor mechanisms in very challenging situations such as after nerve or muscle transpositions. After transposing the gastrocnemius (G) muscle, an ankle extensor, to the distal tendon of the ankle flexor tibialis anterior (TA), it was found that the gastrocnemius, which normally discharges during stance, continued to discharge in stance despite its being now an ankle flexor (Forssberg and Svartengren 1983; Gordon et al. 1986). This was taken as evidence that the basic spinal locomotor circuitry had no significant plasticity, in support of the notion of an innate hardwired locomotor network. This is in agreement with the classical work of Sperry on tendon transposition in rats. After transposition of ankle antagonists muscles (Sperry 1941) or nerves (Sperry 1940) in the hindlimbs or muscles and nerves in the forelimbs (Sperry 1942), there was no adaptation even several months after the transposition and despite various attempts at training to force the proper use of the transposed muscles. Even during basic motor behaviors such as locomotion, rats contracted the gastrocnemius during stance, causing the ankle to flex. Similarly during swing, TA contracted and extended the ankle. Although the transposed muscle apparently did not adapt, other muscles at the thigh and hip



. 7. Comparison of EMGs in control, neurectomized, and neurectomized-spinal states of *cat 1*. These values were from several step cycles (see Fig. 8 for number of cycles values). All gains are the same for each muscle in the 3 itions.

lified their contraction pattern to prevent scraping the on the floor during swing. Sperry concluded that rats ved no plasticity in the function of transposed muscles. the other hand, monkeys eventually were able to learn, ral months after a transposition of elbow antagonist mus- to use the transposed muscles properly in certain particu- asks (Sperry 1947), suggesting that primates, including , have more potential for plasticity than rats. Similar ings were made after muscle transposition in the cat limb in which some adaptive discharges were found miya et al. 1979). For instance, the palmaris longus cle, which normally discharges during stance, now is ve mainly during swing and only little during stance. here is thus conflicting evidence on whether or not the S is capable of sufficient plasticity to reroute its command als so that transposed muscles can be recruited in accor- ce with their new and opposite biomechanical functions. is a very demanding situation, which may be at the limit of adaptive mechanisms. On the other hand, it is r, as shown in the present results, that it may be a more nomical strategy to develop compensatory movements of r joints to maintain the locomotor function when one t cannot function properly, as is the case here, with the tic reduction of ankle flexion due to the denervation. question then is where are these compensatory changes nized and controlled?

s of functional plasticity

Whereas the cats had adapted very well to the neurectomy n otherwise intact, the same cats showed a disorganized motor pattern soon after spinalization. The gait was characterized by irregular cycles with marked hyperflexions especially of the knee. The synchronous hip and knee flexion did not bring the limb forward but the limb rather was stepping in place. There was no plantar foot placement, and therefore the weight was supported mainly by the other hindlimb, leading to a quite asymmetrical gait. On the other hand, when the neurectomy was performed in a spinal cat that already had recuperated a stable locomotion, the locomotor pattern remained rather symmetrical and regular although there was no significant ankle flexion nor proper plantar foot contact. In other words, neurectomy after spinalization did not have the remarkable disorganizing effect that spinalization had on cats having recuperated from the same neurectomy when their spinal cord was intact.

We interpret these data to mean that, when an animal modifies its locomotor pattern to compensate for a lesion of its neuro-muscular peripheral apparatus, long-term changes occur both at the spinal and the supraspinal levels. We postulate that, initially, step-by-step and asymmetrical descending signals from supraspinal structures are provided to the spinal cord to compensate for the peripheral deficits. These may, after some time, induce some long-term changes in the spinal cord that, for instance, could subserve the hip/knee hyperflexion seen as one of the main compensating mechanisms. Where could these changes occur? Part of the compensation, such as the increased flexion of the knee and/or the hip joints, as well as the subsequent placement of the paw may result from compensatory supraspinal inputs, such as the motor cortex, which has a cyclical discharge and is especially active during voluntary gait compensation, such as stepping over obstacles (Armstrong and Drew 1984, 1985; Drew 1993; Widajewicz et al. 1994; Yumiya et al. 1979). Given the extensive

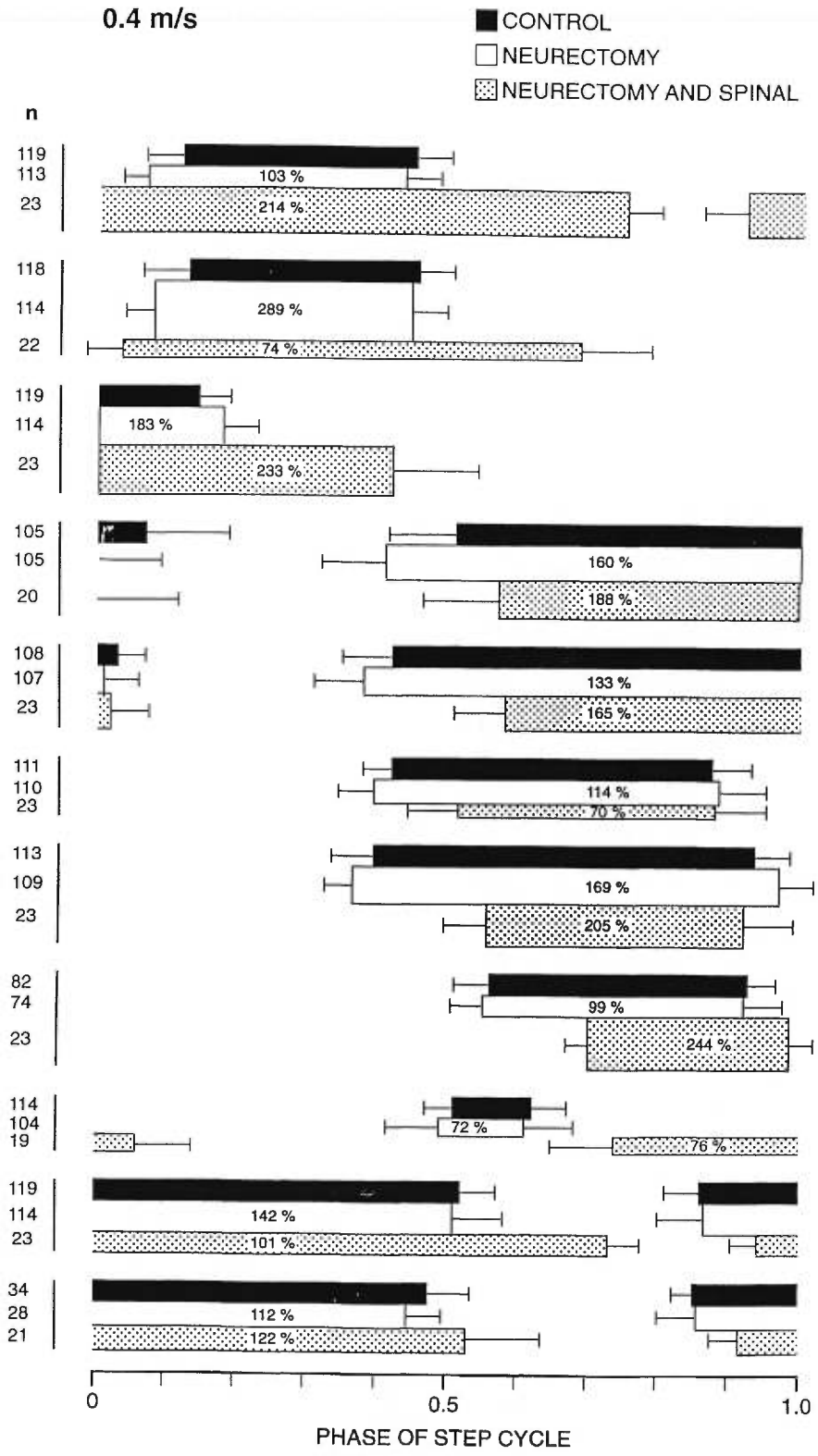


FIG. 8. Bar diagram of EMG recordings in control, neurectomized, and spinal states at 0.4 m/s. EMG bursts are displayed as horizontal rectangles whose width represents average onset and offset of bursts + 1 SD in 1 normalized step cycle and whose height represents normalized amplitude of burst (normalized amplitude = integrated burst divided by its duration). *n* values beside muscle names indicate numbers of burst used to calculate these averages.

ɛrgence of supraspinal and peripheral afferent inputs on l interneurons (Baldissera et al. 1981), it is interesting ɜtulate that long-term facilitation mechanisms in the corpinal pathway (Iriki et al. 1990) could lead to long-term ɣes in the excitability of interneurons closely related to ɔinal pattern generator. Increased descending inputs on ide of the lesion could induce changes in excitability that could persist after spinalization, leading to the abnormal hyperflexions seen at the hip and knee.

When these correcting supraspinal signals are removed by spinalization, the spinal pattern is expressed on its own without descending compensation. This pattern clearly expresses hyperflexion of the more proximal joints on the ipsilateral side and is asymmetrical. On its own, this modified

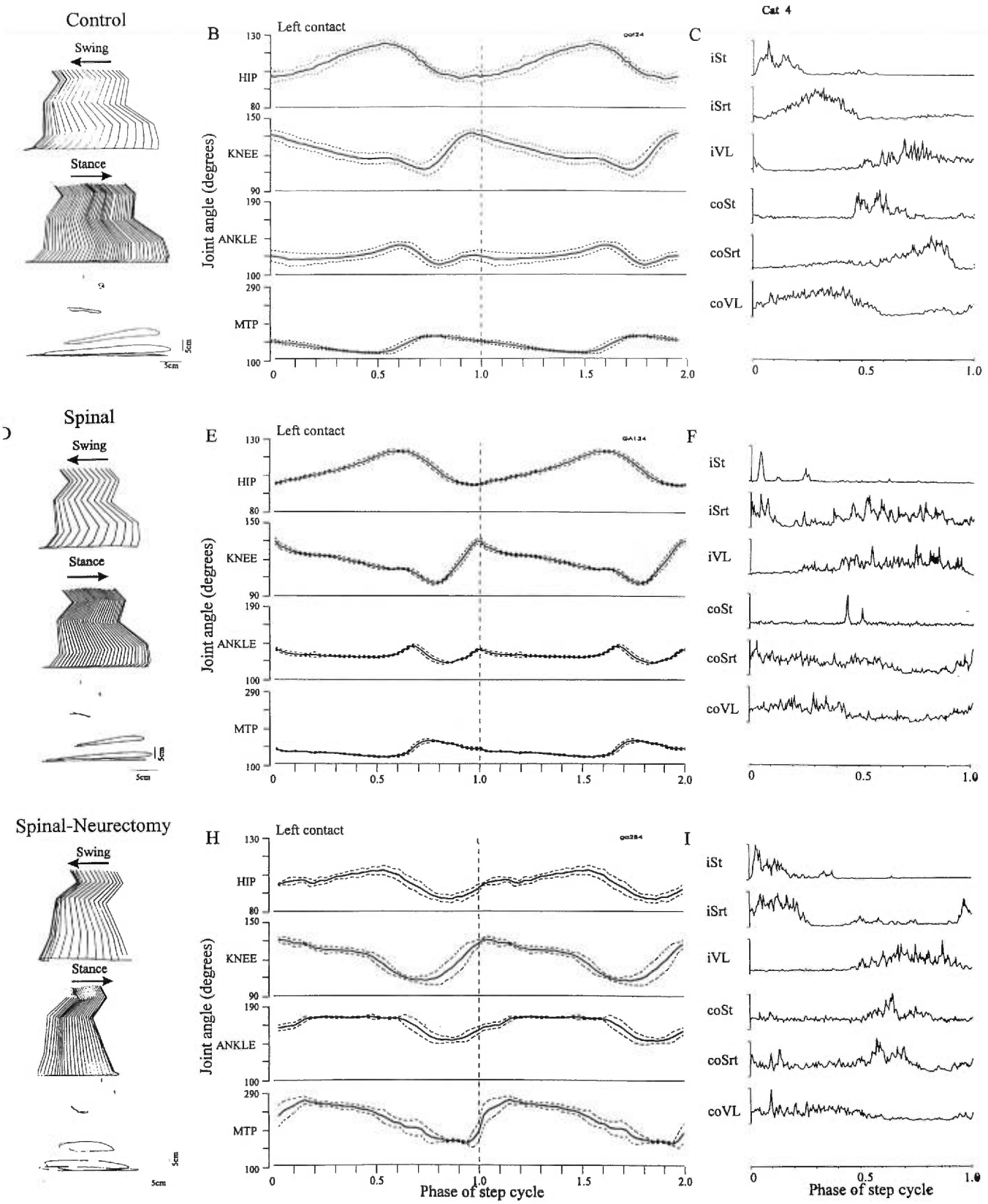


FIG. 9. Kinematics and EMGs recorded from *cat 4* during control, post spinal (day 21), and spinal + neurectomy (day 29) ıdmill locomotion at 0.4m/s: *A, D,* and *G*: stick figures of swing and stance of a representative step cycle. Related trajectories lifferent joint markers are illustrated *below* each stick figure. *B, E,* and *H*: average of angular displacement of hip, knee, ankle, MTP joints in 6–10 consecutive cycles. Normalized step cycle starts at left foot contact and is displayed twice. *C, F,* and *I*: ıted EMG activity presented after averaging (n = 5–10) and normalization. After spinalization, cat recuperated an organized, ımetrical locomotion. Note that sartorius anterior (Srt) muscle also displayed some activity in extension. After neurectomy cat ıtinues to walk regularly but lacks ankle flexion. Note that Srt pattern has come back to a pattern closer to control period.

inal pattern is, however, neither adequate nor sufficient to mpensate for the peripheral lesion. The conclusion that e spinal cord has been modified on a long-term basis was ached because when the neurectomy is performed after inalization, at a time when the cat has recuperated a stable comotor pattern, the spinal pattern expressed does not in-ude the significant ipsilateral hyperflexions of the proximal ints nor the marked asymmetry seen in the other cats that ere spinalized only after the neurectomy. Their locomotor ttern is basically the one seen in normal (i.e., without urectomy) cats after spinalization except that there is only tle ankle flexion and therefore an improper foot placement.

Do the changes observed in cats spinalized after neurec-my represent some form of spinal "learning"? The idea spinal learning has been around for many decades (see erry 1945; Wolpaw 1994 for review of early and more cent literature). There are indications that the monosynap-reflex can change with operant conditioning and that ese changes persist after spinalization (Wolpaw 1994). milarly, there are indications that the flexion reflex can be nditioned classically in spinal cats (Durkovic 1975, 96). Finally, the spinal cord appears capable of "learn-g" more complex and specific tasks (Hodgson et al. 94). The evidence presented here does not suggest that e spinal cord has "learned" a complete locomotor strategy compensate for the denervation of the pretibial muscles cause the movements performed in the spinal state are adequate and do not compare with the fine compensation en before spinalization. However, these experiments do ggest that some changes have occurred in the spinal cord cats, which previously had adapted to the lesion, because e locomotor pattern expressed after the spinalization is ite different from the pattern normally expressed by spinal ts and also quite different from the locomotor pattern seen nen the denervation is performed after the spinalization.

In summary, this work suggests that there is some plastic-in the spinal locomotor network and that this plasticity uld perhaps be used within the context of locomotor reha-itation in patients with spinal injuries as suggested in the TRODUCTION. Whereas the specific spinal circuitry in-lved in the control of specific muscles and their antago-sts may have to be hardwired as suggested above, other ntrol mechanisms can be modified to preserve the overall nction of locomotion. Whether functional locomotor plas-ity can be achieved after complete spinalization remains be elucidated. The present work, however, suggests that ch spinal plasticity could be meaningful after incomplete inal cord lesions, which leave some of the descending mmand tracts intact and potentially capable of producing ng-term changes in the spinal cord.

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Locomotor capacities after complete and partial lesions of the spinal cord

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Abstract. This paper first reviews some of the observations made on the locomotor capabilities of several animal species with a special emphasis on cats and including primates and man after complete spinal lesions. We show that animals can perform well-coordinated walking movements of the hindlimbs when they are placed on a treadmill belt and that this locomotion is also adaptable to speed and perturbations. Cats with partial spinal lesions of the ventral and ventrolateral parts of the cord can perform voluntary quadrupedal locomotion overground or on the treadmill albeit with deficits in weight support and interlimb coordination. We also show that some drugs such as clonidine (an alpha-2 noradrenergic agonist) can be used to trigger locomotion in early-spinal cats and discuss the effects of various neurotransmitter systems on the expression of the locomotor pattern in both complete and partial spinal cats. It is concluded that a pharmacological approach could be used, in combination with other approaches, such as locomotor training and functional electrical stimulation, to improve locomotor functions after spinal cord injuries in humans.

Review

Key words: locomotion, spinal cord, spinal pathways, interlimb coordination, locomor pharmacology, clonidine, partial spinal lesions

INTRODUCTION

Information on the locomotor capabilities of animals subjected to spinal lesions is of great interest in the context of gait rehabilitation of patients with spinal cord injuries (Barbeau and Rossignol 1994). This knowledge can be very helpful in orienting the study of patients as well as in the design of rehabilitation approaches (Rossignol and Barbeau 1995). In the first section of this paper, we will summarize some observations obtained on the locomotor abilities of cats with complete spinal transection at T13 and also introduce more recent work on cats with partial ventral and ventrolateral lesions of the cord at the same level. In a second section we will briefly discuss the effects of agonists and antagonists of different neurotransmitter systems in cats with total or partial spinal lesions. This is aimed at better understanding how the activation or blockade of different receptors of these neurotransmitters could participate in various aspects of the control of locomotion such as its initiation and the modulation of its timing and amplitude characteristics and how they could be used to improve gait rehabilitation in patients. For some general background on locomotor mechanisms, several reviews can be helpful (Grillner 1981, Armstrong 1986, Grillner and Dubuc 1988, Pearson 1993, Rossignol and Dubuc 1994, Rossignol 1996).

LOCOMOTOR CAPABILITIES OF ANIMALS WITH SPINAL LESIONS

Complete spinal lesion

HISTORICAL PERSPECTIVE AND GENERAL DESCRIPTION

At the turn of the century, it was shown that, one or two days after a spinal section, cats and dogs can perform spinal standing (dogs better than cat because more weight is supported by forelimbs). Sherrington (1899, 1910a,b) described the locomotion of spinal animals (dogs and cats) as "the postural act of standing upon which there are grafted rhythmic flexion-extension movements of each limb in turn, resulting in locomotion". In the decapitated cat (high spinal), he showed that stimulation of the perineum or a foot or "faradization" of an afferent nerve can elicit stepping, as well as the stimulation of the cut end of the bulb or spinal cord which can evoke mainly unilateral stepping on the stimulated side. Sherrington writes that "the rhythmic response of the musculature is referable to a rhythm not resident in the stimulus or in sense-organs of the skin, but developed in the spinal centres occupied with the reflex action. In other words, in these centres there arises a rhythmically recurrent refractory phase... Indeed, the burden of shunting from flexion to extension and vice versa is thrown greatly upon mechanisms wholly intrinsic to the cord (Sherrington 1910b). This is already a clear statement on the generation of locomotion within the spinal cord, a view strongly defended by Brown (1911) an thoroughly reviewed elsewhere (Grillner 1981, Rossignol 1996).

Since these celebrated accounts on reflexes, walking and standing in spinal animals, there have been several reports on the reflex and motor capabilities of animals with a complete transection of the spinal cord. Table I lists some key references on locomotor functions after chronic lesions made at various levels in different animal species and at various ages. The following description summarizes some aspects only of this topic.

After a spinal transection, dogs were shown to eventually redress voluntarily by supporting their weight on the forelimbs and lifting their hindquarters (Philippson 1905, Ten Cate 1939, Kellogg et al. 1946). These movements were reported to be better when the animal was allowed to move every day for a few hours (similar results have been reported recently on walking and air stepping in chronic adult spinal dogs; Naito and Shimizu 1991, Shimizu 1991). Spontaneous walking for long distances on all 4 limbs could be performed even after a second transection (see however Sherrington 1899). Shurrager and Dykman (1951) reported observations in kittens spinalised at the age 2 days to 12 weeks and 1 dog. In both the cats and dog, they reported unaided

TABLE I

List of references related to locomotor studies after complete spinal lesions at different levels at various species. For man, anatomically complete refers to surgical or to Magnetic Resonance Imaging confirmation of the completeness of the lesion

Chronic complete spinal section in different species

Species	Level of transection	References
MAN	Anatomically complete at various levels	(Holmes 1915, Kuhn 1950, Dietz et al. 1994, Dietz et al. 1995)
MONKEYS	thoracic (undefined)	(Philippson 1905, Freeman 1952)
MONKEYS	Th 6-9	(Eidelberg et al. 1981b, Eidelberg 1983, Vilensky et al. 1992)
DOGS-pups		(Freeman 1952)
DOGS-adults	6-10 thoracic	(Sherrington 1899, Philippson 1905, Sherrington 1910b, McCouch 1947, Shimizu 1991)
DOG-adults	L1-L3 + S2-S3 for (Ten Cate, 1939)	(Ten Cate 1939, Kellogg et al. 1946, Shurrager and Dykman 1951, Naito et al. 1990, Shimizu 1991)
CAT-Kittens and infants	Th12 to L1 and L3	(Shurrager and Dykman 1951, Freeman 1952, Forssberg et al. 1980a, Forssberg et al. 1980b, Smith et al. 1982, Goldberger 1986, Robinson and Goldberger 1986b)
CAT-ADULT	C1	(Miller and Van der Meche 1976, Zangger 1981)
CAT-ADULT	Th 3-4	(Ranson and Hinsey 1930, Kozak and Westerman 1966)
CAT-ADULT	Th 6-10	(Ranson and Hinsey 1930, McCouch 1947, Eidelberg et al. 1980)
CAT-ADULT	Th13-L1	(Sherrington 1910a, Ranson and Hinsey 1930, Ten Cate 1962, Kozak and Westerman 1966, Afelt 1970, Baker et al. 1984, Goldberger 1986, Lovely et al. 1986, Robinson and Goldberger 1986a,b, Barbeau and Rossignol 1987, Barbeau et al. 1987, Giuliani and Smith 1987, Rossignol et al. 1989b, Lovely et al. 1990, Barbeau and Rossignol 1991, Edgerton et al. 1991, Roy et al. 1992, Barbeau et al. 1993, Belanger et al. 1996)
RAT-neonatal and weanling	Th 4-11	(Stelzner et al. 1975, Weber and Stelzner 1977, Meisel and Rakerd 1982)
RAT-ADULT	mid-thoracic	(Freeman 1952, Freeman 1954, Meisel and Rakerd 1982, Bregman et al. 1993, Kunkel-Bagden et al. 1993, Zhang et al. 1994)
RABBITS- YOUNG	Th 12	(Fayein and Viala 1976, Viala et al. 1986)
RABBITS-ADULTS	Th 12	(Laughton 1924, Hinsey and Cutting 1932, Ten Cate 1964, Viala et al. 1986)
OPOSSUM	low thoracic-upper lumbar	(Hinsey and Cutting 1936)
PIGEONS	intumescentia lumbosacralis	(Ten Cate 1960, Ten Cate 1962)
FROGS		(Afelt 1963)

walking movements overground. In one cat, a second transection above the first spinal section did not change the locomotor behaviour. They insisted much on daily training as well as the fact that young animals performed much better than older animals.

Ten Cate also undertook a later study on spinal pigeons (Ten Cate 1960, 1962) and spinal cats (Ten Cate 1962). Using specially designed carriages, he showed that spinal pigeons could walk with normal extension of the toes during stance and were able to

propel the body forwards; it seems that, once initiated, they could maintain this walk through proprioceptive inputs from the moving legs themselves. Perineal stimulation and other stimuli modulated the frequency of stepping. Cats on the other hand could generate hindlimb movements only when the body was pulled forwards and the hindlimbs stretched, due to forelimb movements.

Laughton (1924) reported air stepping in chronic spinal rabbits which had the typical alternating configuration seen in dogs (Mark-time reflex) but not the in-phase coupling typical of rabbit locomotion. Ten Cate also described the locomotion of chronic spinal rabbits (1964) in a special carriage. Both synchronous bilateral and alternate movements could be elicited in these rabbits but could not be maintained for more than a few steps. In spinalised young rabbits (Fayein and Viala 1976) it appears easier to get both alternate and non-alternating gaits. Infant rabbits initially have normally an alternate pattern; by 20 days, they become exclusively in-phase (Viala et al. 1986). These authors made the important observations that rabbits spinalised 2 days after birth could be trained to have either a predominant alternate or non-alternating gait. These patterns were maintained after a second spinal transection. These respective patterns were also maintained in fictive conditions, suggesting that the training has had a major effect of the locomotor pattern which is expressed; this in turn implies a certain plasticity in the locomotor network, at least before descending pathways complete their connections with the cord (around day 18).

STUDIES IN CHRONIC SPINAL CATS

Work in the cat was rare before or around the seventies (Sherrington 1910b, McCouch 1947, Kozak and Westerman 1966, Afelt 1970, 1974). However, in 1973, two very influential papers appeared (Forssberg and Grillner 1973, Grillner 1973) describing that acute low spinal cats could walk with their hindlimbs on a treadmill when injected with an alpha-2 noradrenergic agonist, clonidine and that kittens, spinalised within a few days after birth (thus before they had expressed any locomotor pattern) could walk and gallop with their hindlimbs on a treadmill. These spinal kittens not only could walk with the hindlimbs on a treadmill with correct foot placement and support of the hindquarters but could also place their paw on a surface when the dorsum of the foot touched an edge (placing reaction; Grillner 1973, Forssberg et al. 1974) and could hop sideways.

A more complete account of the findings in chronic spinal kittens was published later (Forssberg et al. 1980a,b, Grillner 1981) and showed not only that the kinematics and the EMGs of spinal kittens were very similar indeed to normal cats but also that the spinal kittens had the ability to adapt their locomotion to the various speeds of the treadmill and even to asymmetrical treadmill speed. This emphasized the importance of peripheral afferent signals in adapting the locomotor pattern to external conditions. Further, these chronic spinal cats were shown to adapt to perturbations applied during the swing and stance phase and generate specific reflex compensatory responses in the various phases (see (Rossignol et al. 1988) for a review). This suggests that the spinal cord not only generates the locomotor pattern but that it can adapt it to external perturbations.

A report on adult chronic spinal cats (Eidelberg et al. 1980) stated that 2/3 of the cats had quasi normal stepping movements of the hindlimbs on the treadmill while 1/3 had persistent abnormal movements which could not even be described because they were so erratic. None of the cats were capable of weight support and a sling under the belly was used to test locomotion of all 4 limbs on the treadmill. It was shown that these cats had no weight support and no coordination between the forelimbs and hindlimbs and that even the spinal stepping had abnormal features such as a greater variability in hindlimb coupling, foot drag during swing and uncoupling between the knee and ankle. Further, once established, the locomotor pattern did not improve with repeated trials. This altogether rather negative report testing spinal cats on all 4 limbs obscured the fact that adult spinal cats could indeed

'alk with their hindlimbs on a treadmill, much as ıe spinal kittens.

Along these lines of work, it was shown that the ge of spinalisation and the amount of training on ıe treadmill (30 min 5 times a week) had important ffects on the locomotor pattern (Smith et al. 1982, regman and Goldberger 1983, Robinson and oldberger 1986b). Animals spinalised at 2 weeks f age had a much better locomotor performance ıan those spinalised at 12 weeks of age. Training ı 12 week old cats had an observable effect on eight bearing during locomotion. The EMG pat-rn appeared normal in many respects, except that lonus was frequently observed. Even so, defects in ıe locomotor pattern such as uncoupling between ıe knee and ankle as well as an absence of yield in 2 phase of the step cycle were reported.

Work in adult chronic spinal cats (Rossignol et l. 1982, 1986, 1989a, Barbeau and Rossignol 987, Belanger et al. 1996) has clearly established ıat the quality of locomotion is indeed improved y training and that the spinal locomotor pattern volves with time. For instance, as time progresses, ats make larger steps at the same treadmill speed; ıis is achieved by a greater lengthening of the ex-ensor burst relative to the flexor burst, a structure hich resemble more the situation in the intact. In cats it was found that cycle duration increased as function of time but reached a plateau at around months. All cats made plantigrade foot contact nd could maintain the weight of the hindquarters y the third week. The important joint uncoupling escribed before and the absence of yield in E2 were ot confirmed (see also: Lovely et al. 1990). How-ver, foot drag was present in most cats during the irst part of swing. This was interpreted as being due ɔ a decrease in the delay between the onset of the nee flexor and the hip flexors so that both start nore or less simultaneously in the spinal cat where-s normally the knee flexor first removes the foot rom ground before it is brought forwards. It was lso observed that Tibialis Anterior, an ankle flexor, lso tended to be recruited earlier than in the intact hich again could result in a foot drag if the foot is ıot already lifted from ground. In many other as-pects however, the EMGs observed after spinalisation were similar to those observed before in cats chronically implanted with EMG electrodes before the spinalisation (Belanger et al. 1986, 1987, 1988a,b,c, 1989, 1996). Lovely et al. (1990), using force transducers placed on Soleus and Gastrocnemius Medialis, further emphasized that, although the force generated by spinal cats reaches the same level as normal cats, it declines rapidly during stance. This decrease in force may facilitate the premature onset of swing (Duysens and Pearson 1980) which could result in foot drag. On the other hand the force level recorded, at least in Soleus, would be large enough to participate in propulsion as suggested for the spinal kittens (Forssberg et al. 1980a).

The importance of regular daily training (even starting as late as 1 month after transection) was further emphasized by others (Lovely et al. 1986, 1990, Edgerton et al. 1991, Roy et al. 1992). This is of course of great interest in the clinical human situation, especially if training can be accelerated through the use of pharmacotherapy (see later). Another remarkable effect appears to be in the specificity of training. In contrast to spinal cats which had locomotor training, those that were instead trained only to stand for several months had a very poor locomotor performance on the treadmill (Edgerton et al. 1991). Since the difference between the behaviour of the two groups can hardly be explained by the state of the neuro-muscular apparatus, it is suggested that training of spinal locomotion is a form of spinal "learning" akin to the learned modifications of the H-reflex seen in monkeys after spinalisation (Wolpaw and Lee 1989).

Partial spinal lesions

Based on lesion studies, it was generally considered that pathways of the ventral and ventro-lateral quadrants of the spinal cord are important for the control of locomotion. Subtotal lesions of the spinal cord also point to the importance of the medial and mediolateral pathways in the control of locomotion (see: Eidelberg 1981, for a review of early literature on different subtotal lesions in primates

and non-primates). Sparing of at least part of a ventrolateral quadrant in the cat, and the associated labelling by HRP of neurones in the pontine and medullary formation, were claimed to be essential for recovery of locomotion in chronically lesioned cats (Afelt 1974, Eidelberg et al. 1981a, Contamin 1983) and monkeys (Eidelberg et al. 1981b). Evidence is however mounting that cats (Górska et al. 1990, 1993a,b, Brustein et al. 1993, 1994) and monkeys (Vilensky et al. 1992) can walk with the hindlimbs even after complete section of these pathways, although there are changes in the forelimb-hindlimb coupling. Extensive work by the group of Górska has been performed on this subject (Górska et al. 1990, 1993a,b, Zmysłowski et al. 1993, Bem et al. 1995). The recent work in our group (Brustein et al. 1993, 1994, 1995) on chronically implanted cats confirm that one of the main deficits of these cats with massive lesions of the ventral and ventrolateral tracts is the lack or instability of hind-fore-

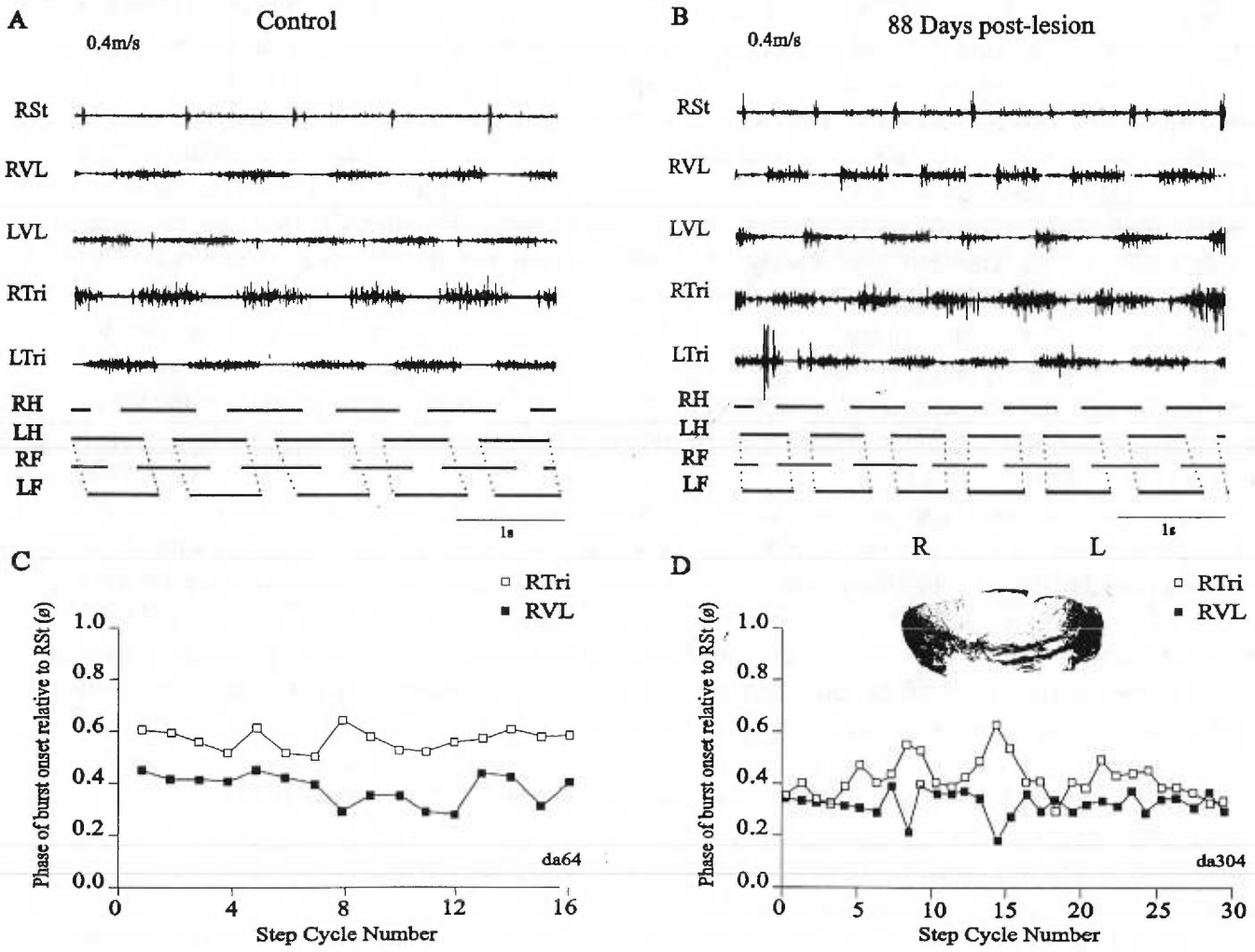


Fig. 1. EMG and foot fall pattern .n a cat with a bilateral ventral and ventrolateral spinal cord lesion (the histological section is shown as an insert in D). A, control period. EMGs during walking at 0.4 m/s. St, Semitendinosus; VL, Vastus lateralis; Tri, Triceps brachii, lateral head. R, right; L, left. H, hindlimb; F, forelimb. B, same but 88 days after the lesion shown in D. C. phase plot of the onset RVL in the hindlimb and RTri in the forelimb to indicate the coupling between the fore- and hindlimb. The values are expressed as phases of the step cycle defined by the onset of RSt. Note the phase difference between the two is in the order of 0.25. D, same as C but 88 days post-lesion; note the tendency for a reduced phase coupling (tendency to pace). The insert in D is a cresyl violet staining of the lesion at its maximum.

nb coordination. Cats tend to increase the control ver the forelimbs and also tend to pace even several weeks after the lesion (see Fig.1). After such assive lesions of the ventral and ventrolateral ithways, the remaining dorsolateral pathways are us capable of triggering voluntary hindlimb ovements and in part coordinate all 4 limbs fauring the most stable locomotor pattern (increase the number of feet on the ground at all times). We ive not found any foot drag in these cats, although sions of the dorsolateral funiculus (Jiang and rew 1996) produce such foot drag much as in the mplete spinal cat.

About a week after a spinal hemisection at the w thoracic level, cats can readily walk overground ith both hindlimbs; after a second transection at e midthoracic level contralaterally to the first sec-)n, cats still can regain voluntary locomotor func-)ns overground, although the fore- and hindlimbs)upling may be lost (Kato et al. 1984). After a longitudinal split of the lumbar cord from L2-3 to L7-S1, cats can, after about 1 month, stand and walk with bilateral hindlimb coordination suggesting that the interlimb coordination can be assured by descending pathways (Kato 1988). After such a split and a further unilateral hemisection, the isolated spinal cord can eventually step even though it is isolated from supraspinal and contralateral inputs. However, it is difficult to assess the quality of such locomotion without further EMG data and especially kinematic data (Kato 1989, 1991). A list of some of the pertinent references on partial spinal lesions in relation to locomotion can be found in Table II.

Locomotor capabilities of primates and humans after spinal lesions

Philippson (1905) made some laconic comments on 3 spinal monkeys. Although it is clearly stated

TABLE II

List of references related to locomotor studies in various species after partial spinal lesions. In man, neurologically incom-)te means that the patients still have some sensory-motor functions whereas neurologically complete means that the patients ve no sensory-motor functions although the spinal cord is not completely severed

Chronic partial spinal section in different species		
)ecies	Type and level of lesions	References
AN	neurologically incomplete: various levels	(Fung et al. 1990, Wainberg et al. 1990, Stewart et al. 1991, Norman and Barbeau 1992, Barbeau and Fung 1994, Calancie et al. 1994, Barbeau and Rossignol 1994, Nathan 1994)
AN	neurologically complete: various levels	(Bussel et al. 1988, Stewart et al. 1991, Wernig and Muller 1992, Dietz et al. 1994, Dietz et al. 1995, Wernig et al. 1995)
ONKEYS	various quadrants	(Eidelberg et al. 1981b, Vilensky et al. 1992)
ONKEYS	hemisection	(Aoki et al. 1991)
ATS	various quadrants	(Eidelberg and Stein 1974, Eidelberg 1981, Eidelberg et al. 1981a, Eidelberg et al. 1985)
ATS	ventral and/or ventrolateral	(Afelt 1974, Górska et al. 1990, Brustein et al. 1993, Górska et al. 1993a, Brustein et al. 1994, Brustein et al. 1995, Bem et al. 1995)
ATS	dorsal columns and/or dorsolateral and/or lateral funiculi	(Windle et al. 1958, English 1980, Contamin 1983, English 1985, Górska et al. 1993b, Zmyslowski et al. 1993, Jiang and Drew 1996)
ATS	simple and serial hemisections	(Kato et al. 1984, Masamichi et al. 1984, Kato et al. 1985, Kato 1988, Kato 1989, Kato 1991, Helgren and Goldberger 1993)

that locomotor movements were observed, we have unfortunately very few details. A major study was made by Eidelberg et al. (1981b) on macaque monkeys (spinalised at T8-T9). In contrast to the situation in cats (Grillner and Zangger 1979), DOPA did not induce locomotion in acute spinal monkeys. In the chronic state (up to 4 months), it was impossible to elicit locomotor movements even after clonidine. In monkeys with partial spinal lesions, it was concluded that the ventrolateral cord sector was crucial for locomotion and that the spinal stepping generator in monkeys was more heavily dependent on supraspinal inputs.

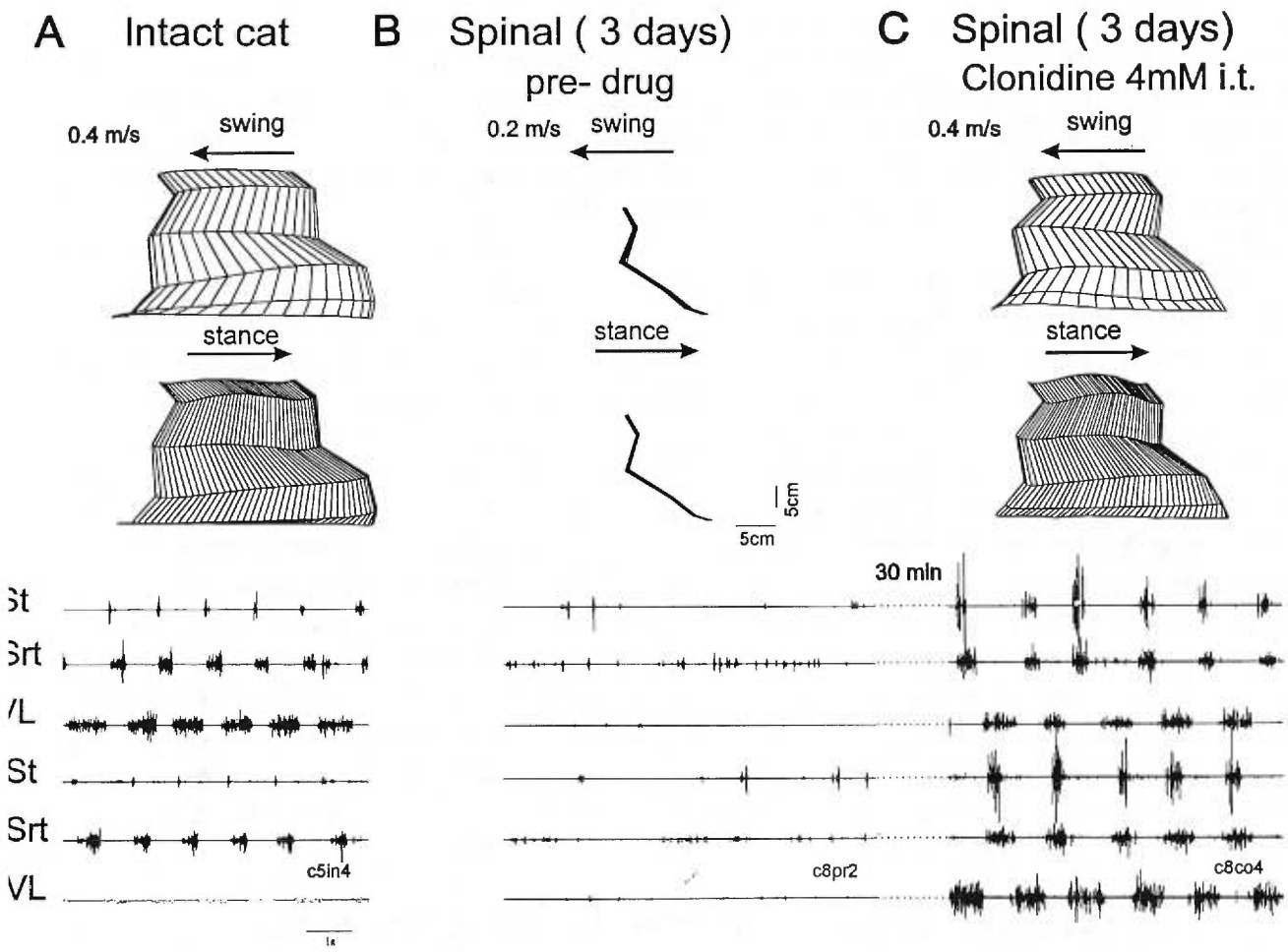
A reappraisal of the same data 10 years later (Vilensky et al. 1992) indicates that locomotion is possible in primates after very extensive but incomplete spinal lesions, that there is no specific correlation between the sparing of specific tracts and the recovery of locomotion (namely the ventrolateral tracts), and that there is some limited evidence of rhythmic hindlimb movements in the chronic spinal monkey. Recent evidence (Hultborn et al. 1993) on the marmoset, considered to be a relatively primitive primate in evolutionary terms, indicates that fictive locomotion can be induced after paralysis (clonidine or DOPA). Chronic hemisections in monkeys (Aoki et al. 1991) led to recovery of function after several months, apparently due to collateral sprouting of the contralateral cortico-spinal tract.

Evidence in Man is also not very clear (see Vilensky et al. 1992). Holmes (1915) and Kuhn (1950) describe such rhythmic locomotor movements of the lower legs in several patients having sustained a spinal cord injury during both world wars. Some more recent observations (Roby-Brami and Bussel 1987, Bussel et al. 1988, Dobkin et al. 1992, Calancie et al. 1994, Dietz et al. 1994, 1995, Wernig et al. 1995) also suggest that there might be some spinal circuits in man capable of generating a basic locomotor rhythmicity. Electrical stimulation of the spinal cord in complete anatomical paraplegic can evoke well organized rhythmic activity in the lower limbs (Rosenfeld et al. 1995). There is thus the possibility in man to generate involuntary rhythmic locomotor movements probably through circuits implicating the spinal cord and perhaps some brain stem structures. Other reports on brain death patients (Mandel et al. 1982, Hanna and Frank 1995) also suggest that such locomotor movements can be present for several days before the actual death, again suggesting that involuntary mechanisms may be implicated in the generation of locomotor rhythmicity in man. It is thus possible to think that such mechanisms could be facilitated through locomotor training and perhaps pharmacotherapy (see later). The work of several authors on humans suggest that this is a real clinical possibility (Barbeau and Rossignol 1994, Rossignol and Barbeau 1995).

PHARMACOLOGY OF LOCOMOTION AFTER SPINAL LESIONS

Given the fact that there are control mechanisms within the spinal cord and the brain stem which are crucial for locomotion, to what extent can we act on these mechanisms through the activation or inactivation of the receptors of the various neurotransmitter systems? Our attempts at initiating and modulating the spinal locomotor pattern in early spinal cats (within the first week or so after the lesion) and in chronic spinal cats that have already regained the ability to walk with the hindlimbs when placed on a treadmill have been summarized recently and most of the earlier references can be found in these reviews (Rossignol and Barbeau 1993, Barbeau and Rossignol 1994, Rossignol et al. 1995).

In brief, it appears that it is only the activation of the alpha-2 noradrenergic receptors (through agonists such as clonidine, tizanidine, oxymetazoline, the precursor DOPA or the transmitter itself, norepinephrine) which can trigger locomotion in early complete spinal cats. All other systems that we have tested (glutamatergic, serotonergic, dopaminergic) have failed in our hands to trigger sustained locomotion in the early spinal cat although they may do so in other mammal species or preparations, i.e. 5-HT and NMDA in neonatal rats (Cazalets et al. 1992),



g. 2. Early-spinal (3 days) cat injected with intrathecal clonidine (4 mM). A, walking on a treadmill at 0.4 m/s during the ntrol period before spinalisation. B, three days after spinalisation before injecting clonidine. C, thirty min. after i.t. clonidine. uscles: Srt, Sartorius anterior; other abbreviations as in Fig. 1. Note that the gains of EMG are the same in the three panels. rows indicate the direction of the movement. The distance calibration in X is twice that in Y because the hip point is displaced the amount of the displacement of the foot. Note in the stick figure of C a foot drag in the initial part of swing, a frequent fect in spinal cats which is enhanced by clonidine.

MDA in decerebrate cats fictive locomotion ouglas et al. 1993), 5-HT and dopamine in rabbits iala and Buser 1969).

Figure 2 illustrates the effect of an intrathecal t.) injection of clonidine in a complete spinal cat. ıe left panel illustrates the locomotor pattern of ɜ cat during the control period before spinalisa- ın. Three days after spinalisation the cat is virtually ɔtionless on the treadmill, the limbs being passive- dragged on the treadmill belt. A few minutes after ɜ i.t. injection of clonidine, the cat can step with its ıdlimbs on the treadmill in a well coordinated pat- ın and continue to do so for several hours.

When the animals have recovered the ability to spontaneously walk with the hindlimbs on the treadmill belt without any external help (late-spinal cats: Barbeau and Rossignol 1987), then the different neurotransmitters may exert modulatory effects on the expression of the locomotor pattern. For instance, clonidine may markedly increase the duration of the step cycle (Barbeau et al. 1987, Barbeau and Rossignol 1991, Rossignol et al. 1995), whereas serotoninergic agonists may increase the amplitude of electromyographic activity (Barbeau et al. 1987, Barbeau and Rossignol 1990, 1991). Although NMDA does not appear to trigger locomo-

tion in the early-spinal cat, it does greatly increase the excitability of the spinal cat during walking as evidenced by frequently interspersed episodes of fast paw shake. AP5, an NMDA blocker may block locomotion of the chronic spinal cat which may then be reinstated with an intrathecal injection of NMDA (Chau et al. 1994).

The above results should be clearly interpreted within the context of a complete spinalisation where there is a complete disappearance or major reduction of the neurotransmitters below the lesion and in which receptor hypersensitivity may develop. This is important because the effect of the drugs may differ in animals with partial lesions walking voluntarily on all 4 limbs. In such animals, we have found that clonidine may be detrimental to walking by significantly decreasing the ability to sustain the weight of the hindquarters. In this context, it is worth mentioning that spinalisation may unmask excitatory alpha-1 effects, whereas without spinalisation or with incomplete spinal lesions, the inhibitory alpha-2 effects may predominate (Kehne et al. 1985). On the other hand, drugs that may exert an excitatory effects on motoneurones, such as alpha - 1 noradrenergic agonists (Methoxamine) or 5-HT agonists (quipazine) may be more helpful in improving the ability of these partially lesioned animals to walk with better weight support for longer period of time.

Figure 3 illustrates the locomotor EMG pattern of a cat 9 days after a severe (but as yet undocumented) lesion of the ventral and ventrolateral funiculi. The cat could hardly sustain its weight and had a disorganized locomotor pattern. After i.t. injections of noradrenaline, the EMG amplitude was much increased and the cat was able to walk steadily for a relatively longer time period and double its maximal walking speed (0.2 m/s to 0.4 m/s). This suggests that the enhanced excitability of the cord produced by noradrenaline does not interfere with voluntary control of the animal but rather that the animal can utilize this increased spinal excitability to achieve a better locomotor performance, which is really the main goal and hope of locomotor pharmacotherapy in spinal cord injured patients (Barbeau and Rossignol 1994).

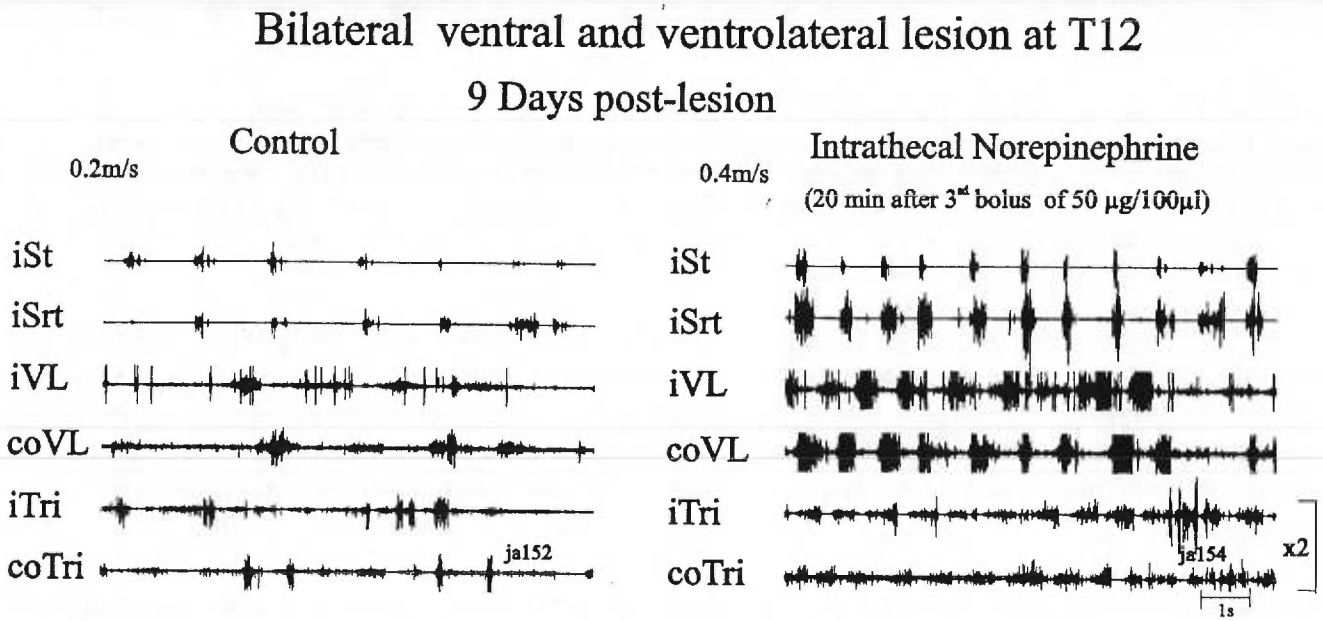


Fig. 3. Modulation of locomotion in a partially lesioned cat by i.t. administration of noradrenaline (NE) at 9 days post-lesion (large but yet undocumented spinal lesion because the cat is still alive). On the left is shown the EMG pattern of the cat walking at 0.2 m/s (its maximal speed performance) before the drug administration. On the right, the cat is walking at 0.4 m/s, 20 min after the 3rd dose of 50 µg/100 µl of NE i.t. (The other injections were given 1 and 1.5 h before). Abbreviations: are the same as in Figs. 1 and 2. Note saturation of the signal in the extensor muscles VL and that the rhythms of the hindlimb and forelimbs are different.

CONCLUSIONS

From these studies, we can conclude that, in ıost species, the locomotor program is innate and enerated centrally in the spinal cord. After spinali-ation, the locomotor capabilities change with time nd training (plasticity) and locomotion can be dapted to speed and perturbations. Locomotion an be triggered by alpha-2 noradrenergic stimula-on in early-spinal cats. In late-spinal cats, all neu-ɔtransmitter systems can on the other hand ıodulate the expression of the locomotor pattern. ı normal conditions, it is possible that the locomo-ɔr program can be triggered through the action of ɛveral descending pathways. We have no indica-on that any particular descending pathway is nique or essential for triggering locomotion. Even ats with massive ventral and ventrolateral lesions an perform voluntary quadrupedal locomotion al-ıough there are major deficits in weight support nd interlimb coordination. It is therefore possible) conclude that there is a spinal circuitry implicated ı the generation of the locomotor rhythm, that this ircuitry has some degree of plasticity which is es-ɛntial for any potential benefit of locomotor train-ıg, that activity in this locomotor circuitry can be iggered or modulated by neurotransmitters as well ; sensory inputs and finally that different descend-ıg pathways in the dorsolateral or the ventral-ven-olateral quadrants can trigger or modulate this rcuitry. It is believed that these concepts are im-ɔrtant and necessary for the design of locomotor ɛhabilitation strategies in patients with spinal cord ıjuries, especially when different approaches such ; locomotor training, pharmacotherapy and func-ɔnal electrical stimulation are combined (Barbeau ıd Rossignol 1994).

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