Université de Montréal

STUDIES ON THE PHARMACOLOGY OF LOCOMOTION IN ADULT CHRONIC SPINAL CAT

par

Connie W. Chau

Département de Physiologie
Faculté de Médecine

Thèse présentée à la Faculté des études supérieures
en vue de l'obtention du grade de
Philosophiæ Doctor (Ph.D.)
en science neurologiques

Avril 1998

Université de Montréal
Faculté des études supérieures

Cette thése intitulée:

Studies on the pharmacology of locomotion in
adult chronic spinal cat

présentée par:

Connie W. Chau

a été évaluée par un jury composé des personnes suivantes:

Dr. Tomas Reader
President-rapporteur

Dr. Serge Rossignol
Directeur de recherche

Dr. Hugues Barbeau
Co-directeur de recherche

Dr. Réjean Dubuc
Membre du jury

Dr. Paul Bédard
Examinateur externe

Thèse acceptée le 27 Avril 1998

Never give up on your dreams

Rick Hansen

SUMMARY

The goal of the present project was to study the enhancement of recovery of locomotion of the hindlimbs after spinalisation through training and/or pharmacological agents.

Locomotion has been shown to be generated by a locomotor circuitry within the spinal cord which normally interacts with peripheral afferents and descending inputs to adjust the pattern to changes of the environment or perturbations. Following a low thoracic complete spinal transection in adult cats, which eliminates all descending commands, cats initially loose their ability to support the weight of the hindquarters or walk on a treadmill. However, with time and especially locomotor training (practised stepping on a treadmill belt), spinal cats can recover the ability to fully support the weight of the hindquarters and walk on the treadmill with the hindlimbs which in a manner resembles the normal cat in many ways. This evolution after spinalisation implies that the spinal cord circuitry responsible for locomotion is capable of some plastic changes. It is not known, however, if locomotor training done sooner after spinalisation would further promote or accelerate the locomotor recovery process. The first series of experiments address this question. In order for locomotor training to be possible during the early post-spinalisation period (within the first week when the cat could not initiate any stepping), Clonidine, a noradrenergic agonist known to trigger locomotion, was

given. Electromyogram (EMG) of the hindlimb muscles synchronized to video images of the hindlimbs during treadmill locomotion were recorded. We found that cats that received daily injection of Clonidine followed by early and daily locomotor training showed a progressive improvement of locomotion and an acceleration of recovery of locomotion (6-11 days) as compared to previously reported (3 weeks).

Another goal of this project was to investigate the specific roles of various pharmacological agents in enhancing locomotor recovery. In the literature, the noradrenergic system was the most effective neurotransmitter system among others (serotonergic, dopaminergic, or excitatory amino acids) in triggering locomotion in adult spinal cats soon after transection. For instance, Clonidine was shown to trigger locomotion soon after spinalisation and modulate the well established locomotor pattern in adult spinal cats by increasing the step cycle duration. While different types of noradrenergic drugs (α1- and α2-agonists) have been shown to be able to mediate different physiological effects, little information is available on the effects of noradrenergic agonists other than Clonidine. The second series of experiments therefore explored the role of two other α2-agonists (Tizanidine and Oxymetazoline), an α1-agonist (Methoxamine), and noradrenaline itself, on locomotion and reflex transmission in adult spinal cats using an intrathecal catheter. Selective α2- and α1-antagonists (Yohimbine and Prazosin) were also used to block the effects. We found that all α2-agonists initiated locomotion within the first week post transection, whereas the α1-agonist was less consistent. All α2-agonists

reduced cutaneous excitability while α1-agonist increased the cutaneous excitability.

Therefore, our results obtained in cats would suggest that pharmacotherapy could be used as an adjunct to locomotor training to optimize the recovery of locomotion in patients soon after injury. It is also possible to incorporate the use of drugs in targeting specific postural and locomotor deficits as these drugs are capable of interacting with spinal locomotor center to affect locomotor ability, cutaneous reflex and muscle tonus.

RÉSUMÉ

L'objectif de cette étude était d'explorer la possibilité d'entraîner des chats à marcher sur tapis roulant, très tôt après une lésion spinale complète grâce à des agents pharmacologiques. Le rythme locomoteur est généré par une circuiterie neuronale intrinsèque à la moelle épinière, connue sous le nom de générateur central de patrons rythmiques, chez une variété d'espèces animales allant des invertébrés aux primates.

Le contrôle de la locomotion implique un système tripartite qui englobe l'interaction entre les centres supraspinaux, les circuits intraspinaux et les afférences sensorielles périphériques. Dans la littérature, il a été démontré qu'un chat adulte qui a subi une section complète de la moelle épinière est incapable, tôt après la lésion, de marcher sur tapis roulant et de supporter son poids, car les fonctions supraspinales n'agissent plus sur le centre locomoteur spinal. Avec le temps et de l'entraînement locomoteur sur tapis roulant, le chat spinal adulte retrouve la capacité locomotrice des membres postérieurs, avec support de poids complet, placement du pied et adaptation à différentes vitesses. Ceci suggère que la circuiterie spinale responsable de la locomotion a une certaine plasticité et est capable d'apprendre par l'entraînement locomoteur. Malgré l'importance de l'entraînement sur la récupération de la locomotion, aucune étude n'a été faite sur l'impact de l'entraînement très tôt après la spinalisation. Après une lésion spinale,

il est d'un grand intérêt d'entraîner le plus tôt possible car c'est probablement la période la plus propice pour obtenir une récupération maximale en profitant d'une certaine fenêtre de temps ou la plasticité est maximale.

Durant la première semaine post-lésion, l'entraînement sans drogue est presque impossible car les animaux n'ont pas de placement de pied et les mouvements alternés sont de courte durée. Cependant , des agonistes noradrénergiques (ex: Clonidine) administrés dans les premiers jours post-lésion, peuvent déclencher la locomotion chez l'animal spinal, ce qui permet des sessions d'entraînement plus longues et de meilleure qualité. Par conséquent, l'objectif de notre première série d'expériences était d'étudier l'effet de la Clonidine sur l'entraînement, dès les premiers jours post-lésion.

Pour la première série d'expériences, nous avons utilisé cinq chats adultes spinaux chroniques. Tous les chats étaient implantés à l'aide d'électrodes électromyographiques intramusculaires excepté un animal qui a reçu des électrodes sous-cutanées. Pour l'étude de la cinématique, nous avons enregistré sur cassette vidéo les séquences de marche sur tapis roulant, et l'utilisation de pastilles réflectrices placées sur des protubérances osseuses au niveau des articulations de la patte postérieure gauche a permis la reconstitution des mouvements.

Grâce à l'injection de Clonidine, dont l'effet dure de 4 à 6 heures, les animaux ont été entraînés tous les jours 1-5 fois et pour 15-20 minutes par session pour les premiers 10 à 11 jours post-spinalisation, et nous avons pu obtenir des périodes d'entraînement assez longues avec de vrais rythmes locomoteurs. Chaque

expérience a été documentée à l'aide de vidéos synchronisés avec l'activité musculaire, avant et après l'injection de Clonidine pour pouvoir suivre l'évolution de la récupération ainsi que l'effet immédiat dû à la Clonidine. Nous avons observé que l'injection quotidienne de Clonidine suivie d'entraînement intense dès le 2^{e} ou 3 ^{e} jour post-spinalisation accélère la récupération de la marche. De plus, après 6-11 jours d'entraînement, selon les animaux, la marche avec un support de poids et un placement du pied est réapparue. Dans la littérature on parle de délais beaucoup plus longs (14 jours, 24 jours et 3 mois). Nous avons également remarqué une amélioration quotidienne du patron locomoteur, une augmentation de la durée du cycle de marche, de l'excursion angulaire et de l'activité électromyographique.

Un autre objectif de ce projet était d'étudier les rôles spécifiques de plusieurs agents pharmacologiques sur la récupération de la fonction locomotrice. Jusqu'à maintenant, le système de neurotransmission le plus étudié dans le contrôle de la locomotion chez le chat spinal adulte tôt après la spinalisation est le système noradrénergique, car il s'est avéré être le plus efficace par rapport aux systèmes dopaminergiques, sérotoninergiques et certains acides aminés excitateurs. Il y a peu d'information quant à l'effet de d'autres agonistes alpha-2 (mis à part la Clonidine), ou d'agonistes alpha-1 sur le contrôle de la locomotion. En explorant différents agonistes noradrénergiques sélectifs, nous aurons probablement une meilleure compréhension de l'activation de différents récepteurs et ceci nous permettra de trouver le plus approprié pour une application clinique spécifique.

Cette information sera fondamentale pour nous permettre d'intégrer en toute connaissance de cause la pharmacologie et l'entraînement pour la récupération de la locomotion chez des patients.

Donc, pour la deuxième partie du projet, une série d'expériences a été faite chez cinq chats, ayant les muscles des membres postérieurs implantés ainsi qu'une canule intrathécale pour permettre l'administration des drogues directement sur la moelle lombaire. La spinalisation complète élimine tous les récepteurs pré-synaptiques en enlevant les terminaisons noradrénergiques descendantes, donc les récepteurs activés sont situés post-synaptiquement. Nous avons comparé les effets sur la locomotion et sur les réflexes, après l'administration intrathécale d'agonistes alpha-2 (Clonidine, Tizanidine et Oxymetazoline), et d'agonistes alpha-1 (methoxamine) et de la noradrénaline. Nous avons également utilisé des bloqueurs comme le Prazosin (antagoniste alpha-1) et la Yohimbine (antagoniste alpha-2). Dans tous les cas, la capacité locomotrice a été évaluée dès les premiers jours post- lésion. Après chaque injection, les effets ont été évalués à des intervalles réguliers, pour pouvoir déterminer la durée de l'effet de cette dernière. L'effet des drogues sur les réflexes cutanés chez les chats spinaux après récupération a aussi été analysé. Nous avons trouvé que les agonistes alpha-1 et alpha-2 agissaient différemment sur l'initiation de la locomotion chez le chat spinal aigu et sur la modulation de la locomotion et des réflexes cutanés chez le chat spinal chronique.

Tôt après la spinalisation, les trois agonistes alpha-2 étaient capables

d'initier la locomotion. L'effet de l'agoniste alpha-1, methoxamine, était moins constant. Il a déclenché une locomotion soutenue avec support de poids chez un chat spinal seulement et cette locomotion a été bloquée par le Prazosin, un antagoniste alpha-1 suggérant un rôle des adrénorécepteurs alpha-1 dans l'initiation de la locomotion. Dans trois chats testés, la methoxamine induit des épisodes de locomotion, quelques heures après l'injection, mais pas de façon aussi spectaculaire qu'après la Clonidine.

Chez les chats spinaux au stade chronique, les agonistes alpha-2 augmentent la durée du cycle de marche mais diminuent l'activité des extenseurs (ces effects sont bloqués par un antagoniste alpha-2, la Yohimbine). Les agonistes alpha-1 et la noradrénaline ne modulent pas la synchronisation du cycle de marche, mais augmentent le tonus extenseur des membres postérieurs. La Clonidine réduit l'excitabilité cutanée et augmente le trainement du pied, tandis que la methoxamine et la noradrenaline augmentent l'excitabilité cutanée. Parmi les agonistes alpha-2, des différences sont observées quant au temps d'action, au degré de modulation de l'excitabilité cutanée ainsi qu'au support de poids des membres postérieurs. La Clonidine et la Tizanidine ont des effets à court terme (quelques heures). L'Oxymetazoline par contre a un effet beaucoup plus prolongé jusqu'à 2-3 jours. L'effet de la methoxamine dure jusqu'à 24 heures chez le chat spinal aigu. Nos résultats suggèrent des effets différents des agonistes alpha-1 ou alpha-2 , les alpha-1 semblent plus importants dans le contrôle de l'activité musculaire et les alpha-2 jouent un rôle dans les aspects temporels du patron locomoteur.

Nos résultats tendent à démontrer que la pharmacologie peut jouer un rôle important dans la récupération de la locomotion. Par l'injection de différents agonistes noradrénergiques, nous pouvons obtenir des effets spécifiques sur l'initiation et la modulation du patron locomoteur, l'excitabilité des réflexes et aussi sur le tonus musculaire. Les progrès rapides obtenus suggèrent qu'un entraînement quotidien avec l'administration de drogues comme la Clonidine dès les premiers jours suivant une transection complète de la moelle épinière accélère la récupération de la marche sur tapis roulant en augmentant la durée et la qualité de l'entraînement dès les premiers jours post-transection. L'usage d'agonistes noradrénergiques pourrait nous permettre de cibler des déficits spécifiques et d'y remédier. La méthoxamine peut être plus utile que la Clonidine si l'on veut compenser de déficits posturaux car elle augmente le tonus extenseur des membres postérieurs, ce que la Clonidine ne fait pas.

L'usage de drogues comme l'Oxymetazoline et la methoxamine, qui ont un effet prolongé allant jusqu'à trois jours, pourra sûrement présenter des avantages d'un point de vue clinique. Les resultats ce projet pourraient permettre de développer de nouvelles stratégies dans le but d'accélérer la récupération des fonctions locomotrices chez des patients ayant subi une lésion spinale.

TABLE OF CONTENTS

LIST OF FIGURES

(General introduction)

LIST OF ABBREVIATIONS

(General introduction and discussion)

EMG:	Electromyogram
ENG:	Electroneurogram
VL:	Vastus Lateralis
GL:	Gastrocnemius lateralis
Glu:	Gluteus medius
St:	Semitendinosus
Srt:	Sartorius
IP:	Iliopsoas
FRA:	Flexor reflex afferent
EDB:	Extensor digitorum brevis
CPG:	Central pattern generator
MLR:	Mesencephalic locomotor region
PLS:	Pontomedullary locomotor strip
PPN:	Pedunculopontine nucleus
MRF:	Medullary reticular formation
EAA:	Excitatory amino acids
NMDA:	N-methyl-D-aspartate
NMA:	N-methyl-D,L-aspartate
APV:	2-amino-5-phosphonovaleric acid
CNQX:	6-cyano-7-nitroquinoxaline-2,3-dione
NE:	Noradrenaline
5-HT:	Serotonin
5-HTP:	5-hydroxytryptophan
6-OHDA:	6-hydroxydopamine
GABA:	Gamma amionbutyric acid
ACh:	Acetylcholine
AHP:	After hyperpolarization

ACKNOWLEDGEMENTS

I give thanks to God for His grace and rich blessings so abundantly bestowed upon me.

I express my profound gratitude to my research supervisor and mentor, Dr. Serge Rossignol from whom I learned so much. His guidance, insights, inspirations, patience, encouragements, understandings, and unfailing support have been essential for the advancement and completion of my doctoral studies. I am also thankful to have the chance to work in his laboratory.

I am also grateful to my research co-supervisor, Dr. Hugues Barbeau, for his constructive criticisms, understandings and encouragements throughout my doctoral studies.

My sincere thanks to Ms. Janyne Provencher and Ms. France Lebel, my work partners and friends. Their expert help, resourcefulness, enthusiasms, sense of humour and constant encouragements have not only made my graduate studies a rewarding experience but a truly enjoyable one.

I am also indebted to Dr. Jean-Pierre Gossard who has unfailing helped me throughout all phases of my studies. I am grateful that I can always count on him for support and advice.

Many thanks to Dr. Trevor Drew for his availability to answer my never-ending list of "quick questions". I am always thankful for his generous help and his friendliness.

I am grateful to Dr. Tomas Reader for use of pharmacological agents from his laboratory, for his help with all my questions of pharmacology, and for his valuable advice during the writing of the articles. His kindness is also much appreciated.

Sincere thanks are also due to Dr. Vincent Castellucci for his support during my thesis preparation.

I also gratefully acknowledge Mr. Philippe Drapeau, Mr. Gilles Messier, Mr. Claude Gagner and the late Mr. Richard Bouchoux for technical assistance; Ms. Jeanne Faubert for help during surgery; Mr. Daniel Cyr, Mr. Claude Gauthier and Mr. Giovani Filosi for skillful artwork; Ms. Féliciana Cantin and Ms. Jeanne Lavoie for the histology; Ms. Suzanne Cabana and Ms. Chantal Nault for secretarial assistance.

I extend my thanks to my colleagues, Ms. Edna Brustein, Ms. Nathalie Giroux, Dr. Laurent Bouyer and Ms. Iran Salimi for their friendships, encouragements and many interesting discussions.

Last but not least, heartfelt thanks to my family (mom and dad, Sharon, Matthew) and friends (Luke and Nicole) who have sustained me through the years.

DEDICATION

To

Mom and Dad

GENERAL INTRODUCTION

It has been shown, in a wide range of animals and preparations, that locomotion is still possible following a complete spinal cord transection and that the spinal cord itself is capable of coordinating and generating many stereotypic movements including locomotion. The ability of the spinal cat to recuperate locomotion has been shown to depend on the amount of locomotor training which also implies that there is some plasticity in the spinal cord.

There is, however, no information on the effects of very early locomotor training on locomotor recovery. Since pharmacological agents, especially noradrenergic drugs (L-DOPA and Clonidine) can trigger treadmill locomotion soon after a spinal transection, we have used Clonidine daily for an intensive early locomotor training in the first week post-transection. We have also investigated and compared other noradrenergic agonists (α2- and α1-type).

Early locomotor training is of particular interest as early post-lesion may be an optimal time period where training might interact or even shape the ongoing plasticity process thus maximizing recovery. This question will be addressed in detail in the first article. Furthermore, it is important to examine other pharmacological agents that may affect locomotion as this information is the first step towards identifying perhaps the most pertinent drug that could be tested or used for clinical studies. A better understanding of the receptors involved in mediating different physiologic effects is also vital in our hope to use pharmacology to help patients with functional deficits. The effects of different noradrenergic

noradrenergic agonists will be addressed in the second article.

In the general introduction, some general literature pertaining to the understanding of locomotion will be reviewed. First, the characteristics of the normal locomotor cycle shown by kinematic and electromyographic data will be described. A brief literature review on the role of the spinal locomotor center in generating the basic locomotor pattern and on the importance of locomotor training on the recovery of locomotion in cats with complete spinal cord transection will be made. The evidence of plasticity of the spinal cord underlying learning and recovery will be presented. A brief review on the role of peripheral afferents and supraspinal inputs which adapt and regulate locomotion will be made. We will also describe some previous works on the pharmacological effects of various drugs on locomotor functions, with particular emphasis on the descending noradrenergic system.

Intact locomotion

To better understand how drugs or other manipulations can change locomotion, it is important to know first the kinematics and electromyographic (EMG) characteristics of locomotion in normal cats.

Kinematics

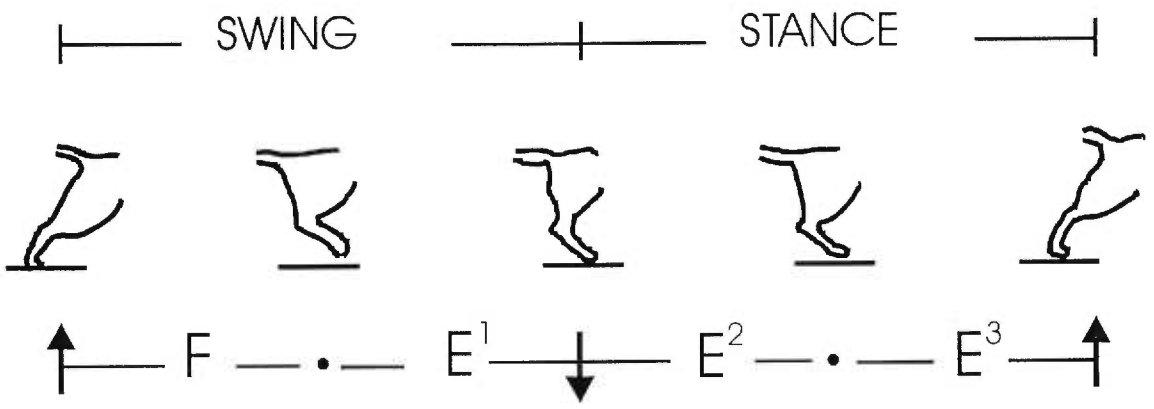
A complete step cycle during locomotion is composed of a stance and swing phase (Goslow et al. 1973; Rasmussen et al. 1978; Halbertsma 1983; Kuhtz-Buschbeck et al. 1994). The swing duration is usually 40% of the total step cycle duration whereas stance occupies about 60% (Goslow et al. 1973). With increasing treadmill speed, however, the stance duration shortens considerably whereas the swing duration, in real time, remains relatively unchanged (Halbertsma 1983).

Throughout the stance phase, the paw, by definition, is in contact with the support surface (support phase) and during the swing phase, the limb is brought forward again (transfer phase). According to Philippson (1905), the step cycle can further be subdivided into one flexion phase (F) and three extension phases (E1, E2, and E3) based on events occurring at the knee and ankle, as shown in Fig.1.

Figure 1.

Schematic diagram illustrating the subdivision of the locomotor step cycle by Philippson (1905) (Rasmussen, S., Chan, A.K., and Goslow, G.E.J. *J.Morphol.* 155:253-270, 1978.).

F: flexion; E^1 E^2 E^3 : first, second and third extension phases. Upward arrows indicate of foot-lift and downward arrow indicates foot contact.


SWING
STANCE
F
E1
E2
E3

The swing phase can be subdivided into F and E1. The F phase starts with lifting the lifting of the foot when the hip, knee and ankle all flexes and ends with E1 which starts when the knee and ankle begin to extend while the limb is still the air and the hip was still flexing. The stance phase can be subdivided into E2 and E3 phase. E2 starts with touch down and the hip joint only begin to extend after touch down (Engberg and Lundberg 1969). Soon after the foot strikes the ground, the knee and ankle joint yield (flexed slightly) under the body weight. E3 starts when there is extension of all joints. During stance, there is a slow and steady increase in extension of the hip, knee, ankle and MTP joints (Goslow et al. 1973).

Transition to swing phase At the end of the third extension phase (E3) while the hip and knee extension continues (Goslow et al. 1973) there is a rapid extension of the ankle joint, accompanied by a rapid plantarflexion of the MTP joint. The maximal ankle extension is reached at the transition phase and the maximal MTP extension is reached ~50 ms before lift-off (Kuhtz-Buschbeck et al. 1994). All these events participate in the body propulsion.

Placement of the foot at foot contact This event has been described as "*the single most important event in the stride cycle* " by Halbertsma (1983). Indeed, the control of a proper foot placement would ensure that the animal succeeds in weight acceptance during stance and prevents the animal from falling. A high covariance at the hip, knee and ankle joint has been found suggesting that there is a precise

position control of the foot at touch down (Halbertsma 1983). In addition, a rapid flexion of the distal joint, the proximal interphalangeal joint (PIP) joint at touch down (Kuhtz-Buschbeck et al. 1994), through active muscle contraction or passively induced by the braking force at contact (Halbertsma 1983) might also serve to ensure proper anchorage of the foot on the ground.

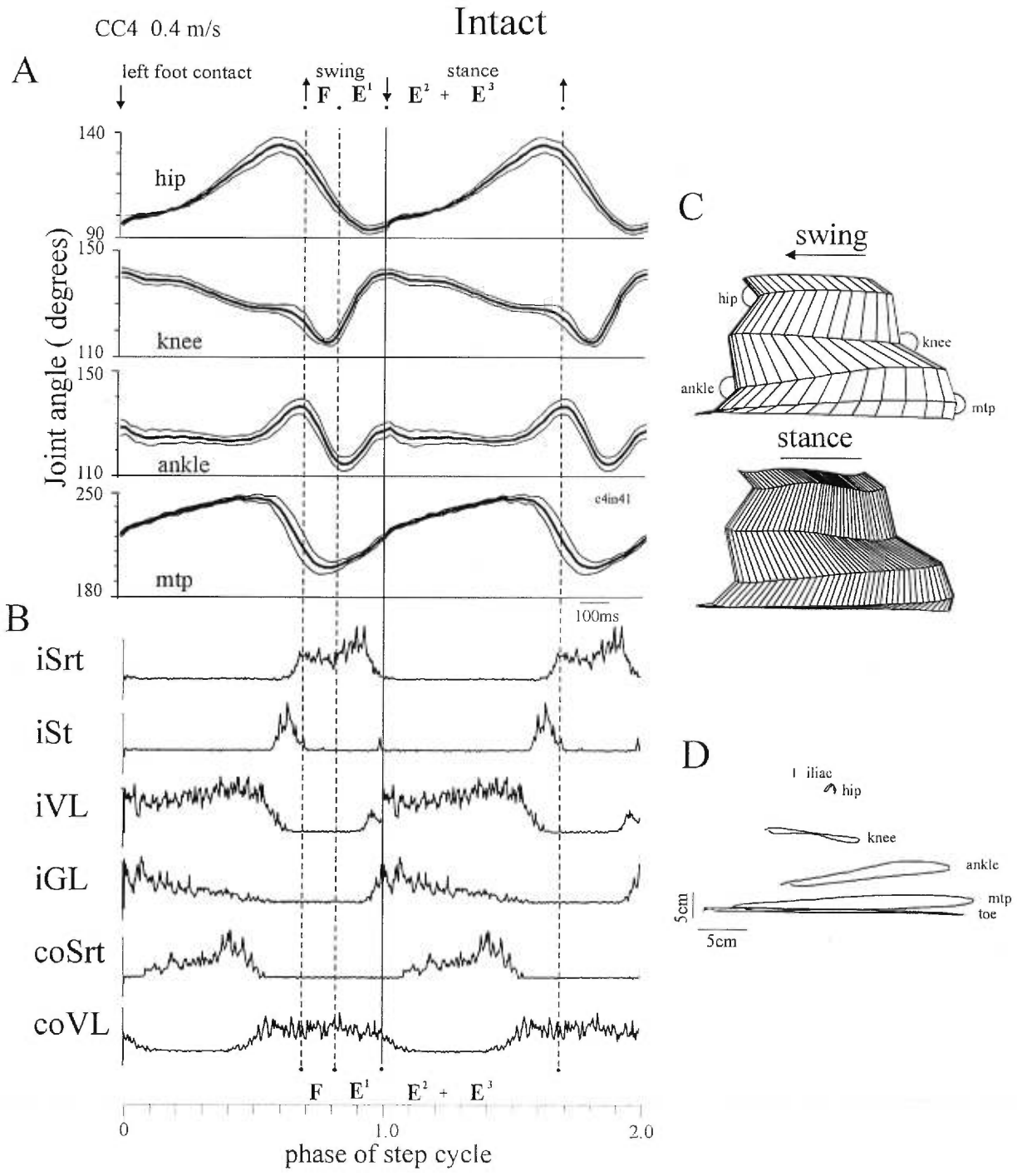
The subdivision of the Philippson's cycle is useful for describing the locomotor cycle but it is not absolute. In the case of spinal locomotion, for example, uncoupling of the hip and knee joints is often seen which therefore is sometimes difficult to describe it using Philippson's terminology.

Electromyograms (EMG)

While undoubtedly all hindlimb muscles are involved in controlling locomotion, only the hindlimb muscles that we have recorded will be described. Figure 2 shows an example of the joint angular changes coupled with EMGs during normal treadmill locomotion in one of the intact cat (CC4) used in the experiments.

Figure 2.

Angular excursion, stick diagrams, trajectories and EMG of the hindlimb during intact locomotion of a cat CC4. Treadmill speed was 0.4m/s. Averaged angular plots (n=10) are synchronized with averaged hindlimb EMG activity (n=10). On the angular plots, an increase in angle value indicates extension while a corresponding decrease indicates flexion. The stance and swing phase are subdivided into Philippson's F, E^1 E^2, and E^3 phases. Upward arrows indicate foot lift and downward arrows indicate foot contact. The stick figures with the corresponding joint trajectories represent the stance and swing phase of 1 step cycle. i: ipsilateral, co:contralateral, Srt: Sartorious, St: Semitendinosus, VL: Vastus Lateralis, GL: Gastrocnemius lateralis.


CC4 0.4 m/s
Intact
A
left foot contact
swing
stance
F
E1
E2 + E3
140
90
hip
150
110
knee
150
110
ankle
250
180
mtp
Joint angle (degrees)
c4im41
100ms
B
iSrt
iSt
iVL
iGL
coSrt
coVL
F
E1
E2 + E3
0
1.0
2.0
phase of step cycle
C
swing
hip
knee
ankle
mtp
stance
D
iliac
hip
knee
ankle
mtp
toe
5cm
5cm

Extensors In general, the extensor muscles always have their main activity in the stance phase, and in most cases preceded by a short lasting activity in E1 (Engberg and Lundberg 1969). Vastus lateralis (VL) and gastrocnemius lateralis (GL) are knee and ankle extensors muscles respectively and they are primarily active during the stance phase to provide support for the animal. VL and GL are recruited before the actual paw contact (~ 40 ms) and are active until the E3 phase just prior to paw lift. It is believed that the cessation of activity just before paw lift is to allow the unloading of the limb to prepare for the swing phase (Engberg and Lundberg 1969; Rasmussen et al. 1978). The profile of VL and GL is different. While VL activity has a gradual onset and plateau during E3 phase, the GL activity seems to have a sharp onset but progressively decreased during the E3 phase. Gluteus medius (Glu) is a hip extensor. It fits the typical extensor muscle pattern where it is active during the support phase. Its prime function is to stabilize the pelvic girdle and prevent it from sagging during stance thus supporting the limb in a more rigid alignment.

Flexors In general, the activity of the individual muscle in the flexor group does not conform to a single pattern as in the case for extensors (Engberg and Lundberg 1969). Sartorius (Srt) and iliopsoas (IP)(not shown) are primarily hip flexors. IP and Srt are recruited just before F and their activity are maintained throughout the entire swing phase (F, E1) until paw contact. Semitendinosus (St) exhibit two EMG burst during walking or trotting. St is recruited in late E3 phase and is active in initial

swing involving paw lift, a second smaller burst of St can be seen during E1, just prior to paw contact. St is a biarticular muscle (primarily a knee flexor, but also a hip extensor) (Engberg and Lundberg 1969; Rasmussen et al. 1978). The second burst of St increases at speeds (0.35-2.5m/s) and may be attributed to the greater need of a flexor torque to decelerate the hip and knee at the end of swing (Smith et al. 1993; Wisleder et al. 1990).

Neural control of locomotion

Stepping has been proposed by Brown (1911; 1914) to be generated by a central spinal mechanisms involving a mutual inhibitory connection between flexors and extensors half-centers. The activity of one half center (flexor) would silence the other center (extensor), and later, due to "fatigue" the activation will be shifted to the other half-center, thus giving rise to rhythmic reciprocal activity of the 2 half centers (for review, see Lundberg, 1981). The existence of a spinal circuitry compatible with the "spinal half-centers" was later suggested by Lundberg and colleagues (Jankowska et al. 1967). In acute spinal cats, after i.v. L-DOPA injection, they found that while the usual short latency reflex evoked by the flexor reflex afferent (FRA) was inhibited, a long lasting discharges was seen. The scheme of Fig. 3A indicates that DOPA (through the release of noradrenaline) inhibits the transmission of the short latency FRA reflex (pathway A) thus releasing pathway B from tonic inhibition

pathway B from tonic inhibition (by pathway A), which lead to the long lasting late-discharges (Anden et al. 1966b,c). Subsequent findings showed that in the presence of DOPA, FRA can evoke alternating activity in the ipsilateral flexor and extensor motoneurons resembling the stepping rhythm (Jankowska et al. 1966). Thus, a neuronal network resembling a half-center organization with mutual inhibition between interneurons exciting flexor and extensors was found through the stimulation of FRA (Anden et al. 1966a,c; Jankowska et al. 1966; Jankowska et al. 1967)(Fig. 3B). It was suggested that this network, released by DOPA, could be part of the neuronal circuitry for rhythmic alternating movements such as stepping (Jankowska et al. 1967). They proposed that a simple central program ("Brown's half-centers or DOPA- network") was responsible for the generation of basic alternating flexor and extensor activity, which is modified by proprioceptive reflexes responsible for "sculpting" the details of the locomotor pattern (eg. different timing and durst duration of Srt and St) (Lundberg 1969). In this scheme, the sensory feedback is needed to produce the details of the locomotor pattern.

Figure 3.

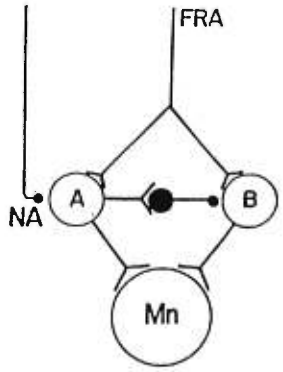
Spinal neuronal circuitry proposed by Lundberg and colleagues (Jankowska, E., Jukes, M.G., Lund, S., and Lundberg, A. *Acta physiol.scand.* 70:369-388, 1967.).

A. Proposed circuitry to explain the late and long-lasting discharges observed after L-DOPA injection in cat.

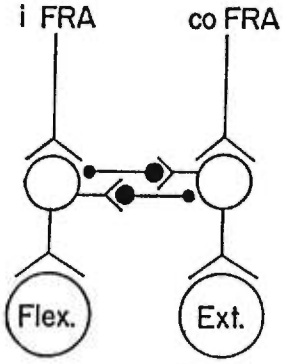
B. Neuronal substrate identified by Lundberg and colleagues which corresponds with Brown's half-center's hypothesis for spinal stepping.

i: ipsilateral; co: contralateral; FRA: flexor reflex afferent.

A



B



Central pattern generator

Later, it has been reported that the spinal cord, deprived of descending and peripheral inputs, was capable of generating a complex locomotor pattern (Grillner and Zangger 1979; Delcomyn 1980; Perret and Cabelguen 1980; Perret, 1983; Dubuc et al. 1986; Pearson and Rossignol 1991). For example, Grillner and Zangger (1979) reported that in acute spinal curarized cat, eliminating all phasic sensory inputs, rhythmic alternating discharge pattern of electroneurographic (ENG) bursts of activity of hindlimb muscle nerves were observed following the injection of the noradrenergic precursor (DOPA) potentiated with nialamide, a monoxidase inhibitor. They showed that the onset of St (knee flexor) and TA (ankle flexor) were also different. The distinct activity pattern of extensor digitorum brevis (EDB, a distal toe-dorsiflexor), which is maximally active during the placing of the foot (E1)(Engberg and Lundberg 1969), was still observed after dorsal root transection in decerebrate cats (Grillner and Zangger 1984) and in acute curarized decorticate cats (Perret and Cabelguen 1980). Thus, a complex detailed locomotor "pattern" could be generated by the neuronal circuitry within the spinal cord itself in the absence of supraspinal or peripheral inputs. The neuronal network for locomotion in the spinal cord was referred to as the "central pattern generator" (CPG) (Grillner, 1981). This "central control mechanism" has also been found to be responsible for a number of rhythmic behaviors such as breathing, chewing, fast paw shaking, wiping and scratching and is found in animals ranging from invertebrates to

vertebrates (Delcomyn 1980). Recent evidences suggest that the spinal locomotor networks might be present in monkeys (Hultborn et al. 1993) and possibly in humans (Calancie et al. 1994).

In normal animals, control of locomotion is probably achieved by constant interactions of the CPG with segmental and supraspinal mechanisms to produce goal-oriented behavior adapted to changing environmental needs. This neural control system for locomotion will be reviewed since it is important for the following chapters. Emphasis, however, will be placed on the segmental control mechanisms as animals in this study have a complete spinal cord transection which deprived the spinal locomotor center of any influence from the descending pathways. On the other hand, peripheral inputs will be described in some details since they are important for the control of locomotion in spinal cats.

Spinal locomotion

Following complete spinal cord transections (Th 12) in kittens during the neonatal period to the first 2 weeks after birth, they were shown to have a remarkable ability to recover hindlimbs locomotion (Forssberg et al. 1980a, b; Goldberger, 1986; Bradley and Smith 1988). These spinal kittens were able to produce some steppings provided that the hindlimbs were touching the moving

treadmill belt, beginning at as early as 1-2 days post-transection, a stage when the kittens' eyes were still closed (Forssberg et al. 1974; Bradley and Smith 1988) suggesting that the spinal locomotor rhythm is innate. The kinematics and EMG pattern of the spinal treadmill locomotion in chronic spinal kittens progressively improved and were found to be similar to that seen in normal cats (Forssberg et al. 1980a, b; Bradley and Smith 1988). There were also, however, some deficiencies such as a lack of equilibrium and a smaller propulsive force. The motor pattern was also found to be more fatiguable (Grillner, 1985), a deficit which in part could be attributed to the loss of descending inputs.

Cats spinalized as adult have been shown to fare not as well as those spinalized as kittens (Smith et al. 1982; Goldberger and Murray, 1985; Goldberger, 1986; Eidelberg et al. 1980). In contrast to cats that were spinalized early at birth, adult spinal cats only exhibited few treadmill-elicited locomotor cycles with shorter stride length (Goldberger and Murray, 1985). In another study, the locomotor capacity of the adult spinal cats (Th 6-8) were also found to limited, when all four limbs were placed on the treadmill (Eidelberg et al. 1980). On the contrary, it has been reported that adult cats that had a complete spinal cord transection were still capable of a great extent of locomotor recovery of the hindlimbs provided that they were given locomotor training following spinalisations (Rossignol et al. 1982; Barbeau and Rossignol 1987; Rossignol and Dubuc 1994; Smith et al. 1982; Rossignol et al. 1996, see appendix B). Later findings also supported the

importance of training in the recovery of locomotion, especially training that involves weight-bearing stepping of the animals (Barbeau and Rossignol 1987; Lovely et al. 1990; Edgerton et al. 1991). The role of training in locomotor recovery will be described in more details.

Locomotor training

In fact, training has long been suggested to play an important role on the functional ability of cats following spinal cord transection. Shurrager and Dykman (1951) reported an improvement in the overground walking behaviors in spinal kittens that received electrical stimulation of the hind limbs (1 hour a day). They found that "walking responses of the hind legs became stronger and more functionally effective as training was continued".

Previous work in our laboratory showed that adult spinal cats (Th 13) that were trained on the treadmill 2-3 times per week for at least half an hour, attained a good locomotor function at three weeks to 1 month following the training (Rossignol et al. 1982; Barbeau and Rossignol 1987). During the training sessions, the spinal cats were led to progressively support more weight at different treadmill speeds. The recovered spinal locomotion was characterized by large steps, bilateral plantar foot placement , weight support of the hindquarters up to 3 minutes, and adapt to the treadmill speed up to 1.0m/s. However, a paw drag was frequently

observed during the initial swing phase. In another set of experiments, adult spinal cats were trained on the day following spinalisation ranging from 15-30 minutes each session, 1-2 sessions per day (Bélanger et al. 1996). However, during the first week, training was not effective as the hindlimbs were extended and the paw could not advance in front of the hip to enable weight acceptance during stepping. Ranging from 14-24 days post-transection (a mean of 20 days), spinal cats were found to be able to fully support the weight of the hindquarters.

It has also been shown that among the 12-week spinal cats (Th 12), the trained cats had superior performance to those that were untrained. Training began on the 3rd week post-transection and was done 5 times a week, 25-30 minutes each. During training, treadmill locomotion at different speeds ranging from 0.2 m/s-1.0 m/s was practiced with the goal of achieving full weight-supported locomotion. Trained and untrained spinal cats were evaluated at the 4th month post-transection. The untrained spinal cats performed poorly with occasional plantar foot placement and were unable to support their weight during treadmill locomotion whereas the trained spinal cats exhibited excellent weight support during locomotion and adapted to treadmill speed up to 0.8 m/s (Smith et al. 1982).

In another study, adult spinal cats were trained 30 min/day, 5 days/week for approximately 5 months. Assessment of the locomotor ability at six months post-training showed that the adult spinal cats were able to fully support the weight of

their hindquarters and generate reciprocal stepping on a treadmill (Lovely et al. 1990; Edgerton et al. 1991; Hodgson et al. 1994). The EMG and kinematic data of the general stepping pattern was found to be remarkably similar to normal (Lovely et al. 1990).

Thus, there are ample evidence confirming that following spinalisation, adult spinal cats can remarkably recover locomotor ability with time. Training appears to be crucial in enhancing this progressive recovery process suggesting that there is some plasticity of the spinal circuitry responsible for generating locomotion.

Spinal plasticity and effects of training

A brief review on some studies examining the ability of the segmental circuitry to undergo plastic changes following injury will be made. This is important as spinal plasticity might be part of the underlying mechanisms of training effect (in animals with complete cord transection) which lead to locomotor recovery.

Initially, it has been suggested that the spinal cord circuitry generating locomotion is hard-wired and has limited capacity to reorganize itself following muscle transposition (Sperry 1940; Sperry 1941; Forssberg and Svartengren 1983). In rats, it was reported that the transposition of flexor and extensor muscle of the shank or crossing of flexor and extensor nerves resulted in a reversal of foot

movement which persisted with time (Sperry 1940; Sperry 1941). The lack of motor readjustment of the hindlimb was interpreted as the limitation of the central mechanism to adapt to altered peripheral input. In adults cat where the ankle extensor muscle (gastrocnemius) was transposed, it was also shown that the transposed extensor muscles retained the same motor pattern during locomotion despite their inappropriate action in the cycle (Forssberg and Svartengren 1983). For example, gastrocnemius was activated concomitantly to soleus in all cats although it had now a mechanical action of an ankle flexor. It was proposed that the central locomotor network, being an innate type of motor pattern, might be rigid and hard-wired and thus only capable of minor modification in response to the transposition of muscles. However, studies have shown that long term functional changes can occur following the transposition of palmaris longus muscles in the cat forelimb. The transposed muscle became active mainly during swing instead of its original activation during stance (Yumiya et al. 1979).

Although it is suggested that the spinal cord circuitry for generation of locomotion is hard wired, increasing evidence emerged in the past decade to demonstrate plasticity at the spinal cord level in response to an external changes, such as changes of the descending supraspinal input or the peripheral inputs, or both (for review, see Wolpaw and Carp 1993). Evidence of spinal cord plasticity can be found in different studies.

Early studies by Shurrager and colleagues (Shurrager, 1955) provided evidence that spinal learning can be achieved through training. They reported that conditioning was still possible after spinalisation. In spinal animals, the conditioned stimulus (CS) was initially neutral, that is, when presented alone did not cause a movement of the hind leg. However, when presented in conjunction with the unconditioned stimulus (UCS), an electric shock, the animal responded to the CS with a movement of the hind leg, that is a conditioned response (CR). The CR persisted and was still present after the CS was presented alone or after a rest period. Also, the CR could be extinguished by successive presentations of the CS without reinforcement by the UCS. It was then suggested that the chronic spinal mammals are capable of acquiring motor conditioned response with training.

Similarly, in a classical conditioning paradigm, Durkovic and colleagues (Durkovic 1983) showed that the flexion reflex of acute spinal cats (Th 10) exhibited facilitation over trials to the first of 2 temporally paired stimuli (conditioned and unconditioned stimuli). They suggested that the spinal cord of the cat has the capacity for functional changes, and the locus of learning might be in the interneuronal pool (Durkovic 1983).

Wolpaw and colleagues conducted a series of studies to examine the adaptive plasticity of the spinal cord (Wolpaw 1983; Wolpaw et al. 1983; Wolpaw and Chong 1989; Wolpaw et al. 1989). Initial experiments were based on studies

of spinal stretch reflex (SSR). The SSR of biceps was elicited in monkey by delivering a short pulse to cause an extension torque to the elbow. The task was to learn to adjust the biceps EMG to a preset level (SSRup or SSRdown mode) without any change in initial muscle length or background activity in order to receive liquid reward. In the SSRup mode, reward was given only if the biceps EMG was greater than a specific value, and in the SSRdown mode, reward was given only if the biceps EMG was lower than a specific value. This task requires continual changes in supraspinal influence on the reflex arc. They measured the SSR amplitude which reflect the number of α-motoneurons that fires in response to Ia afferents due to the sudden muscle stretch. The amplitude of SSR was also subject to changes of the descending activity. They operantly conditioned the descending activity onto this reflex pathway. With time and repeated practice, monkeys can gradually increase or decrease the amplitude of SSR without changes in the initial muscle length or background EMG activity (Wolpaw et al. 1983; Wolpaw 1983). They concluded that spinal reflex function can undergo adaptive change. They hypothesized that SSR change was due to intrinsic segmental alteration. To eliminate the muscle spindle as a possible site for plasticity, the task was modified to operantly conditioned the H-reflex (Wolpaw and Chong 1989; Wolpaw et al. 1989). They measured the triceps surae H-reflex amplitude (HR) in both legs while the reward was controlled only by H-reflex in one conditioned leg, and the other leg served as control. Similar to the SSR change, the operant H-reflex size change (HRup and HRdown) was seen progressively in the conditioned leg while little

changes were observed in the control leg. That is, the change in triceps surae H-reflex amplitude was specific to the trained muscle. Moreover, acute experimentations on these animals revealed that this H-reflex conditioning persists in the spinal cord of a trained animal after the removal of all supraspinal influences by thoracic cord transection (Wolpaw and Chong 1989; Wolpaw et al. 1989). Reflex asymmetries between the conditioned leg and control leg, consistent with the effect of H-reflex conditioning persisted through the 3 days of study where the animal remained anesthetized throughout.

Theses studies provide evidence that plasticity for H-reflex conditioning was indeed located within the spinal cord. To further identify the neuronal changes responsible for the plasticity, Wolpaw and colleagues studied the triceps surae motoneurons properties and Ia EPSPs of the animals using intracellular study (Carp and Wolpaw 1994; Carp and Wolpaw 1995). They suggested that supraspinal sites as well as spinal sites, or an interaction of both may have been responsible for the H-reflex conditioning changes.

Recent work in our laboratory also demonstrated the functional plasticity of the spinal cord following nerve lesion (Carrier et al. 1997). Cats were exposed to 2 subsequent injuries, firstly, a neurectomy of the ankle flexor nerves, tibialis anterior (TA) and long extensor of toes (EDL) in one limb (lesioned), and secondly, a complete thoracic spinalisation (Th 13). The cat was found to recover remarkably well following peripheral neurectomy. Slight adjustments such as increase hip and

knee flexion, was sufficient to compensate for the loss of function at the ankle. However, following the second injury, spinalisation, the locomotion was greatly disrupted. There was an asymmetry between the lesioned and intact leg. The steps were short, the dorsum of the paw was contacting the treadmill belt, the ankle and MTP joint were in hyperextension and the knee showed exaggerated flexion during swing. They suggested that in the neurectomized but otherwise intact cat, the compensation is possibly achieved at the spinal and supraspinal levels. The neurectomy caused new biomechanical constraints to the CPG of the spinal cord. The supraspinal input onto the CPG may have been "readjusted" to provide for the necessary adjustments to maintain the locomotion (such as an increase in flexor EMG). This readjusted descending input may have caused adaptive changes in the spinal cord which became evident after spinalisation when all descending inputs were removed. These findings are in agreement with Wolpaw and Carp (1993) suggesting that exposure of the spinal circuitry to altered supraspinal influences can induce intrinsic and persistent changes in the spinal cord.

In fact, this spinal plasticity can also be reflected by the evolution of the locomotor pattern with time. Barbeau and Rossignol (1991) showed that the locomotor pattern of chronic adult spinal cats improved from d2 to d7 post-transection following Clonidine injection (150ug/kg i.p.). From d2-d7, there was an increase in the step cycle and stance duration for the same treadmill speed accompanied by a prolonged EMG activities of the extensor muscles (Barbeau and

Rossignol 1991). In a preliminary study (Barbeau et al. 1993, see appendix A) and in the present thesis, we show that locomotor changes can be seen at d2 to d9 post-transection as a result of daily Clonidine injection and locomotor training.

Such time-dependent changes were also seen in the central expression of fictive locomotor pattern (Pearson and Rossignol 1991). Using paralyzed decerebrate cats, the fictive locomotor pattern from hindlimb nerves were recorded shortly after spinalisation (early-spinal animal) and later, after they have been trained to walk on the treadmill (late-spinal animals). In early-spinal cats, the expressed fictive pattern, facilitated by Clonidine injection, was found to resemble that of locomotion with alternating flexor and extensor nerves activities. However, the pattern was more rudimentary, for instance, iSt and iSrt bursts had the same duration. In contrast, the fictive locomotor pattern in late-spinal cats was more complex. The bursts duration of different flexors (iSt and iSrt) were clearly different as in the normal. These findings suggested that the spinal cord was capable of modifying the circuits that established the temporal characteristic of the locomotor pattern and that training was a possible contributing factor.

In addition, other studies have shown that training not only can lead to functional changes in the spinal circuitry in generating a motor task, but that the learning was training specific. Viala and colleagues reported that trained spinal rabbits exhibit different motor ability dependent on the training they received (Viala et al. 1986). In rabbits spinalized at 2 days after birth, spontaneous locomotion was

obtained 3-4 weeks post-transection. The spontaneous locomotion, however, was predominantly of an alternating pattern, with very few rabbits displaying exclusive in-phase patterns. Training of the spinal rabbit began when they were 10 days old. Four groups of animals were compared after each group had received different types of training on a motor driven "bicycle" which moves the hindlimbs in-phase and out-of-phase mode. The first group of animals which were trained exclusively on the out-of phase mode (alternating stepping), exhibited an exclusive alternating locomotor pattern upon tail pinching. The second group was alternatively trained on both in-phase and out-of-phase bicycle. The elicited locomotor pattern consisted of 50% of in-phase and 50% of synchronous stepping. The third group was trained exclusively on in-phase bicycle, with hindlimbs unrestrained in between sessions. These animals progressively developed synchronous stepping during locomotion. The last group of rabbits were also trained on in-phase bicycle, as in the third group, but their feet were bound to a pair of connected skis to preclude any spontaneous alternating movements in between session. These animals exhibited exclusive in-phase locomotor pattern after training. Thus, the expressed locomotor pattern in infant-spinal rabbit was clearly training-specific. Peripheral afferent input could strongly affect the plasticity of central locomotor networks during early development.

Edgerton and colleagues compared 3 group of cats, untrained, stepping-trained, and standing-trained spinal cats. They showed that cats that were trained to stand (standing -trained) were capable of maintaining a standing posture for 30 minutes with minimal perineal stimulation. In contrast, stepping-trained cats were unable to successfully maintain a standing posture for any length of time unless a large amount of perineal stimulation was given (Edgerton et al. 1991; Hodgson et al. 1994). On the other hand, although both the untrained and the stepping-trained cats were able to walk on the treadmill, the stepping-trained cat had a better performance than the untrained cat. While the former can adapt to the treadmill speed to 0.8m/s, the latter can only walk up to 0.25m/s. In contrast, the standing-trained cats were only capable of some poor steps at very low treadmill speed (less than 0.2m/s). They suggested that the specificity in learning the motor task may be attributable to some neural plasticity in the spinal cord (Edgerton et al. 1997).

Collectively, these findings show that the spinal cord is capable of learning and is capable of adaptive plasticity when there is a change in the supraspinal and/or peripheral input. Also, the spinal cord is not only capable of learning (through training), there also seems to be a high level of specificity in learning the motor task in the spinal cord.

Peripheral control of locomotion

Peripheral afferents, although not needed for the generation of the basic locomotor pattern, (Grillner and Zangger 1979) play an important role in adapting the central locomotor program to the changing needs of the environment. Peripheral feedback can act on the CPG to regulate the duration and timing of the muscle activities, and are also important in controlling the transition of phases of locomotion (Amos et al. 1989; Grillner, 1981). In chronic spinal cats, proprioceptive and cutaneous inputs originating from the hindlimb placed on a moving treadmill belt are sufficient to initiate stepping, and adapt locomotion to the speed of the treadmill. The CPG also exerts important modulatory effects onto the transmission of these peripheral signals to appropriately respond to unexpected sensory signals (perturbations) in different phases of the step cycle (Pearson 1993; Rossignol et al. 1988; Rossignol, 1996).

Roles of afferents in the transition phases of locomotion

So far, two sensory signals have been shown to be involved in signaling the transition from stance to swing phase. They are the position of the hip (Grillner and Rossignol 1978) and the unloading of the limb at the end of stance (Duysens and Pearson 1980). However, as deafferented cats (Grillner and Zangger 1984) and

paralyzed cats (Perret and Cabelguen 1980; Grillner and Zangger 1979; Pearson and Rossignol 1991) can terminate stance (extensor activity) or initiate swing (flexor activity), these afferent may not be absolutely required for these function, but may be necessary to "fine tune" the function and be essential in adapting to speed.

Manually displacing the limb forward was shown to block the MLR-induced fictive locomotion in decerebrate cats (Orlovsky and Feldman 1972). Hip flexion of the limb also abolished treadmill walking in chronic spinal cats whereas the pattern resumed with hip extension (Grillner and Rossignol 1978). Similar findings were reported in the forelimbs (Saltiel and Rossignol 1988; Rossignol et al. 1993). The initiation of swing phase depends on the hip extending beyond a critical angle (~95°). In fictive locomotion, hip flexion was also shown to abolish rhythmicity of fictive locomotion and hip extension restored the rhythmicity (Pearson and Rossignol 1991). Collectively, it is clear that afferents arising from the hip probably participate in terminating the stance phase of the step cycle (for review, see Rossignol, 1996).

Proprioceptive inputs from the ankle extensor (triceps surae) may be crucial to signal the transition from stance to swing during locomotion (Duysens and Pearson 1980). Using premammillary decerebrated cats, they demonstrated that locomotor rhythm was inhibited either by a maintained stretch of the isolated triceps surae muscle or by a sudden isometric contraction in the ankle extensor. The

periodic rhythm of flexor and extensor only returned after the force of stretched triceps surae fell below 4 kg. Therefore, they concluded that a necessary condition for the initiation of the swing phase is the unloading of the leg extensor muscles at the end of stance. The afferents responsible for regulating the transition from stance to swing was demonstrated to be primarily the group Ib afferents (from Golgi tendon organ) from leg extensor muscles (Duysens and Pearson 1980; Kettler and Jordan 1984; Conway et al. 1987).

Modulation of reflexes

During locomotion, there is an important modulatory (gating) effects on the transmission of peripheral afferents in different phases of the step cycle. The gain of transmission in the different pathways is phasically modulated so that some reflexes are seen only during one phase of the step cycle and not in the opposite phase. For example, stimulation of the cutaneous receptors on the dorsum of the paw in intact cats elicits an increased flexion during swing (corrective stumbling response) (Forssberg 1979), whereas the same stimulus has no effect when delivered during the stance phase. In chronic spinal cats, a similar stimulation (cutaneous receptors) to the dorsum of the foot during swing causes an increased ipsilateral flexion, whereas the same stimulus delivered during stance causes an increased extension (but shortened, followed by a rapid flexion)(Forssberg et al. 1975).

The modulation of cutaneous afferent has been shown in part due to the modulation at different levels, the motoneuronal (Andersson et al. 1978a, b), interneuronal (Schmidt et al. 1989; Hishinuma and Yamaguchi 1990; Degtyarenko et al. 1996; Seki and Yamaguchi 1997; Andersson et al. 1978a, b) and at the primary afferent (Dubuc et al. 1988; Gossard and Rossignol 1990; Gossard et al. 1990; Dubuc et al. 1985; Nusbaum et al. 1997). As will be described later, different pharmacological agents can also change the transmission of the peripheral afferents to the spinal locomotor centers probably by actions at several of these levels.

Therefore, peripheral inputs interact closely with the spinal locomotor center and are important in regulating different aspects of the locomotor pattern such as timing of the muscle activation or stance-swing transition. Pharmacological substances that change the transmission of these reflex pathway may also change the characteristics of locomotion (timing, amplitude, weight support) which depends enormously on these cutaneous and proprioceptive inputs especially in spinal cats. Our present study and previous studies (Barbeau et al. 1987) showed that noradrenergic agonist, Clonidine, reduced the excitability of the short latency cutaneous reflexes in chronic spinal cats. This decrease in excitability in cutaneous transmission (and may be other pathways such as joint afferents) may increase the step cycle duration by a reduced efficacy of signaling involved in the stance-swing transition. Also, the foot drag that is usually seen after Clonidine injection (see later)

can be related to the decrease of cutaneous inputs, which may normally cause a limb flexion when the dorsum of the paw touches the treadmill.

Supraspinal control

Supraspinal inputs are important as a command center in "driving" the CPG and for generating goal-oriented, and anticipatory adaptive changes to locomotion (for review, see Rossignol, 1996). To produce anticipatory corrective changes in the locomotor pattern, the descending inputs exert their actions on the spinal networks. Cortico-, rubro-, vestibulo- and reticulo-spinal neurons have been shown to be active in different phases of locomotion (Drew et al. 1996a,b, for review, see Armstrong 1986; Grillner 1976; and Rossignol, 1996). Recent evidences clearly support the involvement of supraspinal structures in mediating corrective response to a loss of ground support during treadmill walking in chronic spinal cats (Hiebert et al. 1994).

Following a complete spinal cord transection, all supraspinal influences are eliminated, thus resulting in some permanent postural and locomotor deficits. The known role of different supraspinal systems will be reviewed briefly in so far as they can illuminate our understanding of the deficits seen in chronic spinal cats and on the effects of drugs that would mimick the effects of the transmitters mainly used by some of these descending pathways.

These descending pathways have been separated on the basis of the anatomical and functional differences. Pathways with axons located more ventrally and medially within the spinal cord (reticulo- and vestibulospinal tracts) and those that are located more dorsally and laterally (mainly the cortico- and rubro-spinal tracts).

Ventromedial pathways (reticulo- and vestibulo-spinal tracts)

Reticulospinal projections originate from the ponto- and medullary reticular formation (MRF) and terminates in the intermediolateral and ventromedial zone of the cord (Kuypers 1975). The role of the reticular formation should be viewed as having two components in locomotion, one of initiating and the other of controlling locomotion. Reticulospinal neurons in the MRF receive inputs from the mesencephalic locomotor region (MLR), and one of the role of this system is the initiation of locomotion (Jordan, 1991).

MLR was first described by Shik and colleagues (1966), and is a narrow region in the brainstem (below the inferior colliculus) effective in evoking locomotion in acute decerebrate cats. The speed of the evoked locomotion was found to correspond to the stimulus strength. The animal will switch from walking to trotting with increasing stimulus strength (Shik and Yagodnitsyn 1978).

The MLR corresponds to the caudal region of the cuneiform nucleus (Steeves and Jordan 1984) and pedunculopontine nucleus (PPN), it also coincides with the dorsorostral pole of locus coeruleus, the surrounding coeruleus α and parabrachial nuclei, all of which contain noradrenergic neurons (Grillner, 1981). The MLR does not project directly to the spinal cord for the initiation of locomotion, but instead, projects to brainstem structures (MRF and pontomedullary locomotor strip (PLS)) which themselves project to the spinal cord (Steeves and Jordan 1984; Stein, 1984; Jordan, 1991). Neurons in the MRF then project to the spinal cord as the reticulospinal pathways. Neurons from PLS descend to the spinal cord primarily via the dorsolateral and terminates in the dorsal horn (Shik, 1983). Activations of these descending projections are suggested to be crucial for the initiation of locomotion. Reticulospinal tract was shown to make monosynaptic connections with the flexor motoneurons (Grillner and Lund 1968). It has been suggested that the effects of reticulospinal neurons are exerted through spinal interneuronal system involved in the generation and control of locomotion. The neurotransmitter of the reticulospinal pathways appear to be excitatory amino acids (EAA) such as glutamate and aspartate as their actions can be blocked by EAA receptor antagonist in lamprey (Brodin and Grillner 1985a; Brodin et al. 1988). Other functions mediated by the MRF includes the regulation of the amplitude and timing of muscle activities during locomotion (Orlovsky 1972c; Drew 1991b; Drew and Rossignol 1984; Perreault et al. 1994), and regulation of the posture (Mori 1989; Mori et al. 1978; Mori et al. 1992).

As far as the role of the reticular formation in the control of locomotion, reticulospinal neurons have been shown in decerebrate cats to be mainly responsible for increasing the flexor muscles activity (Orlovsky 1972). However, single unit recordings from the MRF neurons during locomotion in chronic intact cat (Drew et al. 1986) and during fictive locomotion (Perreault et al. 1993) showed its role in controlling both flexor and extensor activities. Also, microstimulation of the MRF during treadmill locomotion in thalamic cats (Drew and Rossignol 1984) and intact cats (Drew 1991b), as well as during fictive locomotion (Perreault et al. 1994) was found to evoke activity in flexor and extensors. Stimulation of MRF not only augments the amplitude of flexor and extensor muscle activity but also changes the duration of the flexor and extensor thus altering the timing of the overall step cycle or even reset the rhythm (Drew and Rossignol 1984). Responses to stimulation of the MRF were phase-dependent and reciprocal. During swing, there is an increased in ipsilateral flexor activity and contralateral extensor activity, whereas the same stimulus given during stance increased instead the ipsilateral extensor activity and contralateral flexor activity (Drew and Rossignol 1984; Perreault et al. 1994). Recent study recording from the MRF neurons shows that they were indeed modulated in a phase-dependent manner with maximal response when cutaneous nerve stimulation was given during the swing phase (Drew et al. 1996a).

The role of reticulospinal neurons on postural control has also been studied (Mori et al. 1978; Mori 1989). In acute decerebrate cat, stimulation of the ventral

tegmental field (VTF), corresponding to the medial 2/3 of the reticular formation, increased bilateral extensor activities (tonus) of the hindlimb (Mori et al. 1978). A higher stimulus intensity in the same area elicited locomotion. Conversely, stimulation of the dorsal tegmental field (DTF) which corresponds to the midline dorsal structures of the pons and medulla (including the locus coelureus complex) markedly decreased the postural tonus and thus suppressed locomotion (Mori et al. 1978). DTF stimulation also stopped the MLR-induced locomotion which only resumed a few seconds after the termination of the DTF stimulation suggesting that the neuronal mechanism activated by MLR stimulation requires an adequate background excitation (postural tonus) before locomotion can be induced. In freely moving cats (Mori 1987), DTF stimulation resulted in a sequence of postural alterations from standing to a final lying position. During feeding and locomotion, DTF stimulation suppressed markedly the bilateral postural support of the hindlimbs. VTF stimulation, on the other hand, changed the posture from lying or sitting to a standing position in the cat and then walking commenced if the stimulation persisted.

Vestibulospinal tract has been suggested not to play a primary role in the initiation of locomotion, but rather to participate actively in the control of ongoing locomotion. Vestibulospinal neurons has been shown to enhance the extensor activity (Orlovsky 1972a, b).

Vestibulospinal tracts originate from the lateral vestibular nucleus of Deiters which receives important input from the cerebellum (Kuypers, 1981). The vestibulospinal tract from Deiter's nucleus descends to the spinal cord along the ventral-ventrolateral funiculus and terminates in the ventromedial part of the intermediate zone of the spinal cord (similar to the reticulospinal tract) (Kuypers, 1981). Vestibulospinal neurons from the Deiter's nucleus have been shown to increase activity during locomotion, and also shown to be active "in-phase" during locomotion, particularly during the extensor phase of the step cycle (Orlovsky 1972b). Stimulation of the Deiter's nucleus induce mono- and polysynaptic EPSP in extensor α- and γ-MN (Grillner et al. 1970).

Animals with lesion of the ventro- or ventrolateral pathways have been shown to have major locomotor and postural deficits including a decrease in weight support and lateral stability as well as a disruption of the interlimb coordination during unrestrained locomotion (Gorska et al. 1993; Gorska et al. 1990) or treadmill locomotion (Brustein and Rossignol 1997).

Dorsolateral pathways (rubro- and cortico-spinal tracts)

The rubrospinal tract originates from the red nucleus and descends contralaterally along the dorsolateral funiculus. It terminates at the dorsal and lateral parts of intermediate zone of the spinal cord of cats (Kuypers, 1981).

Rubrospinal neurons exert di- or poly-synaptic effects on motoneruons, also "Ia inhibitory interneurons" (Hongo et al. 1969; Hultborn and Udo 1972), and group II interneurons (Grillner 1976). Similar to the vestibulospinal tracts, the rubro-spinal system has been suggested to participate actively in the control of ongoing locomotion. The activity of rubrospinal neurons correlates with the contralateral flexor muscle during locomotion (Orlovsky 1972a).

The corticospinal tract of cats originates from the motor cortex and descends contralaterally along the dorsolateral funiculus and terminates in the dorsal horn, as well as in lateral parts of intermediate zone (Kuypers, 1981). The pyramidal tract mono- or polysynaptically activates α- and γ-MN, and also facilitates "Ia inhibitory interneurons" which mediate different reflexes (Grillner et al. 1966; Hultborn and Udo 1972)

The precise role of the motor cortex in regulation of locomotion is not clear. Ablation of the motor cortex does not prevent locomotion, but produces long lasting deficits in the ability of the animal to adapt its locomotion to different situations (eg. stepping over obstacle, locomotion on a ladder)(Armstrong 1986; Drew et al. 1996b; Drew, 1991a; Drew 1993; Armstrong and Marple-Horvat 1996). For example, Drew (1993) showed that 67% of pyramidal neurons in the forelimb region increased their firing rate during, or just before the onset of flexor activity (the swing phase) when the cat has to step over an obstacle. It has also been suggested that the motor

cortex are involved with control of more precise control of the distal musculature such as during foot placement of locomotion (Armstrong 1986).

In animals with lesions of the dorsolateral pathways, it has been reported that there was a change in the overall structure of the step cycle (Gorska et al. 1993; Zmyslowski et al. 1993) during unrestrained walking. Recently, it was reported that cats with a complete lesion of the cortico-spinal and rubrospinal tracts retained postural support and control of equilibrium but showed significant changes in the intralimb coordination (hip-knee coupling) and also a persistent paw drag during treadmill locomotion (Jiang and Drew 1996). Therefore, the paw drag during swing which is often observed in spinal cats could stem from lesions of the dorsolateral pathways.

Cerebellum

Cerebellum is generally viewed as a coordinating center to produce smooth, rapid and accurate movements (Armstrong 1986; Armstrong and Marple-Horvat 1996). Cerebellum has also been shown to contribute to the proper timing of the touch down and lift-off of the forelimb during locomotion by controlling flexor and extensor activities in decerebrate and awake walking cats (Udo et al. 1980). Abnormal coupling of the step cycles of different limbs, frequent loss of equilibrium and uneven step length are some characteristics of locomotion following

cerebellectomy (Armstrong 1986). Arshavsky and colleagues (1983) proposed that the role of cerebellum is to compare and regulate the transmission of signals from supraspinal centers and spinal inputs (via VSCT, DCST) thus to co-ordinate different motor synergies (locomotion, scratching) and adapt them to the environment.

In summary, descending inputs exert important control over the locomotion during different phases of step cycle. While they all participate in producing anticipatory adaptations during locomotion, each system has a specific role to play. Reticulospinal system is involved primarily with initiation of locomotion and postural control and interlimb coordination, vestibulospinal and rubrospinal system are involved with the control of extensor and flexor muscles, respectively. The corticospinal system is involved primarily with control of distal musculature and with complex task, and the cerebellum is primarily involved in the overall coordination and timing of hindlimb activity.

Therefore, in cats with a complete spinal cord transection, in which all descending control were eliminated, some permanent deficits are seen including a loss of the voluntary control of the hindlimb, loss of postural control or equilibrium, a decreased weight support, and muscle weakness attributable in part by a decrease in muscle activation.

Pharmacological control of locomotion

Following a spinal cord transection, which removes all descending pathways, including the descending catecholaminergic and serotonergic innervation to the spinal cord, it has been demonstrated that locomotion can be initiated by exogenous applications of pharmacological agents which mimick the neurotransmitters of the descending pathways and act on receptors of intraspinal neurons.

The different substances such as noradrenaline (NE), serotonin (5-HT), dopamine, excitatory amino acids (EAA), inhibitory amino acids (glycine, gamma-aminobutyric acid (GABA)), acetylcholine (ACh) (Cattaert et al. 1995) and neuropeptides (Barthe and Grillner 1995; Grillner and Matsushima 1991) have been shown in different preparations to be effective in either triggering and/or modulating locomotion. Their actions will be summarized here.

Excitatory amino acids

The ability of excitatory amino acids (EAA) to induce locomotion has been demonstrated in different in vitro preparations including lamprey (Poon 1980; Cohen and Wallen 1980; Grillner et al. 1981; Brodin and Grillner 1985a,b), frogs (McClellan and Farel 1985), neonatal rats (Kudo and Yamada 1987; Cazalets et al. 1990; Smith and Feldman 1987), rabbits (Fenaux et al. 1991) and xenopus embryo (Dale and Roberts 1984). Fictive locomotion has also been shown to be evoked by EAA

in cat (Douglas et al. 1993). There is no report on the ability of EAA to initiate locomotion in chronic spinal cats. In fact, our preliminary results show that in chronic spinal cats, EAA could not trigger locomotion (Chau et al. 1994).

Excitatory amino acids (EAA) such as glutamate and aspartate are the neurotransmitters used by the reticulospinal system (Brodin et al. 1988) and thus have been proposed to play a command role in the initiation of locomotion (Grillner and Matsushima 1991). Intraspinal glutamate- and aspartate-immunoreactive neurons have also been identified (Carr et al. 1995). The action of EAA in triggering locomotor activity was suggested to be mediated by activation of N-methyl-D-aspartate (NMDA) receptors and kainate receptors at the spinal cord level (Dale and Roberts 1984; Douglas et al. 1993). The non-NMDA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) blocked hindlimb locomotion and fictive locomotion induced by MLR stimulation in cats. However, kainate and quisqualate were found to be ineffective in triggering fictive locomotion (Douglas et al. 1993). In a spinal cord-hindlimb neonatal rat preparation, bath application of N-methyl-D,L-aspartate (NMA) elicited alternating rhythmic activity in extensor and flexor muscles which was completely blocked by NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (APV) (Kudo and Yamada 1987). In decerebrate and spinal rabbits, systemic injection of MK-801, an NMDA-channel blocker, dose-dependently blocked the spontaneous and L-DOPA -induced fictive locomotion (Fenaux et al. 1991). In the lamprey model, it has been shown that while bath application of NMDA induced fictive swimming, application of the APV (NMDA antagonist)

decreased the spontaneous and NMDA-elicited locomotion (Brodin and Grillner 1985a, b). Also while kainate receptors also induced fictive swimming, the pattern was reported to be faster and less regular than that induced by NMDA (Brodin and Grillner 1985b). It appears that the spinal NMDA receptors and kainate receptors may play a different role in locomotor circuitry. It also appears that the action of EAA on locomotor circuitry is more specific than just a general increase level of excitability.

EAA have also been reported to produce other types of motor rhythm than stepping. It has been shown to produce fictive fast paw shaking behavior in decerebrate cats (Douglas et al. 1993), spontaneous wiping, hindlimb kicking and jumping movements in adult spinal frogs (McClellan and Farel 1985).

Plateau potentials

NMDA receptors have also been implicated in the rhythmic bursting activity of spinal neurons during fictive locomotion (Grillner and Wallen 1985; Wallen and Grillner 1987). The voltage-dependent properties of NMDA receptor-ion channel appears to play a key role in the generation of low frequency, steady burst pattern (Brodin and Grillner 1985a,b; Brodin et al. 1985). Recently, it has been shown that NMDA receptor-induced rhythmical oscillations are also found in spinal neurons surrounding the central canal in the lumbosacral spinal neurons in rat (Hochman et al. 1994). The oscillatory behavior of neurons, or the NMDA-elicited pacemaker

plateau potentials, was potentiated by 5-HT through its depressive action on the after-hyperpolarization (AHP) thus increases the firing rate of the neurons (Wallen et al. 1989).

Plateau potentials have been reported in the motoneurons of spinal cats during fictive locomotion induced by L-DOPA, Clonidine, or a serotonergic precursor, 5-hydroxytryptophan (5-HTP) (Hounsgaard et al. 1988; Conway et al. 1988; Schomburg and Steffens 1996) and interneurons of spinal rats (Kiehn et al. 1996) induced by NMDA and 5-HTP. The dorsal horn neurons (lumbar enlargement) of turtle spinal cord slices was recently found also to be capable of generating plateau-potentials (Russo and Hounsgaard 1996a,b).

Plateau potential are intrinsic properties of the neurons which maintain a prolonged state of depolarization in the absence of synaptic inputs, thus significantly amplifying the level of excitatory output (Kiehn 1991). Plateau potentials have been implicated in postural control, in the generation and shaping of the locomotor rhythm, as well as enhancing the amplitude of the final motoneuronal output (Kiehn et al. 1996; Kiehn et al. 1997). Clonidine and L-DOPA also induce slow rhythmic oscillation of the membrane potential in some motoneurons in acute spinal cord which were superimposed on plateau potentials (Conway et al. 1988). These pacemaker-like oscillations of the membrane potential may also contribute to locomotion. Interneurons in the intermediate gray and around the central canal and whose activity were phase-locked with ventral roots discharges during locomotion (induced by NMDA and 5-HT) have also been found to have bursting capabilities

(Kiehn et al. 1996). Therefore, the ability of different pharmacological agents in inducing these active membrane potential may also play a role in the control of locomotion possibly also in spinal cats.

Serotonergic drugs

Serotonergic drugs alone or with NMDA have been reported to induce locomotor activity in neonatal rat (Cazalets et al. 1992; Cowley and Schmidt 1994; Kiehn and Kjaerulff 1996), leech (Willard 1981) and mollusc (Panchin et al. 1996). While serotonergic drugs did not induce locomotion in chronic spinal cats (Barbeau and Rossignol 1991), lamprey (Harris-Warrick and Cohen 1985), or mudpuppy (Jovanovic et al. 1996), they profoundly modulated the locomotor pattern.

In the *in vitro* neonatal rat spinal cord, 5-HT was found to consistently increase the tonic electroneurographic (ENG) activity of the left and right hindlimb followed by the appearance of rhythmic ENG pattern similar to locomotion in intact rats (Cowley and Schmidt 1994). In the isolated brainstem-spinal cord neonatal rat preparation, 5-HT was shown to generate rhythmic activity of bilateral hindlimbs ventral roots (Cazalets et al. 1992). In another neonatal rat preparation, one hindlimb remained attached to the dissected spinal cord perfused with drugs in an experimental chamber (Kiehn and Kjaerulff 1996). Within minutes after 5-HT was

added to the superfusing medium, hindlimb flexor and extensor EMG activity show rhythmic locomotor-like activity. A more complex pattern was even observed in biarticular muscles such as rectus femoris where double bursting was seen.

Serotonin did not induce locomotor rhythm in isolated lamprey spinal cord or in isolated spinal cord-forelimb attached mudpuppy preparation (Harris-Warrick and Cohen 1985; Jovanovic et al. 1996), neither did its precursor, 5-HTP induced locomotion in chronic spinal cats (Barbeau and Rossignol 1991). However, 5-HT was found to modulate the ongoing fictive locomotion in lamprey by reducing the ventral root firings frequency but increasing the intensity of firing (Harris-Warrick and Cohen 1985). Quipazine (serotonergic agonist) markedly increased the EMG amplitude of all hindlimb muscles in chronic spinal cats, with little effects on the duration of the burst (Barbeau and Rossignol 1991). In mudpuppy, 5-HT dose-dependently increased the cycle duration and EMG burst duration of the NMDA-induced locomotion (Jovanovic et al. 1996).

Following spinal cord transection in rats, it has been found that the motor response (spontaneous level of EMG activity) to 5-HTP increased progressively with time (Bedard et al. 1979). The increased response was found later to be attributed to the specific supersensitivity to serotonin which developed after a spinal cord transection (Barbeau and Bedard 1981).

Dopaminergic drugs

In *in vitro* neonatal rat spinal cord preparation, dopamine induced slower and more irregular rhythmic pattern as compared to the 5-HT- induced pattern (Kiehn and Kjaerulff 1996). In isolated lamprey spinal cord, dopamine was found to modulate the spinal network primarily by altering the sensory function. Intracellular recordings showed that dopamine reduced the late after hyperpolarizing potential (late AHP) selectively in the sensory-related neurons, but not interneurons in the spinal networks (Kemnitz 1997). In acute cats, dopamine was found to selectively depress the transmission of group II muscle afferent to interneurons in the dorsal horn but not those in the intermediate zone of the spinal cord (Skoog and Noga 1995). Apomorphine (dopaminergic agonist), however, was unable to initiate locomotion in the chronic spinal cats (Barbeau and Rossignol 1991). In chronic cats that have recovered locomotion with training, Apomorphine (0.2-0.3 mg/kg) produced a slight change in the cycle duration but markedly increased the amplitude of knee flexor muscles, St, but decreased the amplitude of knee extensor, VL. A higher dose of apomorphine (0.5 mg/kg) caused a hyperexcitation of the flexor muscles which led to a sustained flexor pattern when the paw touches the treadmill, thus interrupting locomotion. This effect was blocked by haloperidol (dopaminergic antagonist, 1.5 mg/kg).

Inhibitory amino acids

Inhibitory amino acids (glycine and GABA) have been suggested to play an important role in mediating the reciprocal organization of the spinal locomotion (Kiehn et al. 1997). In the lamprey, blockade of either $GABA_A$ or $GABA_B$ receptors by Bicucculine or Phaclofen respectively, did not affect the locomotor pattern. However, a combined blockade disrupted the burst pattern (Grillner and Wallen 1980; Grillner and Matsushima 1991).

In *in-vitro* neonatal rat spinal cord preparation, bath application of $GABA_A$ receptors agonist (Muscimol), $GABA_B$ receptors agonist (Baclofen), and glycine abolished the rhythmic locomotor pattern induced by NMDA (Cowley and Schmidt 1995; Cazalets et al. 1994). The GABA uptake inhibitor, Nipecotic also dose-dependently suppressed the rhythmic activity. On the other hand, a blockade of the $GABA_A$, $GABA_B$ or glycine receptors facilitates the expression of locomotor rhythm (Cazalets et al. 1994; Cowley and Schmidt 1995). $GABA_A$ antagonist, Bicucculine, markedly increased the frequency and amplitude of the NMA-induced locomotor activity (Cazalets et al. 1994). Bath application of Bicucculine and glycine receptor antagonist, Strychnine, also transformed a flexor-extensor and left-right activity into a bilateral synchronous rhythmic activity of all hindlimbs in neonatal rat (Cowley and Schmidt 1995; Kremer and Lev-Tov 1997). Thus, the inhibitory amino acids appear to play a role in shaping the timing and output of the locomotor pattern.

Cholinergic drugs

Choline acetyltransferase immunoreactive neurons are found in the spinal cord during fictive locomotion of decerebrate cats (Carr et al. 1995). Acetylcholine or acetylcholinergic drugs have been shown to induce locomotor activity in xenopus embryo (Panchin et al. 1991), in crayfish (Cattaert et al. 1995), and in neonatal rats (Katakura and Chandler 1991). Bath application of ACh with acetylcholinesterase inhibitor, Edrophonium, has also been reported to induce rhythmic motor activity in neonatal rat spinal cord preparation (Cowley and Schmidt 1994; Cowley and Schmidt 1995). The locomotor pattern was characterized by alternating flexor-extensor activity in one hindlimb coupled with extensor-flexor alternation in the contralateral limb (Cowley and Schmidt 1995).

In the *in vitro* crayfish preparation, stable fictive locomotor activity (slow rhythm) was triggered with bath application of cholinergic muscarinic agonist, Oxotremorine. The activation of muscarinic receptors was found to induce plateau potentials in motoneurons which may also participate in the induction of locomotor rhythm (Cattaert et al. 1995).

Neuropeptides

Neuropeptides such as somatostatin and neurotensin have been found in the spinal cord of lampreys (Grillner and Matsushima 1991). Somatostatin was reported to also coexists with a classic neurotransmitter, GABA (Grillner and Matsushima 1991). Neurotensine-like immunoreactive neurons have been found in the ventral part of the dorsal horn and around the central canal (Brodin et al. 1990), a region which has been implicated in subserving locomotion (see later). Recently, neurotensin was found to modulate the NMA-induced fictive locomotion in lamprey. Bath application of neurotensin induced a slowing of the rhythm together with a prolongation of the burst generation, similar to that observed with activation of 5-HT and $GABA_B$ receptors, but probably through different cellular mechanisms (Barthe and Grillner 1995). Much is still not known about the roles of other peptides in the control of locomotion.

So far, we have shown that various neurotransmitter systems can either initiate or modulate locomotion. Each system appears to have a specific function. The role of the noradrenergic system will be reviewed in more details in the following paragraphs since we have studied it more intensively in this work.

Noradrenergic drugs

The noradrenergic system

Histofluorescence studies revealed the existence of 2 major clusters of NE-containing neurons and fibers in the caudal mesencephalon and the lower brain stem region (Dahlstrom and Fuxe 1964; Kuypers, 1981; Cooper et al. 1991). Noradrenergic neurons form extensive ascending and descending projection systems that innervate specific targets within the entire neuraxis including the neocortex, specific thalamic nuclei, the cerebellum, the tectum and the spinal cord.

The NE projection system, although widespread, was found to be structured and have functional specialization. The brainstem NE system has been found to play an important role in controlling a wide spectrum of physiological functions including arousal and attention, and autonomic functions (stress, blood flow). Amidst its diverse roles in mediating different neural functions, the descending NE system also plays an important role in the activation of the spinal circuitry for locomotion.

Brainstem-spinal cord noradrenergic projections

Noradrenergic fibers arising from the locus coeruleus (A6) and subcoeruleus project ipsilaterally in the ventral and ventrolateral funiculi, and innervate the ventral horn, the intermediate gray, and the ventral part of the dorsal horn at all levels of the spinal cord (Fig. 4A). Another source of NE fibers originate from another group of neurons, the lateral tegmental cells (A7,A5), and to some degree the A1 group, project predominantly to the ipsilateral dorsolateral funiculus and innervates the dorsal horn, intermediate gray, and the intermediolateral column (Fig. 4B) (Bjorklund and Skagerberg, 1982; Bjorklund and Lindvall, 1986).

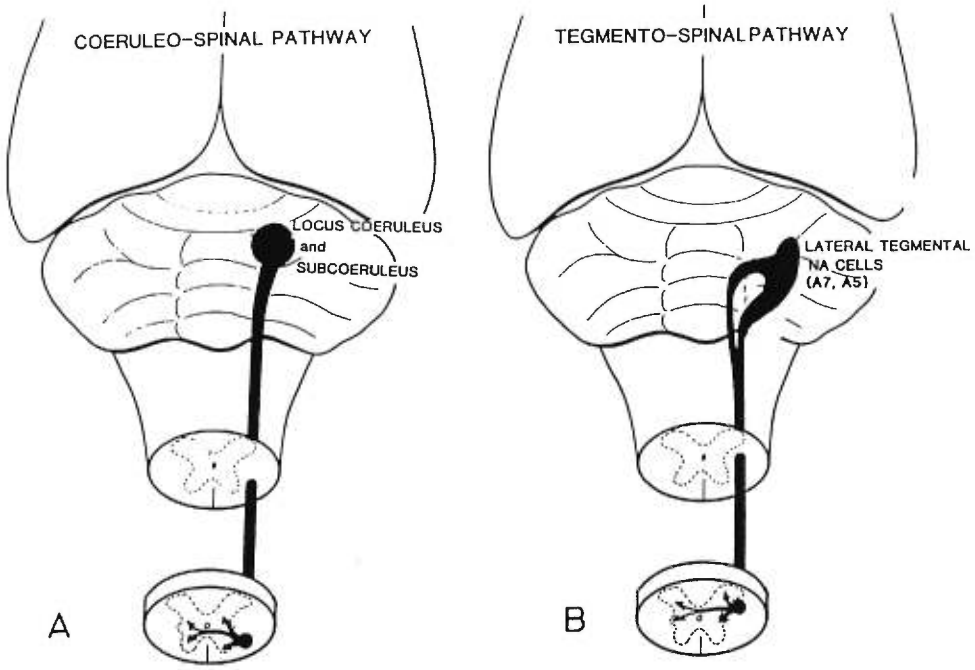
The presence of noradrenaline (NE) in the spinal cord arise exclusively from descending projections (for review, see Bjorklund and Skagerberg, 1982). Following spinal cord transection, almost all catecholamines (NE and DA) caudal to the transection sites disappeared (Carlsson et al. 1964) suggesting that there are no intraspinal noradrenergic neurons.

Figure 4.

Spinal cord projection and termination of the descending noradrenergic pathways (Bjorklund, A. and Lindvall, O. *Handbook of physiology. The nervous system IV.* 1986, p. 155-235.).

A. The noradrenergic projection originated from the locus coeruleus-subcoeruleus complex descends ipsilaterally in the ventral and ventrolateral funiculi and innervates the ventral horn, the intermediate gray and the ventral part of the dorsal horn.

B. The noradrenergic projection originated primarily from the pontine A5 and A7 cell groups descends along the dorsolateral funiculus and terminates the intermediolateral column, the area around the central canal and the outer layers of the dorsal horn.


COERULEO-SPINAL PATHWAY
LOCUS COERULEUS
and
SUBCOERULEUS
A
TEGMENTO-SPINAL PATHWAY
LATERAL TEGMENTAL
NA CELLS
(A7, A5)
B

Role of descending noradrenergic system on locomotion

While the locomotor circuitry is intrinsic to the spinal cord (Grillner, 1981), it can be activated by stimulation to the brainstem MLR (Steeves and Jordan 1984; Grillner, 1981). Noradrenaline was implicated in this MLR activation of the spinal locomotor networks although the precise role of the descending NE system on locomotion was unclear. It has been postulated that MLR-evoked locomotion was due to the activation of descending monoaminergic pathway given the close proximity of locus coeruleus to the MLR, suggesting therefore a «command» role for the noradrenergic system (Steeves et al. 1975). However, MLR-induced locomotion and intact locomotion were still possible after noradrenergic denervation of the spinal cord by 6-hydroxydopamine (6-OHDA) suggesting that the descending noradrenergic system is unlikely to be the only command system for locomotion (Steeves et al. 1980). Instead, the role of the descending noradrenergic system has been proposed to be a "gain setting" one, that is it regulates or sets the threshold for the activation of the spinal circuitry (Grillner, 1973; Grillner, 1981; Eken et al. 1989).

Noradrenergic receptors

Adrenoceptors are membrane associated proteins through which the noradrenergic agonists act to mediate different physiological functions. Adrenoceptors have initially been classified into α and the β types based on pharmacological criterias (Goldberg and Robertson 1983). They have been further subdivided into $\alpha 1$, $\alpha 2$, $\beta 1$ and $\beta 2$ subtypes. In the spinal cord, $\alpha 1$, $\alpha 2$, and β receptors have been identified (Marshall, 1983), and the α noradrenergic receptors will be described here.

The α-adrenergic receptors belong to a large family of receptors that are coupled to guanine nucleotide regulatory proteins (G-protein). In general, $\alpha 1$- and $\alpha 2$- noradrenergic receptors activate different intracellular second messenger system, resulting in different end-organ response. For example, inhibition of adenyl cyclase has been postulated to be a component of the transduction mechanism involving the activation of most $\alpha 2$- adrenoceptors (Ruffolo and Hieble 1994). However, activation of $\alpha 1$-adrenoceptors has been proposed to involve the stimulation of phospholipase C. The proposed second messenger responsible for $\alpha 1$-adrenoceptors are seemingly 1,2-diacyglycerol (DAG) and inositol-I,4,5-triphosphate (ins $(1,4,5)P_3$) (Ruffolo and Hieble 1994).

The classification of α1 and α2 subtypes was originally based on their post- and pre-synaptic location, respectively. The post-synaptic receptor was found to mediate excitation (eg. Vasoconstriction) whereas the pre-synaptic receptors was found to primarily involved in the regulation of the NE release through a negative feedback mechanism (autoinhibition). It was soon realized that the classification of α1 and α2 as post- and pre-synaptic adrenoceptors was inadequate. The existence of α2 receptors was later found outside noradrenergic axons, and also at post-synaptic junction (Timmermans and van Zwieten 1982). Also, post-synaptic α2 receptors have been shown to mediate excitatory response in rat and cat smooth muscles (Ruffolo and Hieble 1994). In light of these problems, the classification of the α1 and α2 receptors is now based on the relative potency of a series of agonists and antagonists, and also on binding experiments. The α2 agonists include Clonidine, Guanfacine, Tizanidine and Oxymetazoline (Ruffolo 1984). The presynaptic actions of α2 adrenoceptor in autoregulating the release of NE was also demonstrated by Clonidine and Oxymetazoline in rat cortex slices (for review, see Timmermans and van Zwieten 1982). Yohimibine, Rauwolscine and Idazoxane are potent antagonists of α2- adrenoceptor (Ruffolo 1984; Timmermans and van Zwieten 1982; Goldberg and Robertson 1983). Selective α1 noradrenergic agonists include Methoxamine and Phenylephrine. Prazosin is a potent antagonist for α1 noradrenergic receptors (Ruffolo 1984; Ruffolo and Hieble 1994; Cooper et al. 1991; Unnerstall et al. 1985; Timmermans and van Zwieten 1981).

In the spinal cord, differential involvement of α1- and α2-adrenoceptors have

been shown in the control of different functions such as spinal reflexes, pain pathways, motoneuronal excitability and autonomic functions (Marshall, 1983). For example, the marked suppression of nociceptive transmission by α2-noradrenergic agonists at the spinal cord has been extensively studied and was shown to be mediated by α2-adrenoceptors (Reddy et al. 1980; O'Neill and Haigler 1985; Davies and Quinlan 1985; Ishizuki and Yanagisawa 1992; Smythe and Pappas 1989) and neither α1-adrenoceptors (Davies and Quinlan 1985) nor imidazoline receptors (Monroe et al. 1995). In the following paragraphs, the localizations of different adrenoceptors in the spina cord and their roles in mediating locomotion, motoneuronal excitability and spinal reflexes will be discussed. While the role of Clonidine (α2 noradrenergic agonist) in locomotion has been extensively studied, no information is available regarding the role of other α2- or α1-noradrenergic agonist on locomotion.

Localization of α1- and α2-noradrenergic receptors in the spinal cord

In the spinal cord, autoradiographic studies in cats using [3H]idaxozan show that the α2 receptors are distributed in high density in the II and III lamina, and X, the central canal region of the spinal cord (Giroux et al. 1997). With [^{3}H]prazosin ligand, α1 receptors were found in high densities in the II, III lamina, also in IX lamina, the motoneuron region, and the X lamina (Giroux et al. 1997).

In rats, α2 receptor densities measured using ligand [^{3}H]rauwolscine were

found highest in the superficial dorsal horn and lowest in the motoneuron area (Roudet et al. 1994). Using [^{3}H]prazosin, densities of α1 receptor was found to be relatively homogenous across the subregions within the gray matter of the spinal cord with the highest density in the central canal areas and lowest in the dorsal horn (Roudet et al. 1993). The presence of α1 receptor in the hindlimb motoneuronal area may indicate the importance of α1 receptors in modulating the motoneuronal excitability.

Recently, it has been shown that there are also noradrenergic imidazoline receptors (I) in the spinal cord. Clonidine, idazoxan, and other α2-agonists with imidazoline structure were capable of interacting with the I receptors (Monroe et al. 1995). These receptors were identified on the basis of their insensitivity to catecholamines with radioligand binding techniques (Monroe et al. 1995). In the spinal cord, they were found to localize at the dorsal horn and the intermediolateral horn, and have been postulated to play a role in sensory processing (Ruggiero et al. 1995). However, their functional role is not well understood yet.

To summarize, α2 receptors are predominantly found at the dorsal horn, substantia gelatinosa, and the intermediate zone, close to the central canal, whereas α1 receptors are found also in high density in the motoneuron area in the ventral horn (Pascual et al. 1992; Roudet et al. 1993, 1994; Giroux et al. 1995).

The distribution of the noradrenergic receptors also coincide with the 2

groups of neurons that have been postulated to be important in the generation of locomotor rhythm. One group of neurons are interneurons in the intermediate and dorsal regions of midlumbar segments (L4) (mostly group II interneurons)(Edgley and Jankowska 1987; Shefchyk et al. 1990). These interneurons receive monosynaptic group II afferents from selective hindlimb muscles (quadriceps, sartorius, gracilis, and pretibial flexors) while also receiving convergent informations from other afferents (group Ia, joint and cutaneous afferents, contralateral group II afferent) and descending inputs (Edgley and Jankowska 1987). Another subset of neurons suggested to be involved in the generation of locomotion are found around the central canal (Hochman et al. 1994; Noga et al. 1995; Dai et al. 1990). In cats, the neurons located around the central canal (medial lamina VII) were identified with locomotor activity-dependent *c-fos* labeling (Dai et al. 1990) and field potential mapping during MLR-induced locomotion (Noga et al. 1995).

Changes in α1- and α2-noradrenergic receptors following spinalisation

Following spinal transection, it has been reported that 90% of the noradrenergic terminals has degenerated at 32 weeks following spinalisation, (Haggendal and Dahlstrom 1973). The gradual disappearance of the noradrenergic terminals below the transection has been suggested to contribute to the development of denervation supersensitivity.

Following complete spinal cord transection (Th 8-9) or selective lesion of the

NE-spinal cord system by 6-OHDA injection, a significant increase in α1- and α2-adrenoceptor densities was seen in rats (Roudet et al. 1993, 1994). A significant increase of α1 receptor densities were detected in all areas of the gray matter suggesting that spinal α1 receptor could become supersensitive after deafferentation and also that the receptors are located post-synaptically to NE fibers or terminals (Roudet et al. 1993). Preferential increase of α2 receptors was found in the superficial dorsal horn but not in the ventral horn in both 6-OHDA treated- and spinalized rats (Roudet et al. 1994).

Recently, Giroux and colleagues (1997) also reported changes in serotonergic and noradrenergic receptors in the spinal cord of the adult spinalized cats. Following spinalisation (Th13), both α1-noradrenergic receptors and α_2-noradrenergic receptors labeling initially increased with time but later return to control level after longer periods of survival. The α2-noradrenergic receptors labeling was increased as much as 88-127% at 15- 30 days following spinalisation The 5-HT_{1A} receptors labeling was also increased at 15-30 days following of spinalisation.

Functional roles of α1- and α2-noradrenergic agonist

1) Roles on Locomotion

The ideas that noradrenergic drugs have an influence on the control of locomotion originated from early works done by Lundberg and colleagues (Anden et al. 1966a,c) where they showed that intravenous injection of DOPA to the spinal cord uncovered a neuronal circuitry related to the generation of locomotion (Jankowska et al. 1967). (see earlier section)

Using a more specific noradrenergic agonist, Clonidine, the ability of activating the spinal locomotor network pharmacologically was reported (Forssberg and Grillner 1973). In acute decerebrated spinal cats (Th 12), locomotion could be evoked after Clonidine injection (200 μg/kg iv). Spinal cats could walk with both limbs when placed on a moving treadmill belt, EMG activities of prime movers were also found to correspond well to the walking of the intact cats. The locomotor pattern could adapt to different treadmill speeds. A nonspecific stimulation such as moderate pinching of the root of the tail was often required to raise the excitability of the spinal circuitry responsible for these movements (Forssberg and Grillner 1973). It was suggested that the descending noradrenergic system could "release" the spinal circuitry for stepping.

In chronic adult spinal cats, intraperitoneal injection of Clonidine (α2 noradrenergic agonist, 150 μg/kg) or L-DOPA (50 mg/kg) preceded by nialamide (monoamine oxidase inhibitor, 12 mg/kg) could trigger treadmill locomotion within the first week of spinalisation but not serotonergic or dopaminergic drugs (Barbeau et al. 1987; Barbeau and Rossignol 1991). The spinal cats were all capable of bilateral plantar foot placement with intermittent weight support of the hindquarters. The elicited locomotor pattern was also capable of adapting to a treadmill speed of 0.8 m/s.

At a later stage post-transection (~3 months), where the chronic cats have already recovered a well coordinated locomotor pattern, noradrenergic drugs modulated the well established locomotor pattern by increasing the timing of the step cycle. For example, Clonidine (30-100 μg/kg) markedly increased the duration of flexor and extensor burst with little effects on the EMG burst amplitude (Barbeau et al. 1987; Barbeau and Rossignol 1991).

Intrathecal application of NE has also been shown to trigger fictive locomotion in paralyzed acute decerebrated spinal cats (Kiehn et al. 1992). Fictive locomotor activity in the flexor (PBST) and extensor nerves (LG-Sol) can be observed with either continuous infusion of the NE (10 mM for 20 min) or after a single bolus injection (20-30 mM). The fictive locomotor effects were blocked by Phentolamine (10 mg/0.5 ml). Thus, it was suggested that a direct activation of the noradrenergic receptors can induce locomotor rhythmic activity in cats.

However, there is no information on the effects on locomotion of noradrenergic agonist other than Clonidine. Differential effects on locomotion were found between α2- and α1-agonists, the details are presented in the second article.

2) Roles on Motoneuronal excitability

Noradrenergic drugs were also found to exert excitatory effects on spinal motoneurons (Marshall, 1983; White et al. 1991). In anesthetized rats, microiontophoretic application of NE produced excitation on the spinal motoneurons. Extracellular recordings from motoneuron in the ventral horn of isolated spinal cord slices from rats also showed that noradrenaline facilitated the cell discharges induced by local stimulation of the ventral horn. The facilitation was found to be mediated by α1 receptor as the effects can be abolished by α1-antagonist, Prazosin and not by β-antagonist, Propranolol or α2-antagonist, Yohimbine. In addition, in the presence of Prazosin, Clonidine reduced the motoneuronal discharges, which can be antagonized by Yohimbine. Thus, it is suggested that the facilitation and suppression exerted by NE was mediated by α1- and α2- receptor, respectively (Hirayama et al. 1988; Ono and Fukuda 1995). In chronically-spinalized rats, Clonidine was also found to reduce the excitability of motoneurons and the tonic activity of the hindlimb muscles (Tremblay and Bedard 1986). In spinal rats, a selective α1 receptor agonist, St 587 was found to increase the tonus of anterior tibialis muscle, indicated by an upward shift of the baseline EMG activities (Rawlow and Gorka 1986).

3) Roles on interneuronal mechanism mediating reflexes

In high spinal decerebrate cats, DOPA and Clonidine were found to depress transmission in both disynaptic and polysynaptic reflex pathways from group II muscle afferents to motoneurons (Schomburg and Steffens 1988) but not the transmission from group I afferents (Bras et al. 1989). This depression was found to occur between the group II muscle afferents and the first order interneurons (Bras et al. 1989). The specific effects of different noradrenergic agonists (α1-, α2-, and β) on the selective depression of the transmission of group II afferents revealed that the depression was primarily mediated by α2-adrenoceptors (Tizanidine, Clonidine) in the intermediated zone of the spinal cord (L4-L5) (Bras et al. 1990). Stimulation of the locus coeruleus/subcoeruleus complex, i.e. descending noradrenergic neurons, also effectively depressed the transmission of group II afferents, but not group I afferents in intermediated zone of the L4 spinal cord (Noga et al. 1992; Jankowska et al. 1993). In light of the interneurons in this region of the spinal cord have properties subserving locomotion (Shefchyk and Jordan 1984; Edgley and Jankowska 1987), and that the peak depression of transmission was short-lasting, it was proposed that the NE exert phasic depressive effects on these L4 interneurons during the step cycle (Noga et al. 1992). In addition, Jankowska and colleagues proposed that L4 neurons may also overcome these depressive actions by reacting to dynamic changes in muscle length and tension during locomotion (group Ia, primary muscle spindles and Ib, tendon organ) which were not affected

by monoamines (Noga et al. 1992; Jankowska et al. 1993).

In anesthetized spinal rats (Sakitama 1993), it has been shown that, while a low dose of NE inhibited the flexor reflex evoked by group II afferents, a higher dose of NE facilitated the reflex. In spinal rats pre-treated with Yohimbine, the effects of low dose of NE shifted from inhibition to facilitation. Also, in these rats, Prazosin antagonized the facilitation of the reflex in a dose-dependent manner. In acutely spinalized rats, it has been shown that St 587, a selective α1-noradrenergic agonist increased the flexor reflex amplitude, an effect which was antagonized by Prazosin (Rawlow and Gorka 1986). Thus, NE facilitated and inhibited the flexor reflex evoked by group II afferents through the activation of α1- and α2- receptors, respectively. Facilitation mediated by α1-receptors is probably through an elevation of the motoneuronal excitability (Sakitama 1993).

In awake, non-anesthetized monkeys, Tizanidine, an α2-noradrenergic agonist, dose-dependently reduced the EMG response of the flexor reflex induced by non-noxious stimulation of cutaneous afferents (Corboz et al. 1991). The effects can be prevented by pre-treatment of Yohimbine suggesting that the depressant effects were indeed mediated by α2 receptors.

However, the effects of Clonidine on the flexor reflex evoked by electrical stimulation of the hindpaw in rats were also reported to change from inhibition to facilitation immediately following spinalisation suggesting that Clonidine's action on α1-receptor have been unmasked (Kehne et al. 1985). Also, another α2-

noradrenergic agonist, Tizanidine, has been reported to enhance flexor reflex in decerebrate spinal rats at higher dosage, an effect which could be blocked by Prazosin (Chen et al. 1987). Thus, the actions of Clonidine and Tizanidine on the α1-noradrenergic receptors following spinalisation could not be completely discounted. This will be further discussed in the second paper.

In summary, α1 and α2 noradrenergic receptors have been suggested to exert differential effects on motoneuronal excitability as well as on interneuronal mechanisms involving flexor reflex. While α1 noradrenergic receptors have been suggested to have a facilitatory effect on both motoneuronal and reflex excitability, α2 noradrenergic receptors have a inhibitory one. Also while α2-noradrenergic agonists, especially Clonidine, effectively initiated and modulated locomotion, no information about the role of α1-noradrenergic agonists on these locomotor functions was available.

RATIONALE

Noradrenergic drugs, especially Clonidine, has been demonstrated to be capable of unmasking the locomotor circuitry in cats following spinalisation. It has also been shown that locomotor training enhanced locomotor recovery in adult cats following complete cord transection suggesting that the spinal cord is capable of undergoing plastic changes following spinalisation. Therefore, it is possible that the spinal locomotor circuitry can be shaped and reinforced by training early after spinalisation. However, during the first week of spinalisation, training is only possible after injection of drugs as the cats are not walking otherwise. The aim of the first series of experiments was then to study in details, after daily injection of Clonidine, the effects of early training and Clonidine on locomotor recovery after spinalisation. The details of the study was presented in the first article.

In addition, noradrenergic agonist acting on different subtypes of α receptors, the α1- and α2-subtypes have been shown to be capable of mediating different functions. So far, there is a scarcity of information on the role of different noradrenergic agonists, other than Clonidine, on the control of locomotion. The second aim of this project is to study in more details the effects of different noradrenergic agonists on the initiation and modulation of locomotion and cutaneous reflexes in chronic adult spinal cats. The details is presented in the second article.

A better understanding of early training and the specificity of noradrenergic agonist-induced locomotor effects is fundamental to our hope of using pharmacological intervention to help patients with locomotor deficits.

ARTICLE #1:

Early locomotor training with clonidine in spinal cats

by

Connie Chau, Hugues Barbeau, Serge Rossignol

Journal of Neurophysiology, 1998 Jan 1; 79(1):392-409

Abstract

Clonidine, a noradrenergic alpha-2 agonist, can initiate locomotion early after spinalisation in cats. Since this effect lasts 4-6 hours, we have injected Clonidine daily, intraperitoneally or intrathecally, and intensively trained 5 spinal cats to perform hindlimb walking on a treadmill starting at day 3 and continuing until 10 days post-transection. Each day, Clonidine was injected to induce locomotor activity and cats were trained to walk with as much weight support as possible and at different speeds during multiple (1-5) locomotor training sessions, each lasting from 10 to 20 minutes, until the effects of Clonidine wore off. Electromyographic (EMG) activity synchronized to video images of the hindlimbs were recorded before and after each Clonidine injection. The results showed, firstly, a day to day change of the locomotor pattern induced by Clonidine from the 3rd to the 11th day including an increase in the duration of the step cycle, an increase in the duration of extensor EMG activity, and an increase in total angular excursion of the hip, knee and ankle joints. Secondly, after 6-11 days of this regimen, there was an emergence of a coordinated locomotor pattern with weight support of the hindquarters that was visible even before that day's Clonidine injection. The results suggested that daily injection of Clonidine followed by early and daily interactive locomotor training can enhance the recovery of locomotion in spinal cats.

Keywords: spinal cord, cat, locomotion, training, Clonidine, recovery

Introduction

It is well established that a few weeks after a complete spinal section at the thoracic level (Th13), adult cats can recover locomotion of the hindlimbs on a treadmill provided that there is adequate interactive training (reviewed in Rossignol (1996)). Training has long been suggested to play an important role in the ability of cats to walk following spinal cord transection. Shurrager and Dykman (1951) reported an improvement in overground walking behaviour of spinal kittens that received electrical stimulation of the hindlimb; "the walking response of the hind legs became stronger, more precise and more functionally effective as training was continued". We have previously studied the recovery of locomotion in cats following spinal cord transection and showed that training played an important role in enhancing the recovery process (Rossignol et al. 1982; Barbeau and Rossignol 1987; Rossignol et al. 1986; Belanger et al. 1996; Barbeau et al. 1993). After 3 weeks to 1 month of training, the adult spinal cat (Th 13) attained good locomotor function with large steps, bilateral plantar foot placement and weight support of the hindquarters for more than 3 minutes (Barbeau and Rossignol 1987). Furthermore, the spinal cat was able to adapt its locomotion for treadmill speeds up to 1.0m/s. Belanger and colleagues (1996) found that spinal cats that received daily training were able to fully support the weight of the hindquarters at 14-24 days post-transection. Smith and colleagues (1982) found in 12-week spinal cats (Th 12) that the exercised group showed a performance superior to that of the non-exercised

group. The untrained spinal cats performed poorly with only occasional plantar foot placement and were unable to support their weight during treadmill locomotion. The trained spinal cats, on the other hand, exhibited excellent weight support during locomotion and adapted to treadmill speeds up to 0.8m/s (Rossignol et al. 1982; Smith et al. 1982). Cats spinalized as adults were able to bear the full weight of their hindquarters, generate reciprocal stepping on a treadmill, and showed EMG and kinematic patterns remarkably similar to those of normal cats (Lovely et al. 1990; Edgerton et al. 1991; Hodgson et al. 1994; Belanger et al. 1996).

Interactive locomotor training during which the experimenter adjusts the weight supported by the animal according to its capacity on any particular day is important during the recovery period (Belanger et al. 1996). However, in the first 7-10 days post-transection the animals make only small hindlimb movements and are unable to advance the hindlimb in front of the hip or to make foot contact with the plantar surface and thus there is little or no weight support. Thus, interactive locomotor training during that period is not optimal. In contrast, pharmacological stimulation during that period is able to induce locomotion. Specifically, noradrenergic $\alpha 2$ agonists have been shown to trigger locomotion with large steps in acutely spinalized cats (Forssberg and Grillner 1973), or in the early post-transection period (Barbeau et al. 1987; Rossignol et al. 1995, see appendix C) in chronic spinal cats. This effects lasts 4-6 hours during which the animal can be trained to walk. Consequently, we planned to evaluate the effect of daily training on the recovery process of locomotion during this early period using Clonidine.

The implication of this approach is that there is some plasticity in the spinal mechanism responsible for generating locomotion. This central plasticity could be reflected by the evolution of the locomotor pattern with time. Barbeau and Rossignol, (1991) recorded locomotion in chronic spinal cats at day (d) 2 post-transection and d7 post-transection following Clonidine injection (150 μg/kg ip) and found that the locomotor pattern seen after Clonidine given on d2 was different from that given on d7. There was an increase in the duration of the step cycle for the same treadmill speed from d2-d7, accompanied by a gradual increase in the duration of stance. Concomitant temporal changes in EMG activity revealed that from d2-d7, the EMG activity of extensor muscles was prolonged and that of the flexor muscles was shortened (Barbeau and Rossignol 1991). In a preliminary study, we showed in one cat a progressive improvement in the locomotor ability from d2 to d9 as reflected by the gradual increase in cycle duration and weight support of the cat during this period.

Similarly, the "fictive" locomotor pattern evoked in early-spinal and late-spinal cat was found to differ, the latter having more characteristics of the normal adult pattern (Pearson and Rossignol 1991). Finally, Edgerton suggested that the spinal cats could be trained specifically to stand or to walk, and their motor abilities were specific to the type of training received (Edgerton et al. 1991; Hodgson et al. 1994).

Since Clonidine can unmask the locomotor circuitry which is capable of undergoing plastic changes following spinalisation, it is possible that this spinal

circuitry can be shaped and reinforced by early training after spinalisation. The aim of the experiments was therefore to study in detail, after daily injection of Clonidine, the effects of early training and Clonidine on locomotor recovery after spinalisation. This study can help determine if early locomotor training in patients with spinal cord injury could be beneficial to enhance their functional recovery.

Methods

Five normal adult cats were trained for periods ranging from 1-4 weeks to walk at constant speeds on a motor driven treadmill belt enclosed by a transparent plexiglas box. All trained animals were capable of maintaining a steady and continuous locomotion at different speeds (0.2-0.7m/s) for at least 20-25 minutes. Following this training period, all animals were prepared to undergo surgical implantation of EMG recording electrodes, and in one cat (CC4) an intrathecal cannula at the time of EMG implantation. After these implantations, the locomotion of the cats was recorded to establish the baseline values of the control period (referred to as Intact), before spinalisation.

Surgical procedures

All operations were performed under general anaesthesia (pentobarbital 35mg/kg) and aseptic conditions. Surgeries performed for these experiments included the following: 1) implantation of EMG electrodes for chronic recording 2) intrathecal catheterization and 3) spinalisation.

1) Implantation of chronic EMG electrodes. Briefly, except for HB6 which was implanted daily with pairs of enamel insulated copper wire electrodes inserted percutaneously into the bellies of a few hindlimb muscles after spinalisation, all

other cats underwent chronic electrode placement. One or two multipin head connectors (TRW Electronic Components Group, Elk Grove Village, IL) were used. Fifteen teflon-insulated stainless steel wires (Cooner Wire Company, Chatsworth, CA,AS633) were soldered to each connector a few days prior to surgery. With the animal secured in a stereotaxic frame, the connectors were placed on its skull using acrylic cement. The stainless steel wires were then led subcutaneously to various muscles. A pair of stainless steel wires was then inserted into each muscle. Unpaired wires, from the last pin of each connector, were placed under the skin of the neck to serve as a ground. Prior to muscle insertion, a small portion of the teflon coating was removed from the stainless steel wires and then the wires were sewn into the bellies of selected flexor and extensor muscles of both hindlimbs. The implanted muscles were the following: Iliopsoas (IP), hip flexor; Gluteus medius (Glu), hip abductor and extensor; Sartorius (Srt), hip flexor and knee extensor; Semitendinosus (St), knee flexor and hip extensor; Vastus lateralis (VL), knee extensor; Gastrocnemius lateralis (GL), ankle extensor and knee flexor; Gastrocnemius medialis (GM), ankle extensor and knee flexor; and Tibialis anterior (TA), ankle flexor.

2) Intrathecal catheterization. An intrathecal cannula (Teflon 24LW tubing) was implanted in one cat, CC4, prior to spinalisation. One end of the cannula was connected to a cannula connector which was cemented on the skull together with the head connectors. The other end of the cannula was inserted into the intrathecal space through an opening in the atlanto-occipital ligament down to L4-L5.

3) Spinalisation. A laminectomy was performed at the Th 13 vertebra. The dura was carefully removed and Xylocaine (2%) was applied topically on the area of spinal cord to be transected. The spinal cord was completely severed with a pair of surgical scissors so that the ventral surface of the spinal canal could be clearly visualized. Absorbable hemostat (surgicel) was then used to fill the space between the rostral and caudal ends of the spinal cord thus helping haemostasis. The wound was then sutured in layers.

Post-operative care

Following all operations, animals were placed in an incubator until they regained consciousness before returning to their cages with ample food and water. Torbugesic (Butorphenol tartrate, 0.05mg subcutaneously) was also given in the first post-operative day (every 6 hours) for analgesia. All spinal cats were placed in individual cages (104cm X 76cm X 94cm). The cages were specially lined with a foam mattress in addition to the usual absorbent tissues to reduce the risk of developing skin ulcers. They were attended to at least twice daily for manual bladder expression, general inspection and cleaning of the hindquarters. All procedures followed a protocol approved by the local ethics committee, and the well-being of the cats was always ensured.

Histology

When the animals were sacrificed with an overdose of sodium pentobarbital, the spinal cord was removed for histological analysis (Kluver-Barrera method) to ensure the completeness of the spinal transection. Sagittal sections of 10 micron thickness were cut, including the area of the transection.

Recording and analysis procedures of locomotor performance

The locomotor performances of the cats were recorded (EMG synchronized to the video images) under the following different conditions; 1) INTACT- following chronic electrode implantation but before spinalisation, 2) SPINAL PRE-DRUG- following spinalisation, just prior to any drug injection, 3) SPINAL POST-DRUG- following spinalisation and at different time intervals following drug injection. The pre-drug and post-drug trials, carried out on the same day, were then compared to the intact trials of the same cat (a within subject design where each animal has its own baseline for comparison).

During recording of the intact locomotion, cats were placed on the treadmill belt enclosed by the plexiglas, and free walking at different speeds was recorded. During recording of spinal locomotion, the forelimbs of the spinal cat were placed on a platform and the hindlimbs on the moving treadmill belt. Early after spinalisation, the experimenter supported the weight of the hindquarters of the cat

and provided equilibrium. With time, the cats were able to walk with complete weight support of the hindquarter and the experimenter only held the tail to provide lateral stability.

Reflective markers were placed on the iliac crest, the femoral head, the knee joint, the lateral malleolus, the metatarso-phalangeal joint (mtp) and the tip of the 3rd toe. The video images of the side view of the cat were captured using a digital camera (Panasonic 5100, shutter speed 1/1000s) and recorded on a video cassette recorder (Panasonic AG 7300). The side facing the camera was the ipsilateral side. Calibration markers (10 cm distance) were placed either on the treadmill frame or on the trunk of the cat to reduce parallax errors.

The EMG signals were differentially amplified (Bandwidth of 100 Hz to 3 KHz). Twelve channels were recorded with a Vetter Digital (model 4000A PCM recording adapter) on a VHS video tape. The frequency response of the tape recorder was 1.2kHz per channel.

The EMG recording was synchronized to the recorded video images by means of a digital SMPTE (Society for Motion Picture and Television Engineers) time code. This time code (Skotel time code generator model TCG-80N) was simultaneously recorded on the EMG tape, the audio channel of the VHS tape and was also inserted into the video images themselves.

The recorded EMG data during locomotion were played back on an electrostatic polygraph (Gould, Model ES 1000) and representative records of the animal's performance before and after drug injections were selected for analysis.

The EMG signals were digitised at 1 kHz and the onset and offset of bursts of activity were detected first automatically, then verified manually and corrected where necessary. The duration and amplitude of the muscle bursts were measured using custom designed software. The mean amplitude was calculated as the integral of the rectified EMG divided by the burst duration. The rectified EMGs signals were then normalized and averaged using the knee flexor iSt as a trigger.

Kinematic analysis of the hindlimb began with the digitization of the selected video images using a 2D PEAK Performance system (Peak Performance Technologies Inc., Englewood, CA). Displacement data, encoded by the X and Y coordinates of different joint markers (iliac crest, femoral head, knee joint, lateral malleolus, mtp joint, and the 3rd toe) were measured at 60 fields per second, (temporal resolution of each images is therefore 16.7ms). Angular displacement data and joint angles were also automatically calculated (e.g. hip joint angle was calculated based on the relative position of the iliac, hip and knee markers). From both X-Y coordinates of the recorded markers, displacement data and the calculated joint angle data, displays of stick diagrams, trajectories or normalized average joint angular displacement plots (filter =3) were generated using custom-made software. Stick diagrams of one step cycle consisted of reconstruction of the actual hindlimb movement during the stance and swing phases (Fig. 1C). Trajectories, or the course of a single joint, were also reconstructed from the X-Y coordinates (Fig. 1D). Normalized average joint angular plots showed the changes in the angular excursion during one normalized step cycle.

A complete step cycle consists of a stance and a swing phase (Fig. 1A). The stance phase begins as soon as the foot contacts the supporting surface, in this case the treadmill belt, and terminates when the foot starts its forward movement. The swing phase begins at the onset of forward movement and terminates as the foot strikes the treadmill belt again. Foot drag is not normally seen but was seen after spinalisation, resulting from an inadequate clearance of the foot during swing, and is defined as the initial period during which the dorsum of the paw touches the treadmill belt during the forward movement of the foot.

Figure 1 shows the normalised average angular plots, with the swing and stance phases subdivided into different components (F, E1, E2, and E3) based on Philippson (1905). The swing phase begins with flexion (F) of all joints, and during late swing, while the hip continues to flex, the knee and ankle start to extend (E1). The stance phase begins when the paw contacts the treadmill (E2), at which point the knee and ankle flex passively (yield) as the hindlimb bear the weight of the body, then the knee and ankle extend again (E3, third extension) to propel the body forward.

The different parameters of locomotion we have investigated are defined below:

1. **Step cycle duration** was defined as the time (ms) elapsed between two successive contacts of the same foot. It was calculated by multiplying the number of video fields by 16.7ms (interval between each field).

2. **Step length** was defined as the horizontal distance (mm) travelled between two

successive contacts of the same foot. Thus, the step length comprised the stance length and swing length. It is important to note that the step length measurement only takes into account the horizontal distance (only x-axis) traversed by the foot from one foot contact to the subsequent foot contact without taking into account the vertical trajectory of the movement. The values at foot contact and foot lift were taken from the displacement data file generated by the Peak Performance calculation program. The stance length was the distance travelled when the foot was in contact with the belt. The swing length was calculated by measuring the distance travelled from foot lift to the most forward horizontal distance reached by the foot.

3. **Joint angular excursion** was defined as the difference in degrees between the maximum and minimum values reached by each joint during a complete step cycle. The angles were calculated by the Peak Parameter calculation program which generated the angular displacement data.

Experimental protocol

Before spinalisation, EMG signals and kinematic patterns were recorded at various speeds on the treadmill. Different recordings were made on several days ranging from 5-14 days. The intact locomotion would later serve as the control reference for each cat.

Following spinalisation, experimentation began at 3 days post-transection, after the cat had recuperated from the surgery. For each following day (for 10-11 days), the locomotor performance of the cat was recorded (spinal pre-drug) to set the baseline recording for that day. Then, Clonidine was injected intraperitoneally (i.p. 150μg/kg-250μg/kg) or intrathecally (i.t. 100 μl of 4mM) and the locomotor performance was evaluated again (spinal post-drug).

During the evaluation of the locomotor performance after spinalisation, the hindlimbs of the cat were placed on the treadmill belt whereas the forelimbs were placed on a platform. The experimenter lifted the hindquarters of the spinal cat to provide for weight support and equilibrium as required. Locomotion was evaluated at various speeds starting from 0.2m/s to 1.0m/s. Perineal stimulation was given to enhance stepping. During pre-drug trials, especially during the early post-transection days, moderate to strong perineal stimulation was given in an attempt to elicit locomotion as much as possible. During the post-drug trials, on the other hand, only light perineal stimulation was needed to enhance stepping. The effect of Clonidine on locomotion lasted for 4-5 hours, thus in this time period some locomotor training was possible.

We did not measure the level of Clonidine in the blood. Pharmacokinetic study has shown that in humans that received an i.v. injection of Clonidine (300μg), the plasma clearance was 1.9-4.7 ml/min/kg of body weight, and that renal elimination of the unchanged drug constitutes 60% of the drug clearance. The half-life averaged 8.5 hours (Davies et al. 1976).

Locomotor Training

Multiple (1-5) short training sessions were given daily following Clonidine injection. These training sessions are additional to the recording sessions that were made at 30-45 mins following Clonidine injection. The length of each training session usually lasted about 10-20 minutes depending on the locomotor capability of the cat on a particular day (see table 1). During the training sessions, the hindlimbs were placed on the treadmill belt and the cat exercised at different speeds as soon as the locomotor pattern appeared after the Clonidine injection. During each training session, the experimenter lifted the hindquarters of the spinal cat to provide some weight support and equilibrium as required, with the goal being to let the animal support its own weight as much as possible during locomotion at all times. Stimulation was also given to the animal by lightly pinching the perineum. As the effects of Clonidine wore off, the ability of the cat to walk consistently on the treadmill decreased and the training periods had to be shortened. Usually, by 4-5 hours after Clonidine injection, it was difficult to elicit proper locomotion and training was stopped. The cats were then returned to their cages and were not trained again until the following day.

Results

Data obtained from 5 spinal cats were used. Table 1 indicates the profile of the experimental cats including the dosage of Clonidine, and the training received. The characteristics and progressive changes of the locomotor pattern observed in intact and spinal cat (both pre-drug and post-drug conditions) were examined.

Intact locomotion

The locomotion of cat CC2 during the intact condition (Fig.1) will serve as a reference for locomotion of the same cat following spinalisation as shown in subsequent figures. The characteristics of the locomotion are reflected in the normalized angular plots of the hip, knee, ankle and mtp joints (the averaged cycle was repeated twice (Fig. 1A), and the stick diagrams representing 1 step cycle (Fig. 1C). Figure 1D shows the trajectory of the different markers during a step cycle. The variability seen in the averaged angular plots was attributed to the cat's difficulty in the intact condition to maintain a steady speed at such a low treadmill speed (0.2m/s). However, it is important to show the locomotion at this speed in order to compare with the locomotor pattern after spinalisation. The averaged EMG activity of the corresponding stepping sequence is shown in Fig 1B. The EMGs signals were synchronized on foot contact. In intact locomotion, the timing of EMG

activity is more complex than a simple alternation between flexor and extensor muscles. For example, the onset of hip flexor, iSrt, was later than the onset of knee flexor, iSt. The onset of ankle extensor was also later than the onset of knee extensor, iVL. Double bursting can be seen in St as well.

Overview of the recovery of locomotion

Clonidine was effective in triggering locomotion in all adult chronic spinal cats a few minutes after the injection and this effect gradually changed with time after spinalisation. To describe this in more detail, the results of one representative spinal cat (CC2) are shown.

At 3d post-spinalisation, prior to Clonidine injection, the cat was only capable of showing some very occasional movements of the hindlimbs when placed on the moving treadmill belt (Fig. 2A) even when strong perineal stimulation was given. The movements were so small that they were almost not seen in the angular traces of the hip, knee, ankle, and mtp joints. The foot was constantly dragging on the dorsum without any plantar foot placement or weight support of the hindquarters. Within 30 minutes following Clonidine injection i.p. 200-250 µg/kg, and with perineal stimulation, despite some foot drag, the spinal cat was able to step consistently on the toes and partially support the weight of the hindquarters (Fig. 2F). All joints participated in these locomotor movements (7-8 step cycles) but the limb tended to

remain behind the hip and therefore there was little efficient weight support in this position. The cat was able to walk at 0.1 and 0.2m/s but could not walk at a speed of 0.3m/s. The cat was then trained to walk at 0.2m/s for 2 sessions of 10 minutes each.

The following day (d4), the effect of Clonidine had completely worn off and there was again very little hindlimb movement before Clonidine injection despite the training of the previous day (Fig. 2B). Following Clonidine injection, the steps were larger but not robust. There was an increase in angular excursion, step cycle duration and step length compared to the pattern on d3 (Fig. 2G). The hindlimbs were placed in a more forward position as compared to that of d3, and the cat was able to accept some weight during each step. The cat was able to walk at 0.3m/s. At d5 and d6 (not shown in the figure), the cat was able to walk at 0.5m/s. A toe drag during swing, indicated by a horizontal line under the foot, was frequently observed following Clonidine injection (Fig. 2 F,G). By d7 post-spinalisation during the pre-Clonidine period (Fig. 2C), after 4 consecutive days of Clonidine injection (d3,4,5, and 6) and 15 training sessions, there was still no apparent improvement in the spontaneous locomotion during this pre-drug period. However, a well-coordinated locomotor pattern was readily elicited a few minutes following Clonidine injection (Fig. 2H). There was an increase in all joint angular excursions, and an hyperflexion during swing, which might be related to the perineal stimulation. The cat was able to walk at 0.6-0.7m/s. Therefore, although there was no apparent carry-over effect of the training from the previous day in the pre-drug period, the

post-Clonidine period clearly indicated that changes were occurring within the spinal cord.

The next day, by d8 (Fig. 2D) prior to the injection of Clonidine, it was indeed possible to obtain a good locomotor pattern with increased joint excursion although the peak values obtained were not as large as those obtained in the intact, pre-spinalisation period (compare Fig. 1A). This was not due to the residual Clonidine because the effects of Clonidine dissipated 6 hours post-injection as seen on previous days. Following Clonidine (Fig. 2I), there was a further increase in cycle duration, step length, and joint angular excursions approaching intact. The cat was able to walk at 1.0m/s. However, there was a marked toe drag during the swing phase as indicated by the horizontal line underneath the stick figures (Fig. 2I).

On d9 (Fig. 2E), the pre-drug locomotor pattern was further improved and more robust as seen by the increase in all joint angular excursions. The locomotion better resembled that seen in the intact condition, indicative of a recovery of locomotion (see Fig. 1). The criteria for locomotor recovery as seen during spinal-pre-drug trials was as follows: 1) consistent stepping, minimum 7-8 consecutive steps, 2) stepping with plantar foot placement, 3) weight support of the hindquarters subjectively assessed by the examiner of the ability of the cat to accept weight during stance. Perineal stimulation was sometimes applied to enhance stepping. The amount of perineal stimulation given was always kept to the minimum; usually, just a light pinch to the perineal area was sufficient. Following Clonidine injection (Fig. 2J), there was a further but small increase in the stance and swing duration as

well as in angular excursion, and the cat was able to walk at speeds up to 1.0m/s.

To summarize, firstly, daily Clonidine injection followed by locomotor training after spinalisation resulted, by d8-d9, in the expression of a coordinated locomotor pattern without any further Clonidine injection. Secondly, the recovery process during the first 9 days post-transection was a progressive one as seen from the gradual improvement in the locomotor pattern from d3 to d9 post-transection following Clonidine injection. The steps were longer and more consistent with time, foot placement became more consistent, weight support increased, and there was a day-to-day increase in the ability to adapt to higher speeds. The maximum speed at which the cat could walk was 0.2m/s at d3; 0.5m/s at d5; 0.7m/s at d7; 1.0m/s at d8-d9. Thirdly, the gradual improvements in locomotion from d3-d7 were only apparent after Clonidine injection and not before, since there was simply no walking from d3-d4-d7 (Fig. 2 A, B, C). Thus, it seems that Clonidine is able to reveal an underlying recovery process in the spinal cord which would not otherwise be apparent. The result of this cat CC2 is representative of all experimental cats used in this study. The results of other cats will be summarized in following figures.

Step cycle duration

In Fig. 3, the step cycle duration in CC2 and CC4 during intact locomotion, spinal pre-drug, and post-drug conditions over the 9d post-transection period is shown. From d3-d7 in CC2, and d3-d4 in CC4, no value was given during the pre-drug trials (note the absence of the grey bar) since there was no locomotion during those periods. There were only some rudimentary rhythmic movements of the hindlimbs with strong perineal stimulation. The hindlimbs were usually extended, with neither plantar foot placement nor any weight support of the hindquarters at all, so there were merely passive back and forth movements of the foot on the treadmill belt due to manipulations of the experimenter. At d8 and d9, the cycle duration of CC2 approached the value obtained during normal intact locomotion (shown as a dotted line) even before Clonidine injection. At d6 of CC4, the cycle duration also approached the intact value. The effects of Clonidine on step cycle duration are clear during the first 7 days (CC2) and 4 days (CC4) post-transection, when there was no locomotion during pre-drug trials. Once the locomotion was elicited (d8 for CC2 and d5 for CC4), the effect of Clonidine became less dramatic; in other words, the relative increase in the cycle duration after Clonidine compared to the pre-drug trial was less.

It is also interesting to note that, at d3, even after Clonidine injection, the cycle duration in CC2 (as well as CC1 and CC3, not shown here) was below the intact value, except in CC4, in which the post-Clonidine cycle duration actually

reached and somewhat exceeded the intact value (see also table 2). For example, at d3, while the cycle duration post-Clonidine for CC1 and CC2 are, respectively, 29.4% and 33.3% *below* their respective intact values, CC4 is 7% *above* its normal value. It is important to note that while CC1, CC2, and CC3 received Clonidine intraperitoneally, CC4 received Clonidine intrathecally, suggesting that the intrathecal injection of Clonidine may exert a more potent effect on the spinal cord during the early post-transectional period (d3). Other findings also support the suggestion that intrathecal Clonidine injection produced a more potent effect than intraperitoneal injection of Clonidine. At d3, following Clonidine injection, cat CC4 was able to walk at 0.8m/s as compared to 0.2m/s observed in CC2.

A closer examination of the changes in the subcomponents of the step cycle duration is shown in Fig. 4 A, B for 2 cats, CC2 and CC4, that received Clonidine i.p. and i.t. respectively. Similar results were obtained. During pre-drug trials, the swing duration remained relatively stable over time, whereas the stance duration significantly increased at d8-d9 approaching normal values. Following Clonidine injection, both stance and swing approached the normal values.

In CC2, following Clonidine injection, the stance duration increased from d3 to d9 with an intermittent peak at d5 whereas the stance duration varied randomly before Clonidine injection when the cat was not walking well. Before Clonidine injection, from d3-d7, the cat exhibited only rudimentary rhythmic movements of the hindlimbs. Following Clonidine injection, the stance duration was increased by 32%

from d3-d4, and by 14% from d4-d5. The progressive increase in the stance duration is one indication of the progressive improvement of the locomotor performance. This progress was apparent only after Clonidine injection as the cats were not walking prior to Clonidine injection. The progressive improvement of locomotion was possibly due to the locomotor training made possible after Clonidine injection.

Step length

Following spinalisation, the step length was very much reduced but Clonidine restored it towards normal values. The relationship between the step length during pre-drug and post-drug trials in 3 spinal cats, CC2, CC3, and CC4 is shown in Fig 5. In Fig. 5A and 5B the step length during the pre-Clonidine trials from 3-7 days post-transection is small as indicated by the nearly horizontal slope, whereas the step length post-Clonidine was increased. From 7-9 days post-transection, there was a sharp increase in the step length in these 2 cats during the pre-Clonidine trials to almost the intact values. Also, the data points formed a cluster along a oblique line indicating that with time, there was increasing effect of Clonidine.

In CC4, that received Clonidine intrathecally (Fig. 5C), notice that on 5 days post-transection, the step cycle length was very similar to that during the intact locomotion (86% of the intact). Also, as early as 6 days post-transection, the step

length during pre-Clonidine trials increased significantly and even slightly more than after Clonidine injection. For all the data shown in Fig 5C, the total dose of Clonidine given each day was the same (100μg i.t.).

Table 2 shows the values of step cycle duration and step length for all of the cats when they were capable of walking without Clonidine (6-11 days). These are the data values used in the histogram (Fig. 3) and x-y plots (Fig. 4 and 5). In the table, the cycle durations during the pre- and post-drug trials were also expressed as percentages of the intact values. In all cats, ranging from 6-11 days post-transection, the cycle duration during pre-drug trials was similar to or lower than that seen during intact locomotion. Following Clonidine injection, cycle duration increased in most spinal cats. The cycle duration reached the intact value except in one case, CC3. The step length during pre- and post-Clonidine trials were all lower than the intact control, and the difference was statistically significant.

Angular excursion

The ranges of joint angles of 1 cat (CC2) during intact, pre- and post-Clonidine at 0.2m/s are shown in Fig. 6. Prior to Clonidine injection, on 3-7 days post-transection, there was very little movement in all joints as illustrated (Fig.6 A-I). Beginning on d8, there was an increase in the movements at all joints. On d9 post-

transection, the angular movements (shown both as ranges or maximum minus minimum angle) of the hip, knee, ankle and mtp (Fig. 6 B,D,F,H), and the step cycle length (Fig. 6I) increased but remained below the intact values (horizontal dotted line). Also, there was a gradual increase in all joint excursions with a parallel increase in step length by d7 post-transection, that was near the intact value by d9 post-transection.

Following Clonidine injection, Fig. 6 J,L,N,P show that there was a significant increase in the angular movements in all joints compared to the pre-drug values. There was an increase in hip (Fig.6K) and ankle (Fig. 6O) joint excursions that exceeded the normal joint movement following Clonidine injection, and tapered off by d8 and d9 but still remained above normal values at d9. Fig. 6R showed that while there was a slight increase in the step length over time, it was still below normal values despite an above-normal increase in the hip and ankle joint excursion as described above.

This paradoxical decrease in step length despite the increase in angular excursion post-Clonidine can be related to the synchronous and exaggerated hip and knee flexion during swing followed by synchronous hip and knee extension prior to paw contact resulting in a decreased forward distance for each step. In normal cats, during the late swing phase (E1) the hip continues to flex while the knee begins to extend to promote forward placement of the paw at contact (see Fig. 1). In spinal cats post-Clonidine, the hip and knee flex, and then extend at the same time before paw contact. A synchronous knee and hip extension results in a

backward placement of the foot, reducing the step length measurement (see Fig. 2H). This observation was common among all experimental cats in this study. The exaggeration in the angular excursions can be partly caused by the perineal stimulation given that was required to initiate locomotion during the early days post-transection.

The values of all these joint angular movement ranges during intact, pre-drug and post-drug trials from 5 spinal cats on selected post-spinal days showing locomotor recovery are shown in Table 3. These values were also expressed as percentages of the intact value for each cat. The values for the post-spinal days shown here represents the first day that the cat was able to walk on the treadmill without need of Clonidine injection. In HB6, CC1, CC2 and CC3, the hip, knee, ankle and mtp angular excursions were increased during the post-drug trials. For example, in CC3 during the pre-drug trials, the hip joint angular excursion was only 73% of the excursion during the intact period. It increased to 119% of the excursion during the intact period following Clonidine injection. For CC2, both d8 and d9 are shown, and an increase in hip, knee, ankle, and mtp joint excursion can be seen from one day to the next even during pre-drug trials. For example, in d8, the hip angular excursion was 77% of the intact value, and on d9, the angular excursion increased to 114% of the intact value. For CC4, which received i.t. Clonidine, the hip angular excursion increased during post-drug trials. The ankle angular excursion during the pre-drug trials was exaggerated (285%) and returned

to a more normal value (160%) after Clonidine injection.

Speed adaptation

All of the cats demonstrated, both pre- and post- Clonidine, a progressive ability to adapt their locomotor patterns to a range of treadmill speeds up to 1.0m/s. As seen in Fig. 7, in the intact condition (hatched area), step cycle duration decreased as the treadmill speed increased. During the intact condition, the locomotion was recorded between 0.2-0.6m/s because this cat did not walk above 0.6m/s. The ability to adapt to treadmill speed was also found to be a progressive process. On d4 (Fig. 7A), following Clonidine injection, the cat was capable of walking up to 0.3m/s, which was an improvement from the previous day (d3, not shown) when the cat could not walk faster than 0.2m/s. On the following 2 days (d5-6), the cat was able to adapt to increasing treadmill speed up to 0.5m/s with Clonidine. By d7, the cat was able to walk at treadmill speeds up to 0.7m/s after Clonidine injection. Until this point (d4-d7), the cat was still unable to walk at 0.3m/s before Clonidine injection (Fig. 7A,B,C,D). On d8 and d9 before Clonidine injection, the cat was able to adapt to treadmill speeds of up to 0.4m/s. Following Clonidine injection, the cat was able to walk at 1.0m/s (Fig. 7E,F).

Thus, there was a progressive improvement in the locomotor ability from d3 to d9 reflected by the cat's increasing ability following Clonidine injection to adapt

to a range of treadmill speeds, from 0.2m/s on d3 to 1.0m/s on d9.

EMG

It is essential to examine the EMG changes accompanying the kinematic changes seen with Clonidine injection and training following spinalisation to better understand the possible underlying neurophysiological changes. Previously, in Fig. 2, we have shown the progressive kinematic changes in the locomotor pattern on different days post-transection; the corresponding EMG activity of the cat CC2 is shown in Fig. 8. All the EMG traces were synchronized to the iSt and all the gains were kept constant to enable comparison of the EMG activity during intact (Fig. 1B), pre- and post-Clonidine conditions. The thin lines of Fig. 8J also shows the EMG signals in the intact state.

On d3 post-spinalisation, prior to Clonidine injection, there was no organized EMG activity (Fig. 8A). There was some tonic activity in the flexors (Srt and St) and no activity in the extensors (VL, GL, and Glu). Following Clonidine injection, clear alternating rhythmic bursting of the flexors and extensors can be seen during treadmill locomotion (Fig. 8F). However, activity of most proximal muscles such as the hip extensor (Glu) was much reduced compared to the intact condition. The burst duration of St was also greatly prolonged and the Srt duration much shorter as compared to the intact pattern. The reduced activation of the proximal flexors

such as iSrt may contribute to reduced flexion, and thus the extended position of the hindlimb throughout the step cycle.

From d5-d7, there was still no organized EMG activity before Clonidine injection. Following Clonidine injection, there was an improvement in the EMG pattern as compared to d3 (Fig. 8G). The emergence of burst activity was seen in both the hip and the ankle extensors, iGlu and iGL, respectively. This increase in extensor activity may have contributed to the improved weight support of the cat at d5. A definite burst activity of a knee flexor (coSt) was also observed although the burst duration was very long.

On d7, following Clonidine injection, there was a change of the EMG pattern as compared to d3 and d5 (Fig. 8H). The burst duration of the hip flexor (iSrt) increased significantly which may explain the increased forward placement of the paw in front of the hip or aligned with the hip as opposed to behind the hip as seen in d3. Also, an increase in hip, knee and ankle extensor (iGlu, iVL, and iGL) activity was seen. This increase in the hindlimb extensor muscle activity presumably contributed to the significant increase in the weight support of the animal at this stage. The burst duration of the contralateral knee flexor (coSt) was reduced compare to that seen on d5. Thus despite the lack of improvement in the EMG pattern before Clonidine injection, a clear progression of the EMG pattern can be seen after Clonidine injection at d7 as compared to d3 post-transection.

By d8, some rhythmic bursting in the flexors and extensors emerged before Clonidine injection. For example, there was a reduction in tonic activity in knee

flexors (St and coSt) as compared to d3 or d7 (Fig. 8D). Extensor (VL and GL) burst activity increased and appropriate St bursts were also seen. However, iGlu activity was still absent. Following Clonidine injection (Fig. 8I), there was an increase in the hip and knee extensor activity (Glu and VL), and a decrease in the GL activity approaching that seen in the intact cat.

At d9, there was a further increase in the activity of all muscles as compared to the pattern seen on d8. The iSt, iSrt and iVL increased in amplitude and showed an appropriate bursting pattern (Fig. 8E). Following Clonidine injection, all EMG activity increased and very clear bursting of each muscle can be seen (Fig. 8J). This Clonidine-induced locomotion at d9 was also compared to the normal locomotion (overlay on Fig 8J, with EMG traces during intact condition shown as thinner lines). The EMG pattern resembled that seen in the intact condition, but some differences were still present. These differences included increased EMG amplitude in all muscles, prolonged St burst duration, synchronous activation of iSrt and iSt as opposed to a later activation of iSrt to iSt in the intact. The difference in timing of muscle bursts can also be seen in Glu, a hip abductor and extensor. In the intact locomotion, the Glu had a much longer burst than it showed following spinalisation which may contribute to a longer step length than that seen in the spinal locomotion.

To summarize, following a daily Clonidine injection followed by training, there was an organized EMG pattern seen as early as d9 post-transection without Clonidine injection. There was a progressive improvement of the EMG pattern from d3 to d9 following Clonidine injection. This progressive improvement included the emergence of an alternating bursting activity of flexor and extensor activity and increased EMG amplitudes.

There was a maturation of the EMG pattern over time. This is shown in Fig. 9 using results from a different cat CC3 as an example. The cat was walking at 0.3m/s. At 7 days post-transection, following Clonidine injection, although there was an alternating pattern between the flexors and the extensors, they were almost of the same duration. The burst duration of hip flexors (iSrt, coSrt) and knee flexors (iSt and coSt) were much longer than was seen during the intact condition. For example, during the intact condition (Fig. 9A), the characteristic of St was a very short double burst, at 7 days post-transection, following Clonidine injection, (Fig. 9B), there was a significant increase in the duration (145%) and amplitude (257%) of the iSt EMG burst, as compared to the intact condition. In extreme cases, the contralateral knee flexor activity, coSt was actually of the same duration as the ipsilateral knee extensor iVL.

On 9 days post-transection, a more detailed and complex EMG pattern can be seen (Fig. 9C). For example, the duration of the flexor bursts (iSt, iSrt, coSt) was much shorter than at 7 days post-transection. A double burst can also be seen in

St. Therefore, the characteristic short flexor and long extensor burst activity patterns were restored.

The changes in the relative EMG amplitude and duration of flexor and extensor muscles over time is shown in Fig. 10. The relative EMG amplitude and duration was calculated as a percentage of the corresponding intact value. In the pre-drug trials, no data were available before d8 as the cat was not walking without Clonidine injection in that period. When the cat began to walk on d8 before Clonidine injection, the data from d8 and d9 are shown by stippled lines. There was an increase of the EMG amplitude of flexor and extensor muscles from d8-d9. On d9, the relative EMG amplitude of these muscles during pre-drug trials were close to the intact values. Following Clonidine injection, the iSt EMG amplitude was initially above normal and stabilized at 150% of the intact value, whereas the iVL amplitude continuously increased from d5 to d9 post-transection.

In Fig. 10B, the EMG burst duration of different muscles following Clonidine injection is shown. It shows that, while the extensor EMG burst duration (iGL and iVL) increased initially with time (d3-d5), the flexor burst duration did not. The progressive increase in the extensor duration would therefore contribute to the increase in the stance duration over time.

Table 4 shows the numeric values of the EMG burst duration and percentage of EMG amplitude of different flexor and extensor muscles of the 5 experimental cats on selected days when the cats were walking prior to Clonidine injection. In general, the EMG burst duration of the hindlimb muscles during the pre-drug trials approached the intact values (CC1,CC2,CC3, and CC4) indicating locomotor recovery. For example, in pre-drug trials of CC2 on d9, the burst duration of iSt, iSrt, iVL, and iGL were 88%, 79%, 97%, and 80% of the intact values, respectively. Following Clonidine injection, the EMG burst duration and the relative EMG amplitude of flexors (iSt or iSrt) were also increased as compared to the pre- drug trials. For example, in CC1, the EMG burst duration of iSt increased from 70% to 113%, and the EMG amplitude increased from 145% to 345%. Changes in EMG burst duration and amplitudes were also seen, although more variable, in the extensor muscles, iVL or iGL.

Discussion

Overview

In the present study, we examined the recovery of locomotion of the hindlimbs after spinalisation using early locomotor training made possible by the injection of Clonidine. We found that 1) the locomotor pattern elicited by Clonidine was rudimentary soon after spinalisation and became more complex with time, 2) a gradual improvement of locomotion during the first week after spinalisation was revealed by daily injection of Clonidine, 3) early locomotor training under the influence of Clonidine resulted in an early recovery (6d-11d) of a locomotor pattern that was similar in many respects to the intact pattern.

Evolution of locomotor recovery

In 5 spinal cats that received daily injections of Clonidine and early locomotor training, recovery of locomotion could be attained as early as 6-11d post-transection with weight support and proper foot contact (see table 1). This recovery period is shorter than that reported in the literature. In a previous study where spinal cats were trained without Clonidine, locomotion with weight support and plantar foot placement at treadmill speeds up to 1.0-1.2m/s was attained only at 3 to 4 weeks of spinalisation (Rossignol et al. 1982; Barbeau and Rossignol 1987). More

recently, in a study by Belanger et al (1996), using intensive training (2 daily sessions, 15-30 mins each) starting on the day following transection, locomotion with full weight support at speeds up to 0.8m/s was observed only after 14 days in 1 cat and after 24 days in another cat post-transection. It should, however, be reminded that in those studies without Clonidine, locomotor training could not really be effective before the cats were able to make plantar foot contact , i.e. within the first week post-spinalisation. It appears then that the combined effects of Clonidine and very early locomotor training of the Clonidine-induced locomotion may contribute to an earlier recovery of locomotion.

Progressive changes in the locomotor pattern were observed over time. The gradual improvement of locomotion from d3-d7 (see Fig. 5,6,7) was apparent only with Clonidine injection since the animal could not walk without Clonidine. There was a gradual increase in the step duration, in the ability to support weight, and in the adaptation to higher speed (see Fig. 5,6,7). This is in agreement with the results of Barbeau and Rossignol (1987) and Barbeau et al. (1993) who reported that in chronic spinal cats that received Clonidine, an improvement in the locomotor pattern (increase cycle duration and weight support) was seen from d2 to d7, and form d7 to d9 post-spinalisation.

The time-dependent improvement was also seen in the central locomotor pattern recorded after paralysis (Pearson and Rossignol 1991). The fictive locomotor pattern was recorded from hindlimb nerves in 2 groups of adult chronic spinal cats. One group was trained to step on the treadmill (late-spinal animals) and

the other was not trained and was examined a short time after spinalisation (early-spinal animals). In early-spinal cats, the fictive pattern, facilitated by Clonidine injection, was often more rudimentary and consisted of merely a simple pattern of alternating flexor and extensor nerve activities of quasi equal duration. In contrast, the fictive locomotor pattern observed in the late-spinal animals was more complex. The burst durations of various flexors were clearly different and the flexor bursts were shorter than the extensor bursts. These finding suggested that the spinal cord was capable of modifying the circuits that establish the temporal characteristics of the locomotor pattern and that training could be a contributing factor to the evolution of the fictive pattern (central locomotor pattern). It is possible that early locomotor training may affect the evolution of the spinal cord undergoing plastic changes after spinalisation. Thus, locomotor training may change, enhance, or guide the underlying plastic changes which will optimise the locomotor recovery. These plastic changes may occur at different levels such as anatomical, physiological, or neurochemical levels.

Anatomical changes

Anatomical changes such as collateral sprouting can contribute to the recovery of function following injury. Collateral sprouting was found during the recovery period in different preparations including partially hemisected animals,

complete unilateral hindlimb deafferented animal, and after partial unilateral rhizotomy or the spared root preparation (Liu and Chambers 1958; Robinson and Goldberger 1986; Murray and Goldberger 1974; Goldberger and Murray 1974; Murray and Goldberger 1986; Zhang et al. 1995; Goldberger and Murray 1982). In the partially hemisected cat, a lesion was made between T12 and L1 of the cat spinal cord sparing the dorsal column. It was found that the use of the limbs for standing and locomotion and the responses to segmental reflex stimulation (but not crossed reflex elicitation) progressively improved beginning at 2 weeks post-hemisection. Using radioautographic methods (injection of ^{3}H-proline) they found evidence of collateral sprouting from dorsal roots at 20 days after hemisection, (Murray and Goldberger 1974). In the spared root preparation, where all dorsal roots caudal to L4 were cut except L6, they found that the L6 roots projected as far as T9 on both sides. That is, the increase in the amount of projection was confined to normal limits (Goldberger and Murray 1982). Also, using electron microscopy, they found morphological changes (complex terminals, originate exclusively form dorsal roots) in the dorsal horn. The number of complex terminals decreased acutely (3 days post-op), representing a loss of terminals from the cut roots. The number returned to normal levels during the chronic stage (3-10 weeks) (Zhang et al. 1995). Therefore, collateral sprouting in the adult lesioned cat can contribute to the recovery of function. In our study, a complete spinal transection was performed in all cats which prevents sprouting of the descending system below the lesion. However, we cannot rule out the contribution of sprouting of neurons such as

primary afferents below the lesion to recovery at a later stage. Collateral sprouting is usually considered as a long process (3-10 weeks) and it is unlikely that the early locomotor recovery (within first 10 days post-transection) observed can be attributed primarily to the anatomical plasticity. However, since it was found that sprouting can begin as early as 4 days after partial cord lesion in rats (Li and Raisman 1994), it is possible that early training may guide or enhance the ongoing sprouting process and promote the recovery of locomotion.

Neurochemical changes

There is also evidence of modification of spinal receptor activity after complete spinal cord transection. For example, specific receptor supersensitivity following spinal cord transection was reported (Barbeau and Bedard 1981). Denervation supersensitivity can be attributed to the gradual disappearance of the noradrenergic terminals below the transection (Haggendal and Dahlstrom 1973). Recently, Giroux et al (1995) reported, in the spinal cord of chronic spinal cats (Th13), an upregulation of 5-HT_{1A} receptors, α_1-noradrenergic receptors and α_2-noradrenergic receptors labelling below the lesion 15-30 days following spinalisation (Giroux et al. 1995). In rats, a significant increase in α_1- and α_2- adrenoceptors densities was also found after a complete transection of the spinal cord at vertebrate level T8-T9 (Roudet et al. 1993; Roudet et al. 1994).

It is possible that the effects of Clonidine in mediating the locomotor recovery may be partly related to these receptor changes following spinal cord transection. Indeed, recent findings in our laboratory show that intrathecal injection of Clonidine in intact cats produced much less pronounced effects than those observed in spinalized animals. Thus, it is suggested that the effect of Clonidine in spinalized animals is related to the changes in the receptor sensitivity. To what extent the daily injection of Clonidine could change the fate of the receptors is still unknown.

Physiological changes

a) Plasticity of neuronal circuitry

Although it has been suggested that the spinal cord circuitry generating locomotor function is hard-wired and has a limited capacity to reorganize itself following injury (Sperry 1940; Sperry 1941; Forssberg and Svartengren 1983), there is some evidence that the spinal circuitry can undergo some physiological changes.

Early studies using spinal kittens and 1 spinal puppy demonstrated that chronic spinal mammals were capable of acquiring motor conditioned responses through training (Shurrager, 1955). Experiments on classical conditioning of the flexion reflex in spinal cats (Durkovic 1983), and on operant conditioning of the H-reflex experiment in monkey (Wolpaw and Chong 1989; Wolpaw et al. 1989) also

showed the capacity for functional changes at the spinal cord level. In these studies, the simple monosynaptic spinal reflex was suggested to have undergone adaptive changes at the segmental level in the presence of supraspinal influences.

In our laboratory, we have also demonstrated the functional plasticity of the spinal cord following a unilateral lesion of the ankle flexor nerves (Carrier et al. 1992; Carrier et al. 1997). In neurectomised cats which have already successfully compensated for the loss of ankle function by an increase of hip or knee flexion, a superimposed spinalisation revealed an asymmetrical spinal locomotor pattern, with large hyperflexion of the knee on the lesioned side. It was suggested that readjusted descending input following neurectomy in the otherwise normal cat may have caused plastic changes in the spinal circuitry to maintain locomotion, and these adaptive changes became evident when all descending inputs were removed as in the case of spinalisation. These findings are in accordance with the suggestion by Wolpaw and Carp that exposure of the spinal circuitry to supraspinal influences can induce intrinsic and long term changes in the spinal cord (Wolpaw and Carp 1993). These studies support the notion that the spinal cord is capable of adaptive plasticity when there are changes in the supraspinal and/or peripheral inputs.

b) Training effect

As mentioned in the introduction, training plays an essential role in the recovery of locomotion in adult spinal cats. It is possible that locomotor training can induce and/or may enhance plastic changes within the spinal cord (deprived of all descending inputs) responsible for the gradual recovery in locomotor function with time.

Edgerton and colleagues reported that training could induce functional changes in the spinal circuitry in generating a motor task (Edgerton et al. 1991; Hodgson et al. 1994). They showed that cats trained to stand (standing -trained) have great difficulty stepping, and the stepping-trained cats have great difficulty in maintaining a standing posture. Since the musculature between cats that were trained to stand and cats that were trained to walk were similar, they suggested that the training effect on locomotor recovery was of neural origin rather than of muscular origin.

Viala and colleagues (1986) also showed that spinal rabbits exhibit different locomotor stepping pattern depending on the types of training they received. Infant spinal rabbits that were trained on a motor driven "bicycle" which moves the hindlimbs either synchronously or alternatively exhibited synchronous stepping or alternating stepping locomotor pattern, respectively, following training.

Thus, these findings not only suggest that the spinal cord is capable of learning (through training), but they also demonstrate a high level of specificity in

learning the motor task in the spinal cord. These studies also suggest that peripheral afferent inputs could strongly affect the plasticity of central locomotor networks during early development.

In the present study, the amount of peripheral afferent input may also help determine the outcome of training. For example, the cat that received Clonidine intrathecally (CC4) fared better than cats that received Clonidine intraperitoneally (HB6, CC1,CC2, and CC3). CC4 was capable of generating a well-organized locomotor pattern at 6 days post-transection as opposed to 9-11 days in the other cats. It is possible that 100µg Clonidine i.t. was a high enough dose to activate the locomotor pattern more strongly soon after spinalisation as compared to the other cats. The relatively more forceful locomotor pattern elicited by Clonidine may then provide a more adequate afferent inflow to the spinal network generating locomotion early on rather than a rudimentary locomotor pattern, and this may have contributed to the earlier recovery in CC4.

Clinical significance

Studies involving the recovery of locomotor functions of incomplete paraplegic patients showed that treadmill training with a body weight support system improves significantly the locomotor pattern in these subjects (Visintin and Barbeau 1989; Barbeau et al. 1992; Fung et al. 1990; Wernig and Muller 1992; Dietz et al. 1994; Dietz et al. 1995). After 1-7 months of training, marked improvements were

seen in weight support capability, the walking speed, and the timing and coordination of the EMG pattern (Barbeau et al. 1992; Wernig and Muller 1992; Dietz et al. 1994; Dietz et al. 1995). Recent studies using another form of locomotor training, functional electrical stimulation (FES)- assisted walking, have shown improvements of walking speed after 1 year of training in incomplete spinal cord injured subjects (Wieler et al. 1995). Barbeau and colleagues reported improvements in locomotion in 2 subjects with chronic incomplete spinal cord injuries after a treatment regimen which incorporated the combined effects of Clonidine and cyproheptadine (a serotoninergic antagonist) together with a treadmill training program while the subject was supported by a body weight support harness system. The weight bearing ability of the subjects improved, their posture became upright, the flexor spasms decreased, the walking speed and the stride length also increased (Visintin and Barbeau 1989; Barbeau et al. 1992; Fung et al. 1990). Taken together, locomotor training alone or in combination with pharmacological intervention was found to be beneficial in these subjects.

As indicated in the present animal study, it is possible that earlier training started as soon as possible after spinal injury might also be beneficial and need further investigation in spinal cord injured subjects.

In summary, the present results support the idea that the spinal cord undergoes plastic changes after spinal injury and that locomotor training given during the early post-spinal period may be beneficial. The locomotor recovery we

observed might be a result of an interaction between the central plasticity and peripheral input. While Clonidine may uncover or "release" the rudimentary locomotor rhythm, the spinal locomotor network might be further consolidated or molded through the peripheral afferent inflow during locomotor training. Early locomotor training may activate peripheral afferents including cutaneous and proprioceptive which interact with the normal plastic changes (physiological, anatomical or neurochemical) and probably reinforce the spinal locomotor network.

The understanding of adaptive capacity of the spinal cord may lead to new and better approaches to the abnormal segmental function caused by spinal cord injury, stroke, or other central lesions. This study provides a rationale for a treatment strategy which incorporates drug therapy and training and might offer hope to improve the recovery process in spinal cord injured patients.

Abbreviations

EMG: Electromyography

IP: Iliopsoas

Glu: Gluteus Medius

Srt: Sartorius

St: Semitendinosus

VL: Vastus lateralis

GL: Gastrocnemius lateralis

GM: Gastrocnemius medialis

TA: Tibialis anterior

i: ipsilateral

co: contralateral

mtp: metatarso-phalangeal joint

i.p.: intraperitoneal

i.t.: intrathecal

d: day

Acknowledgements

This work was supported by the Canadian Neuroscience Network and a Group grant from the Medical Research of Canada. C.C. was supported successively by fellowships from the Fonds pour la formation de Chercheurs et l'Aide à la Recherche (F.C.A.R.), the Canadian Neuroscience Network, and the Groupe de recherche sur le système nerveux central (GRSNC). H.B. is a scholar of the Fonds de la recherche en Santé du Quebec (FRSQ). We gratefully acknowledge the assistance of Janyne Provencher and France Lebel during surgeries, experiments, analyses and preparation of illustrations. We also wish to thank the late R. Bouchoux for mechanical equipment; P. Drapeau and G. Messier for computer programs; C. Gagner for electronic equipment; and J. Faubert for help during surgery. Many thanks to Drs. J-P Gossard and K. Norman for their helpful comments on this manuscript.

References

Barbeau, H., Julien, C., and Rossignol, S. The effects of Clonidine and yohimbine on locomotion and cutaneous reflexes in the adult chronic spinal cat. *Brain Res.* 437: 83-96, 1987.

Barbeau, H., Dannakas, M., and Arsenault, B. The effects of locomotor training in spinal cord injured subjects: a preliminary study. *Restorative Neurol. and Neurosci.* 12: 93-96, 1992.

Barbeau, H., Chau, C., and Rossignol, S. Noradrenergic agonists and locomotor training affect locomotor recovery after cord transection in adult cats. *Brain Res. Bull.* 30: 387-393, 1993.

Barbeau, H. and Bedard, P. Denervation supersensitivity to 5-HT in rats following spinal transection and 5,7 dihydroxytryptamine injection. *Neuropharmacology* 20: 611-616, 1981.

Barbeau, H. and Rossignol, S. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res.* 412: 84-95, 1987.

Barbeau, H. and Rossignol, S. Initiation and modulation of the locomotor pattern in

the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. *Brain Res.* 546: 250-260, 1991.

Belanger, M., Drew, T., Provencher, J., and Rossignol, S. A comparison of treadmill locomotion in adult cats before and after spinalization. *J. Neurophysiol.* 76: 471-491, 1996.

Carrier, L., Brustein, E., Provencher, J., and Rossignol, S. Adaptation of the locomotor pattern to neuro-muscular lesions in normal and chronic spinal cats. *Soc. Neurosci. Abstr.* 18: 861-no.360.3, 1992.

Carrier, L., Brustein, E., and Rossignol, S. Locomotion of the hindlimbs following a neurectomy of the ankle flexors in intact and spinal cats: a model for study of plasticity. *J. Neurophysiol.* 77: 1979-1993, 1997.

Davies, D. S., Wing, L. M. H., Reid, J. L., Neill, E., Tippett, P., and Dollery, M. B. Pharmacokinetics and concentration-effect relationships of intravenous and oral Clonidine. *Clin. Pharmacol. Ther.* 21: 593-601, 1976.

Dietz, V., Colombo, G., and Jensen, L. Locomotor activity in spinal man. *The lancet* 344: 1260-1263, 1994.

Dietz, V., Colombo, G., Jensen, L., and Baumgartner, L. Locomotor capacity of spinal cord in paraplegic patients. *Ann. Neurol.* 37: 574-582, 1995.

Durkovic, R. G. Classical conditioning of the flexion reflex in spinal cat: features of the reflex circuitry. *Neurosci. Lett.* 39: 155-160, 1983.

Edgerton, V. R., de Guzman, C. P., Gregor, R. J., Roy, R. R., Hodgson, J. A., and Lovely, R. G. Trainability of the spinal cord to generate hindlimb stepping patterns in adult spinalized cats. In: *Neurobiological basis of human locomotion*, edited by M. Shimamura, S. Grillner and V. R. Edgerton. Tokyo: Japan scientific societies press, 1991, p. 411-423.

Forssberg, H. and Grillner, S. The locomotion of the acute spinal cat injected with clonidine i.v. *Brain Res.* 50: 184-186, 1973.

Forssberg, H. and Svartengren, G. Hardwired locomotor network in cat revealed by a retained motor pattern to gastrocnemius after muscle transposition. *Neurosci. Lett.* 41: 283-288, 1983.

Fung, J., Stewart, J. E., and Barbeau, H. The combined effects of clonidine and cyproheptadine with interactive training on the modulation of locomotion in spinal cord injured subjects. *J. Neurol. Sci.* 100: 85-93, 1990.

Giroux, N., Aloyz, R. S., Rossignol, S., and Reader, T. A. Serotonin 1a and α1 and α2-noradrenergic receptors in the spinal cord of spinalized cats. *Soc. Neurosci. Abstr.* 21: 926 no.369.3, 1995.

Goldberger, M. E. and Murray, M. Restitution of function and collateral sprouting in the cat spinal cord: the deafferented animal. *J. Comp. Neurol.* 158: 37-54, 1974.

Goldberger, M. E. and Murray, M. Lack of sprouting and its presence after lesions of the cat spinal cord. *Brain Res.* 241: 227-239, 1982.

Haggendal, J. and Dahlstrom, A. The time course of noradrenaline decrease in rat spinal cord following transection. *Neuropharmacology* 12: 349-354, 1973.

Hodgson, J. A., Roy, R. R., De Leon, R., Dobkin, B., and Edgerton, V. R. Can the mammalian lumbar spinal cord learn a motor task? *Med. Sci. Sports Exer.* 26: 1491-1497, 1994.

Li, Y. and Raisman, G. Schwann cells induce sprouting in motor and sensory axons in the adult rat spinal cord. *J. Neurosci.* 14: 4050-4063, 1994.

Liu, C. N. and Chambers, W. W. Intraspinal sprouting of dorsal root axons. *Arch. Neurol. Psychiat.* 79: 46-61, 1958.

Lovely, R. G., Gregor, R. J., Roy, R. R., and Edgerton, V. R. Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cat. *Brain Res.* 514: 206-218, 1990.

Murray, M. and Goldberger, M. E. Replacement of synaptic terminals in lamina II and clarke's nucleus after unilateral lumbosacral dorsal rhizotomy in adult cats. *J. Neurosci.* 6: 3205-3217, 1986.

Murray, M. and Goldberger, M. E. Restitution of function and collateral sprouting in the cat spinal cord: the partially hemisected animal. *J. Comp. Neurol.* 158: 19-36, 1974.

Pearson, K. G. and Rossignol, S. Fictive motor patterns in chronic spinal cats. *J. Neurophysiol.* 66: 1874-1887, 1991.

Philippson, M. L'autonomie et la centralisation dans le système nerveux des animaux. *Trav.Lab.Physiol.Inst.Solvay.(Bruxelles.)* 7:1-208, 1905.

Robinson, G. A. and Goldberger, M. E. The development and recovery of motor function in spinal cats. II. Pharmacological enhancement of recovery. *Exp. Brain. Res.* 62: 387-400, 1986.

Rossignol, S., Barbeau, H., and Provencher, J. Locomotion in the adult chronic spinal cat. *Soc. Neurosci. Abstr.* 8: 163, no.47.1, 1982.

Rossignol, S., Barbeau, H., and Julien, C. Locomotion of the adult chronic spinal cat and its modification by monoaminergic agonists and antagonists. In: *Development and plasticity of the mammalian spinal cord*, edited by M. Goldberger, A. Gorio and M. Murray. Padova: Fidia Research Series III, Liviana Press, 1986, p. 323-345.

Rossignol, S., Barbeau, H., and Chau, C. Pharmacology of locomotion in chronic spinal cat. In: *Alpha and gamma motor systems*, edited by A. Taylor, M. H. Gladden and R. Durbaba. New York, London: Plenum Press, 1995, p. 449-455.

Rossignol, S. Neural control of stereotypic limb movements. In: *Handbook of Physiology, section 12. Exercise: regulation and integration of multiple systems.* edited by L. B. Rowell and J. T. Sheperd. American Physiological Society, 1996, p. 173-216.

Roudet, C., Savasta, M., and Feuerstein, C. Normal distribution of alpha-1-adrenoceptors in the rat spinal cord and its modification after noradrenergic denervation: A quantitative autoradiographic study. *J. Neurosci. Res.* 34: 44-53, 1993.

Roudet, C., Mouchet, P., Feuerstein, C., and Savasta, M. Normal distribution of

alpha-2-adrenoceptors in the rat spinal cord and its modification after noradrenergic denervation: a quantitative autoradiographic study. *J. Neurosci. Res.* 39: 319-329, 1994.

Shurrager, P. S. Walking in spinal kittens and puppies. In: *Regeneration in the central nervous system*, edited by W. F. Windle. Springfield: C.C. Thomas, 1955, p. 208-218.

Shurrager, P. S. and Dykman, R. A. Walking spinal carnivores. *J. Comp. Physiol. Psychol.* 44: 252-262, 1951.

Smith, J. L., Smith, L. A., Zernicke, R. F., and Hoy, M. Locomotion in exercised and non-exercised cats cordotomized at two or twelve weeks of age. *Exp. Neurol.* 76: 393-413, 1982.

Sperry, R. W. The functional results of muscle transposition in the hindlimb of the rat. *J. Comp. Neurol.* 73: 379-404, 1940.

Sperry, R. W. The effects of crossing nerves to anatogonistic muscles in the hindlimb of the rat. *J. Comp. Neurol.* 75: 1-19, 1941.

Viala, D., Viala, G., and Fayein, N. Plasticity of locomotor organization in infant

rabbits spinalized shortly after birth. In: *Development and plasticity of the mammalian spinal cord*, edited by M. Goldberger, A. Gorio and M. Murray. Padova: Liviana Press, 1986, p. 301-310.

Visintin, M. and Barbeau, H. The effects of body weight support on the locomotor pattern of spastic paretic patients. *Can. J. Neurol. Sci.* 16: 315-325, 1989.

Wernig, A. and Muller, S. Laufband locomotion with body weight support improved walking in persons with severe spinal cord injuries. *Paraplegia* 30: 229-238, 1992.

Wieler, M., Stein, R., and Dai, R. Multi-center clinical testing of functional electrical stimulation systems to assist walking. *Proceedings of the 12th International Congress of the World Confederation for Physical Therapy* 773, 1995.

Wolpaw, J. R., Carp, J. S., and Lee, C. L. Memory traces in spinal cord produced by H-reflex conditioning: effects of post-tetanic potentiation. *Neurosci. Lett.* 103: 113-119, 1989.

Wolpaw, J. R. and Carp, J. Adaptive plasticity in spinal cord. *Adv. Neurol.* 59: 163-174, 1993.

Wolpaw, J. R. and Chong, L. L. Memory traces in primate spinal cord produced by

operant conditioning of H-reflex. *J. Neurophysiol.* 61: 563-572, 1989.

Zhang, B., Goldberger, M. E., Wu, L. F., and Murray, M. Plasticity of complex terminals in lamina II in partially deafferented spinal cord: the cat spared root preparation. *Exp. Neurol.* 132: 186-193, 1995.

Table I.

Profile of the experimental cats used for early locomotor training experiments. The control condition represents the time period before spinalisation when the cat was being trained to walk on the treadmill and when recordings of intact locomotion were made for subsequent comparison. The time scale was set such that the day of spinalisation was day 0. The cats were sacrificed at various days post-transection as indicated. Locomotor recovery ranges from 6-11d as indicated by the ability of the cat to walk on the treadmill with weight support of the hindquarters and foot placement even before Clonidine injection.

Table I. Profile of the experimental cats used for early locomotor training experiments.

Cat	gender	condition	time (in days)	daily Clonidine doses and (# of days when injection were given consecutively)	number of training sessions each day of injection and (# of min/session)	post spinal day with weight support during locomotion without Clonidine
HB6	male	Spinalisation	0	50-100µg/kg i.p. (7)	1-2 (12-20)	9
		Sacrifice	28			
CC1	male	Control	-28	150-215µg/kg i.p. (10)	1-2 (15)	11
		Spinalisation	0			
		Sacrifice	24			
CC2	female	Control	-15	200-250µg/kg i.p. (8)	3-5 (15-20)	9
		Spinalisation	0			
		Sacrifice	12			
CC3	female	Control	-15	200µg/kg i.p. (8)	3-5 (15-20)	9
		Spinalisation	0			
		Sacrifice	12			
CC4	male	Control	-5	100µl of 0.4-4mM i.t. (8)	3-5 (15-20)	6
		Spinalisation	0			
		Sacrifice	70			

Table II.

Summary of the numeric values of cycle duration and step length for all cats on the day of locomotor recovery when locomotion during pre-drug trials was observed, which ranges from 6-11 days post-transection (see column 'Days'). The pre-drug and post-drug value is also expressed as a percentage of the intact value. Student t-tests were performed to compare if the pre- drug or the post-drug values were significantly different from the intact condition. * $p \leq 0.05$. ** $p \leq 0.01$. Treadmill speed 0.2m/s.

Table II.

A summary of the numeric values of the cycle duration and step length on the day of recovery of spontaneous locomotion

Cat	Condition	days	n	Cycle duration (ms ± s.d.)	%	Step length (mm ± s.d.)	%
HB6	intact			n/a		n/a	
	pre-drug	7d	4	1029 ± 122		194 ± 55	
	post-drug		6	1536 ± 157		308 ± 37	
CC1	intact		3	1389 ± 173		477 ± 138	
	pre-drug	11d	5	1056 ± 229	77	236 ± 43**	50
	post-drug		4	1017 ± 78*	73	161 ± 12**	34
CC2	intact		6	1475 ± 184		396 ± 54	
	pre-drug	8d	4	1220 ± 101*	83	235 ± 31**	59
	post-drug		4	1379 ± 37	94	314 ± 18	79
CC3	intact		15	1471 ± 218		345 ± 43	
	pre-drug	9d	9	883 ± 112**	60	210 ± 26**	61
	post-drug		14	1102 ± 68**	75	252 ± 22**	75
CC4	intact		5	1176 ± 78		503 ± 30	
	pre-drug	6d	9	1114 ± 168	95	373 ± 49**	81
	post-drug		8	1091 ± 75	93	334 ± 44**	73

Table III.

Numeric values of the range of joint angular excursion, when the cat was able to walk without Clonidine. The averaged angular excursion (degree ± s.d.) was obtained during intact, pre-drug trials and post-drug trials. The angular excursions during pre- and post drug trials are also expressed as a percentage of the intact values. Student t-tests were performed to compare if the pre- drug or the post-drug angular excursion were significantly different from the intact condition. * $p \leq 0.05$. ** $p \leq 0.01$.

Table III. Numeric values of the range of joint angular excursion on the day of recovery of spontaneous locomotion.

				Hip		Knee		Ankle		MTP	
Cat	Condition	day	n	range (degree±sd)	%	range (degree±sd)	%	range (degree±sd)	%	range (degree±sd)	%
HB6	Intact			n/a		n/a		n/a		n/a	
	Pre-drug	7d	4	35 ± 14	n/a	25 ± 12	n/a	64 ± 18	n/a	56 ± 27	n/a
	Post-drug		6	42 ± 9	n/a	35 ± 8	n/a	55 ± 19	n/a	50 ± 15	n/a
CC1	Intact		3	20 ± 4		29 ± 4		33 ± 9		45 ± 14	
	Pre-drug	11d	5	27 ± 10	135	20± 8	70	58 ± 23	175	27 ± 9	60
	Post-drug		4	35 ± 13	175	40 ± 12	138	91 ± 24**	276	29 ± 8	64
CC2	Intact		6	22 ± 5		39 ± 7		26 ± 9		55 ± 15	
	Pre-drug	8d	4	17 ± 6	77	16 ± 9**	41	27 ± 19	104	21 ± 10**	38
	Post-drug		4	27 ± 6	123	22 ± 5**	56	48 ± 12**	185	69 ± 20	125
	Pre-drug	9d	5	25 ± 5	114	24 ± 7**	62	37 ± 10	142	46 ± 42	84
	Post-drug		5	31 ± 6*	141	33 ± 5	95	53 ± 12	204	43 ± 13	78
CC3	Intact		6	26 ± 3		24 ± 3		21 ± 4		47 ± 10	
	Pre-drug	9d	9	19 ± 5**	73	16 ± 5**	67	31 ± 9*	148	42 ± 43	89
	Post-drug		14	31 ± 4*	119	39 ± 5**	163	46 ± 8**	219	47 ± 30	100
CC4	Intact		5	28 ± 10		35 ± 9		20 ± 7		49 ± 32	
	Pre-drug	6d	9	32 ± 6	114	34 ± 7	97	57 ± 15**	285	62 ± 18	126
	Post-drug		8	34 ± 3	121	26 ± 4*	74	32 ± 8*	160	50 ± 14	102

Table IV.

Numeric values of the EMG burst duration and amplitude of flexor (iSt and iSrt) and extensor muscles (iVL and IGL) in 5 experimental cats when the cat was able to walk during pre-drug trials (6-11d). The burst durations during pre- and post-drug trials are expressed in milliseconds and as a percentage of the intact values. The burst amplitude is expressed as a percentage of the intact values.

Table IV. Numeric values of the EMG of flexor and extensor muscles.

Cat	Day	0.2 m/s		Burst duration (ms ± s.d.)					Burst Amplitude (% of control)	
			n	intact	n	pre	n	post	pre	post
HB6	9	iSt		n/a	7	300±87	7	624±148	n/a	n/a
		iSrt		n/a		355±72		337±86	n/a	n/a
		iVL		n/a		67±227		497±141	n/a	n/a
CC1	11	iSt	5	253±40	8	178±49	13	286±60		
						70%		113%	145%	345%
		iSrt		252±34		424±80		482±98		
						168%		191%	132%	204%
		iVL		1105±138		554±122		597±114		
						50%		54%	100%	164%
		iGL		879±146		746±106		483±157		
						85%		55%	123%	354%
CC2	9	iSt	6	298±99	14	262±95	9	245±87		
						88%		82%	174%	224%
		iSrt		426±67		335±154		276±85		
						79%		65%	63%	110%
		iVL		725±279		701±197		814±80		
						97%		112%	156%	267%
		iGL		1031±258		827±166		822±87		
						80%		80%	110%	185%
CC3	9	iSt	16	229±80	10	159±49	16	182±50		
						69%		80%	120%	300%
		iSrt		352±65		N/A		202±33		
								57%	N/A	147%
		iVL		926±177		664±94		669±85		
						72%		72%	287%	342%
CC4	6	iSt	15	122±47	11	100±20	9	188±43		
						82%		154%	338%	192%
		iSrt		285±33		154±35		191±33		
						54%		145%	247%	106%
		iVL		815±100		839±169		671±103		
						103%		82%	131%	138%
		iGL		678±118		883±195		736±117		
						130%		109%	284%	190%

Figure 1.

A. Averaged joint angular displacement (mean ± s.d.) of hip, knee, ankle and mtp joint for 6 normalized step cycles (the cycle is repeated twice) of a cat during the intact condition (before spinalisation). See text for definitions of F, E_1, E_2, E_3. The upward arrows indicate paw lift and the downward arrows indicate paw contact.

B. Rectified, normalized and averaged EMG recordings during the same 6-step sequence synchronized to foot contact of the hip, knee, and ankle extensor muscles, Glu, VL, and GL, respectively, occurred during the stance phase. The hip and knee flexor muscles, Srt and St, respectively, were activated during the swing phase. The onset of hip flexor iSrt was later than the knee flexor iSt. There was also a double burst of activity seen in coSt. **C**. Stick figures of the hindlimb illustrating the swing and stance phases of 1 step cycle. Each stick figure was formed by drawing lines between the different reflective markers. The stick diagrams shown were reconstructions of the actual hind limb movements during the stance and the swing phases. Each frame was displaced from the previous frame by the distance travelled by the foot. Thus, the stick figures are «spread out» horizontally to allow a better illustration of the limb movements. Note that the calibration of the x-axis is twice that of the y-axis.

D. The trajectory of each marker point, as indicated, during a complete step cycle.

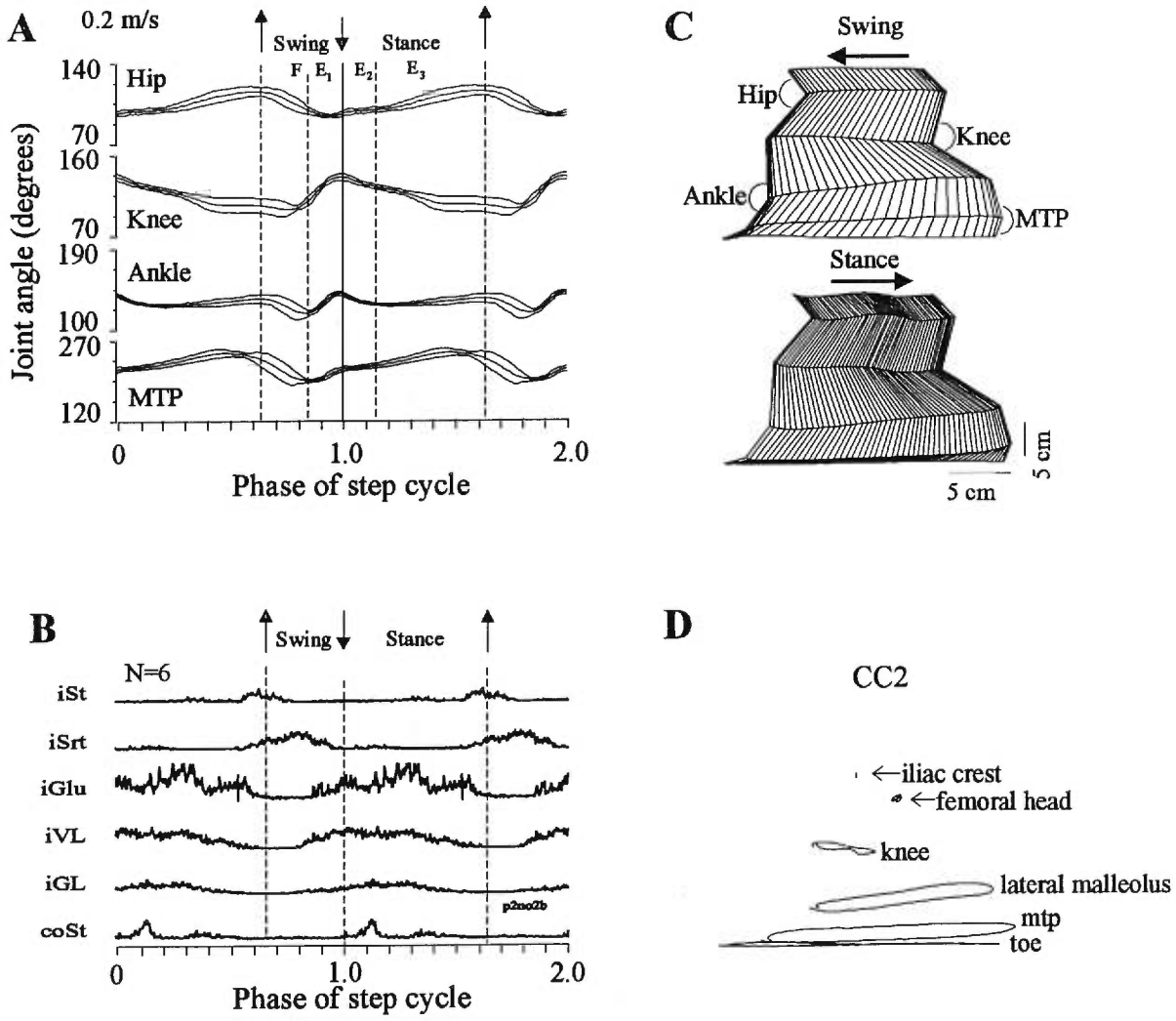

A
0.2 m/s
Swing
Stance
F
E1
E2
E3
140
Hip
70
160
Knee
70
190
Ankle
100
270
MTP
120
Joint angle (degrees)
0
1.0
2.0
Phase of step cycle
B
Swing
Stance
N=6
iSt
iSrt
iGlu
iVL
iGL
coSt
p2no2b
0
1.0
2.0
Phase of step cycle
C
Swing
Hip
Knee
Ankle
MTP
Stance
5 cm
5 cm
D
CC2
iliac crest
femoral head
knee
lateral malleolus
mtp
toe

Figure 2.

A,B,C,D,E. The progressive recovery of locomotion (cat illustrated in Fig. 1) following spinalisation, before Clonidine injection at 3, 4, 7, 8, and 9d post-transection.

F,G,H,I,J. The corresponding locomotion of the spinal cat at 3, 4, 7, 8, and 9 d following Clonidine injection. Left arrows indicate swing, right arrows indicate stance. A small horizontal bar under the stick diagram in F, G and I represents the presence of foot drag during the onset of swing phase.

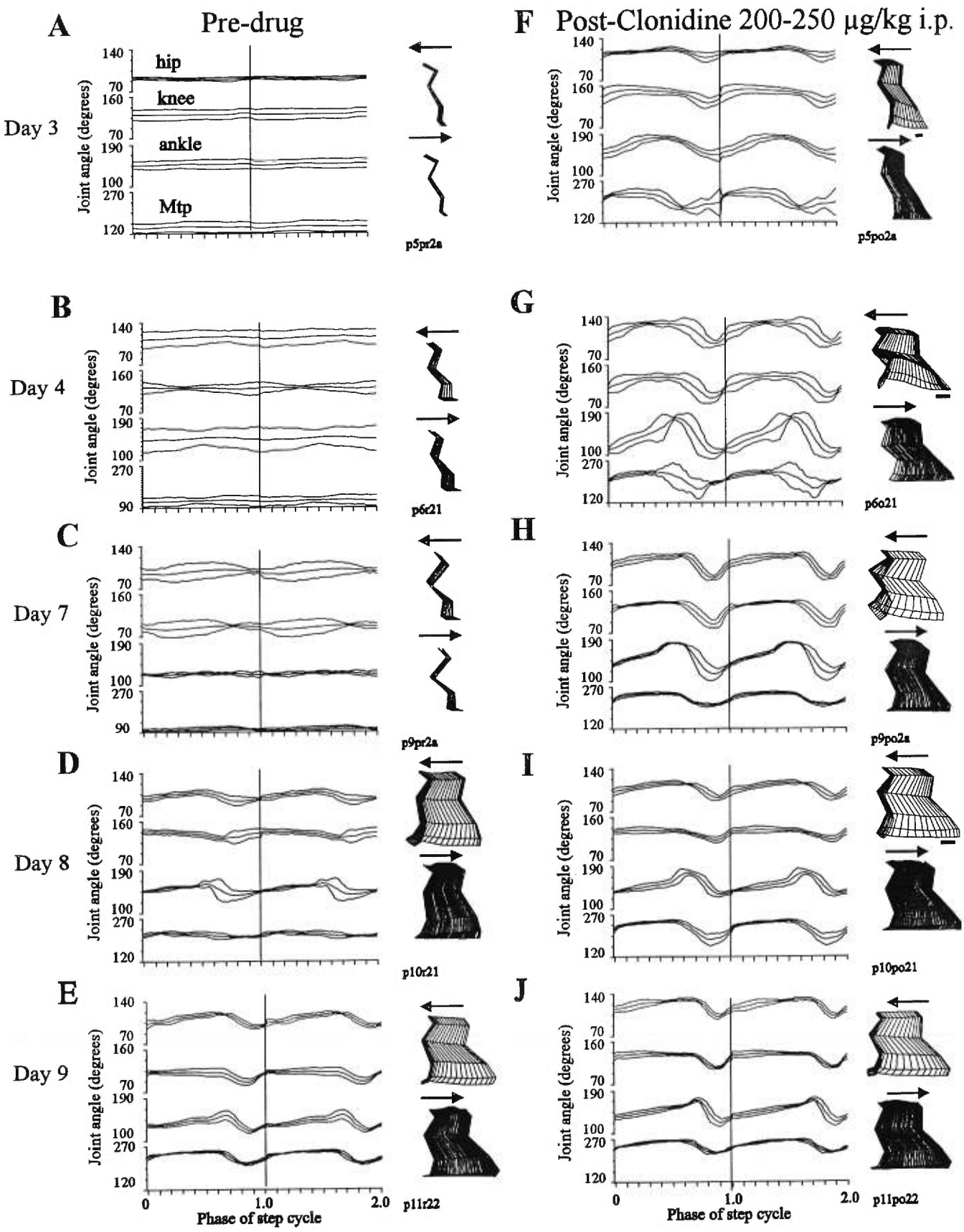
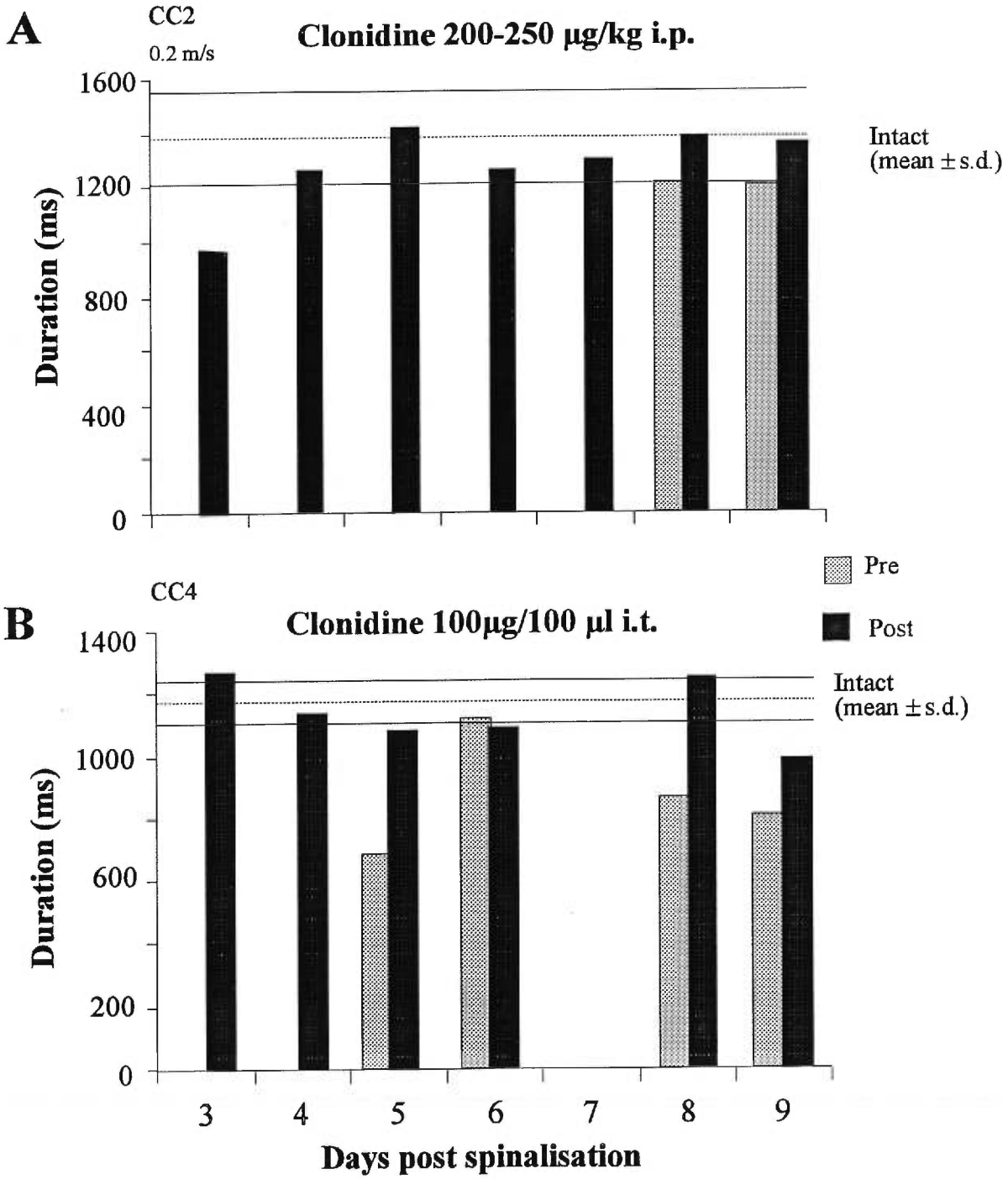

A
Pre-drug
F
Post-Clonidine 200-250 µg/kg i.p.
Day 3
hip
knee
ankle
Mtp
Joint angle (degrees)
p5pr2a
p5po2a
B
G
Day 4
p6r21
p6o21
C
H
Day 7
p9pr2a
p9po2a
D
I
Day 8
p10r21
p10po21
E
J
Day 9
p11r22
p11po22
Phase of step cycle

Figure 3.

Cycle duration as a function of days post-transection in 2 cats, CC2 and CC4. The mean step cycle duration (at 0.2m/s) during the intact condition is represented by a dotted horizontal line with the standard deviation (± s.d. illustrated by 2 thin horizontal lines), during spinal pre-drug condition represented by grey bar and during post-drug condition represented by a black bar.


A
CC2
0.2 m/s
Clonidine 200-250 µg/kg i.p.
1600
1200
800
400
0
Duration (ms)
Intact
(mean ± s.d.)
Pre
Post
B
CC4
Clonidine 100µg/100 µl i.t.
1400
1000
600
200
0
Duration (ms)
Intact
(mean ± s.d.)
3
4
5
6
7
8
9
Days post spinalisation

step1

Figure 4.

Stance and swing duration as a function of time post-transection in 2 cats CC2 and CC4. The dotted lines indicate values of pre-drug trials, solid lines indicate post-drug trials. A horizontal line with shaded area indicates the intact value with standard deviation. Square symbols denote stance duration, and round symbols denote swing duration. Following Clonidine injection, both stance and swing (solid lines) are very close to the normal values.

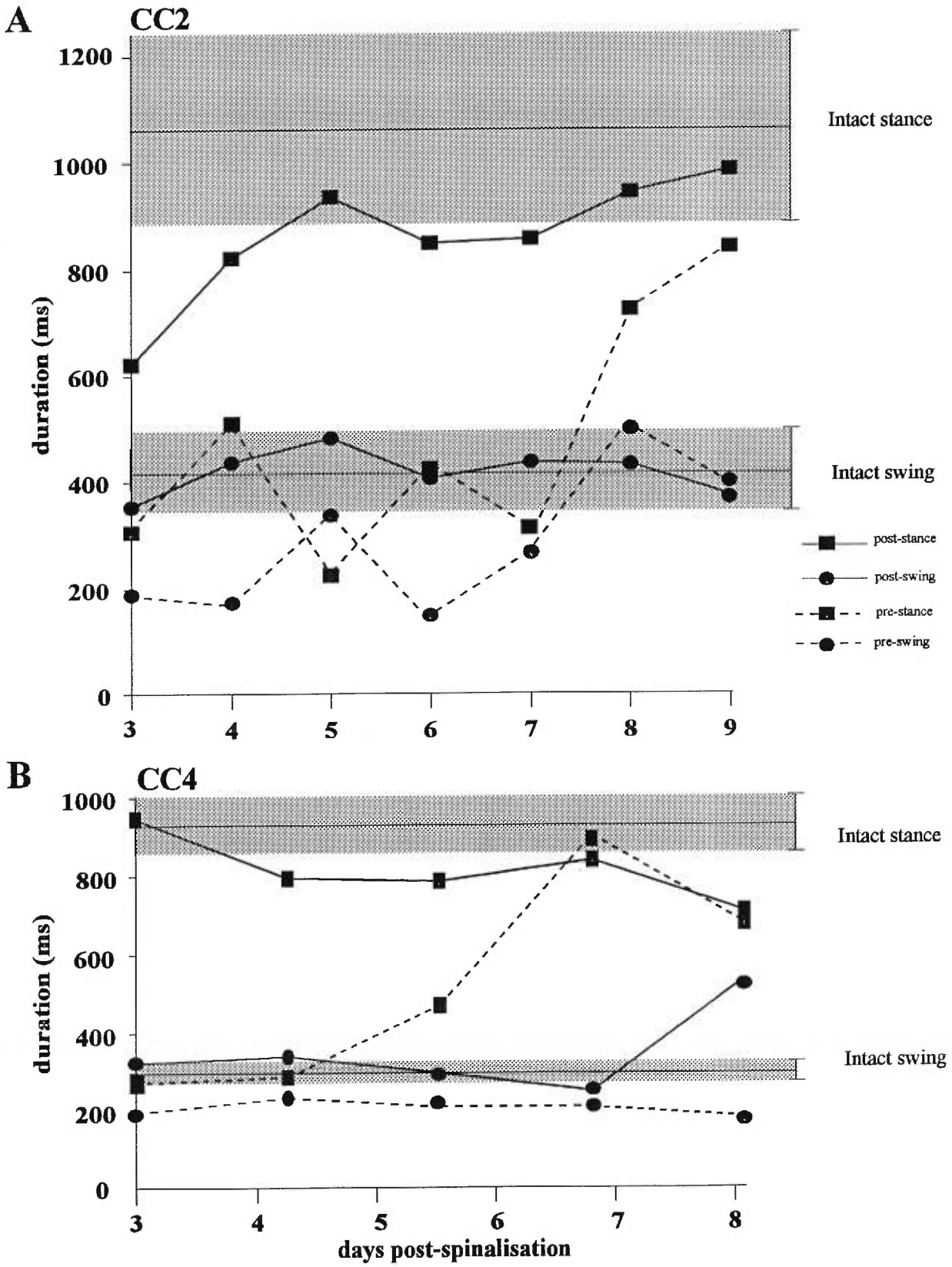

A
CC2
1200
1000
800
600
400
200
0
duration (ms)
Intact stance
Intact swing
post-stance
post-swing
pre-stance
pre-swing
3
4
5
6
7
8
9
B
CC4
1000
800
600
400
200
0
duration (ms)
Intact stance
Intact swing
3
4
5
6
7
8
days post-spinalisation

Figure 5.

The relationship between the step length during pre-drug and post-drug trials in 3 spinal cats, CC2, CC3, and CC4. The Y-axis indicates the step length pre-Clonidine injection, the X-axis indicates the step length post-Clonidine injection. The numbers in the squares indicates the number of post-spinal days. The step length during the intact condition is indicated by the square in the upper right corner. A vertical line and a horizontal line connect the intact values to the X-axis and the Y-axis, respectively, forming a rectangle. Any data point that falls within the rectangle indicates that the value is less than that observed during intact condition. A diagonal line also connects the origin to the intact data point. A data point that falls on the 45 degree line indicates the step length during pre- and post-Clonidine trials are the same. Any data point that falls below the diagonal line indicates that the step length during post-Clonidine trials exceeds the pre-Clonidine values. Any data point found above the diagonal line indicates that the pre-Clonidine step length exceeds the post-Clonidine values.

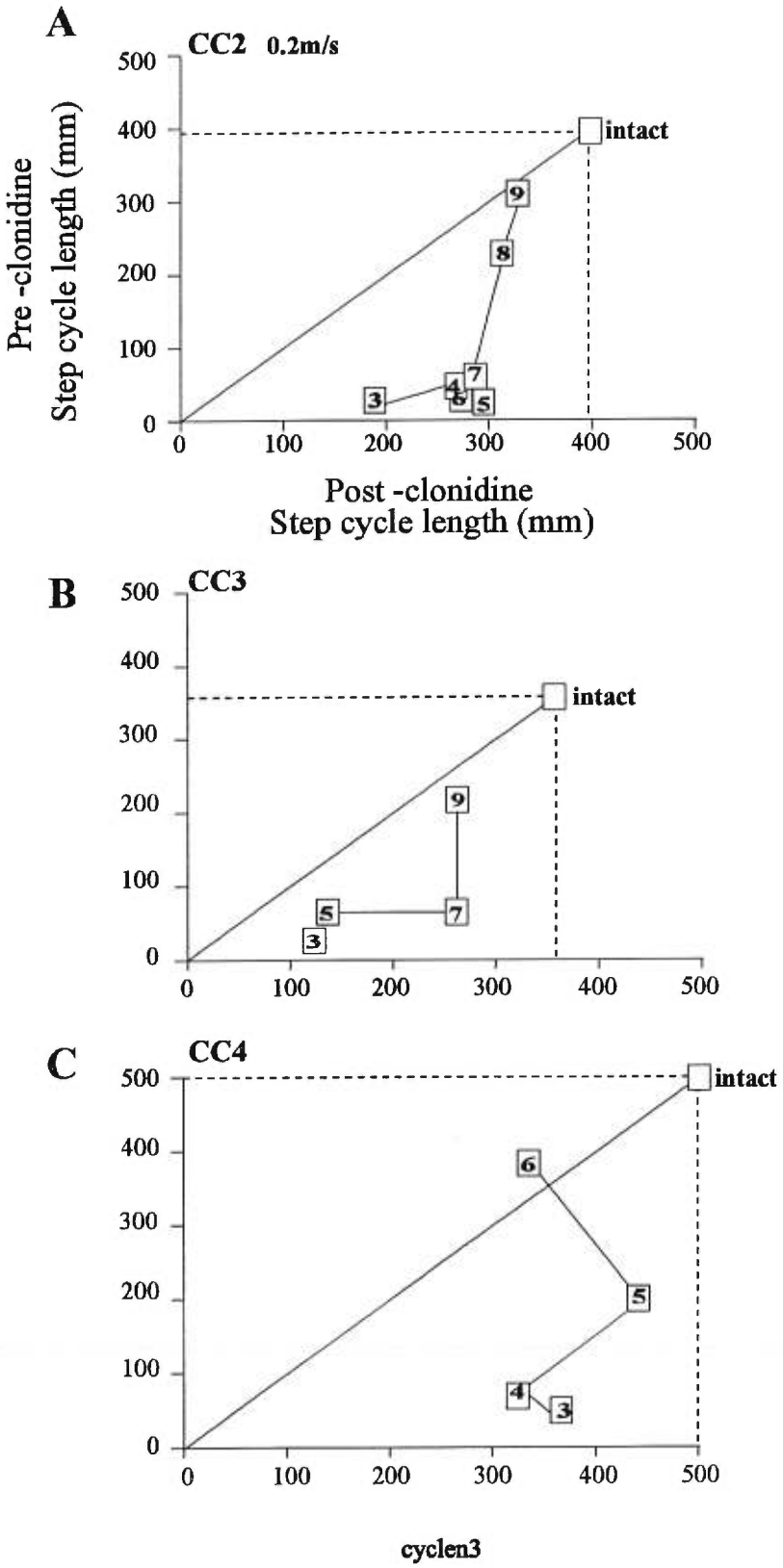

A
CC2 0.2m/s
intact
Pre -clonidine
Step cycle length (mm)
Post -clonidine
Step cycle length (mm)
B
CC3
intact
C
CC4
intact
cyclen3

Figure 6.

A,C,E,G. Display of the hip, knee, ankle and mtp joint movement as a function of days post-transection during pre-drug trials. Note that the first range represents the intact condition.

B,D,F,H. The hip, knee, ankle, and mtp joint angular excursion (maximum-minimum angles) during pre-drug trials. The intact angular excursion is always shown as a horizontal line with the first data point on the left hand column of each graph.

I. The step cycle length at the different corresponding post-transection days during pre-drug trials.

J-R shows the corresponding joint movement, angular excursion, and step cycle length following Clonidine injection.

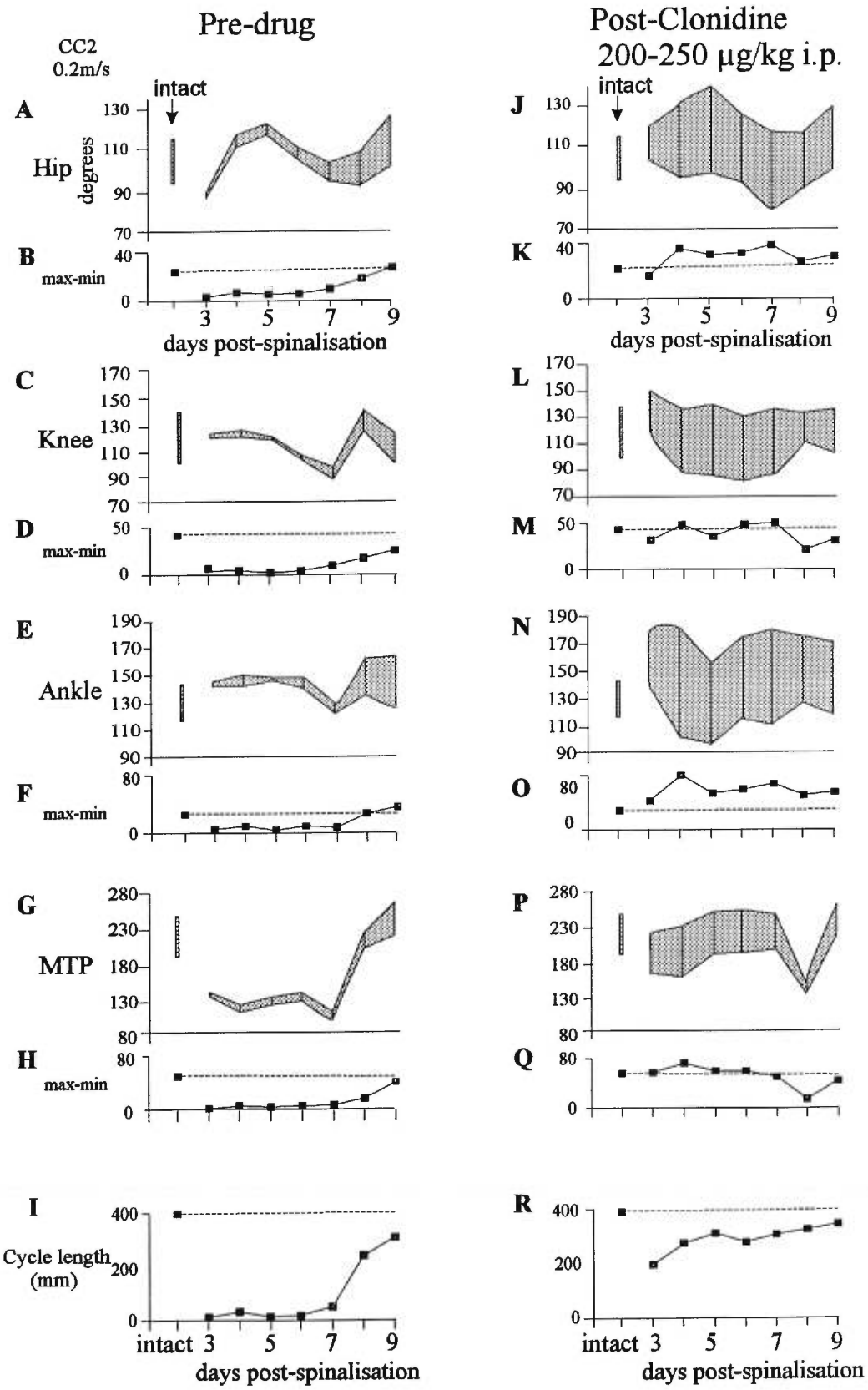

Pre-drug
Post-Clonidine
200-250 µg/kg i.p.
CC2
0.2m/s
intact
A
Hip
degrees
130
110
90
70
B
max-min
40
0
3
5
7
9
days post-spinalisation
C
Knee
170
150
130
110
90
70
D
max-min
50
0
E
Ankle
190
170
150
130
110
90
F
max-min
80
0
G
MTP
280
230
180
130
80
H
max-min
80
0
I
Cycle length
(mm)
400
200
0
intact
J
K
L
M
N
O
P
Q
R

Figure 7.

Day to day changes in cycle duration in one cat (CC2) as a function of treadmill speed post-spinalisation, with and without Clonidine. The hatched areas indicate the values obtained during intact condition, the dotted lines indicate values obtained during the pre-drug condition, and the thick lines indicate the post-Clonidine condition.

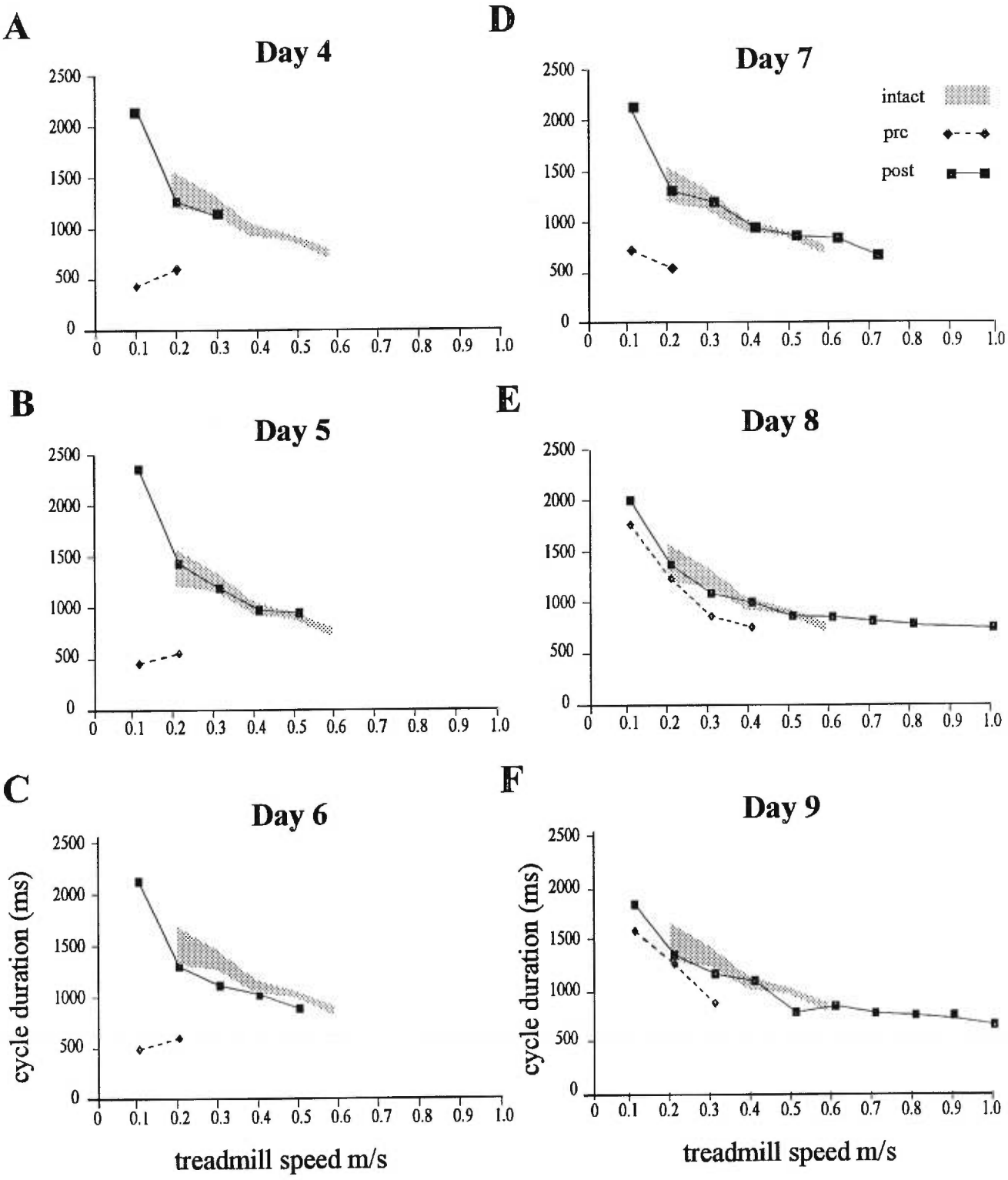

A
Day 4
B
Day 5
C
Day 6
D
Day 7
intact
prc
post
E
Day 8
F
Day 9
cycle duration (ms)
treadmill speed m/s
2500
2000
1500
1000
500
0
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Figure 8.

Rectified, averaged and normalized EMG activity of the cat CC2 (same cat as in Fig 2) synchronized to iSt at 3, 5, 7, 8 and 9 days post-spinalisation, during pre-Clonidine and post-Clonidine trials. Note that the EMG signals in **8J** (9 days post-spinalisation) are superimposed with the intact EMG signals (as seen in Fig. 1B) indicated by the thin lines.

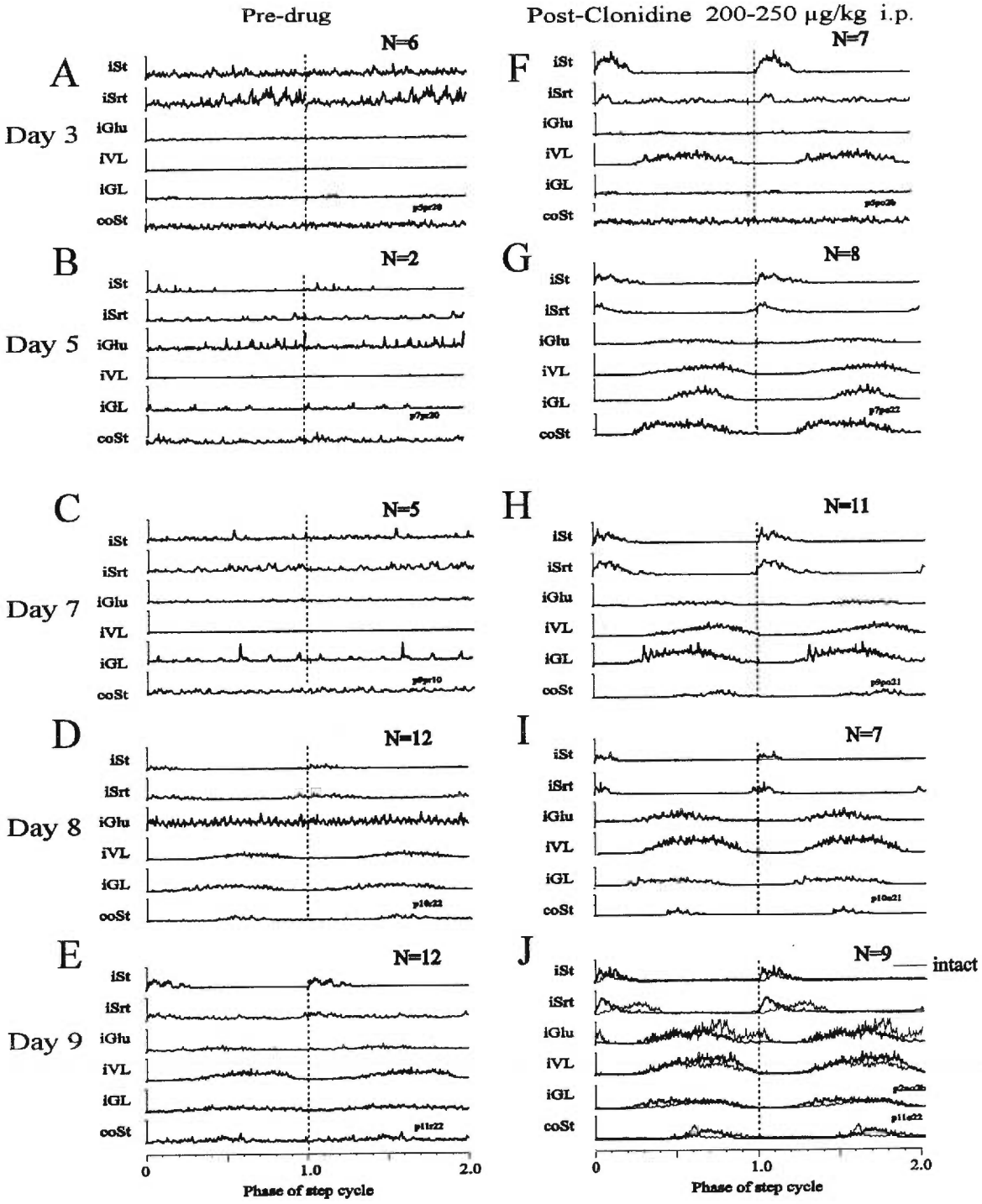

Pre-drug
Post-Clonidine 200-250 µg/kg i.p.
A
Day 3
N=6
B
Day 5
N=2
C
Day 7
N=5
D
Day 8
N=12
E
Day 9
N=12
F
N=7
G
N=8
H
N=11
I
N=7
J
N=9
intact
iSt
iSrt
iGlu
iVL
iGL
coSt
0
1.0
2.0
Phase of step cycle

Figure 9.

Raw EMG activity of hindlimb flexor and extensor muscles during locomotion in the different cat (CC3) during intact condition, 7 days and 9 days post-transection, following Clonidine injection. The treadmill speed was 0.3m/s.

CC3 0.3m/s

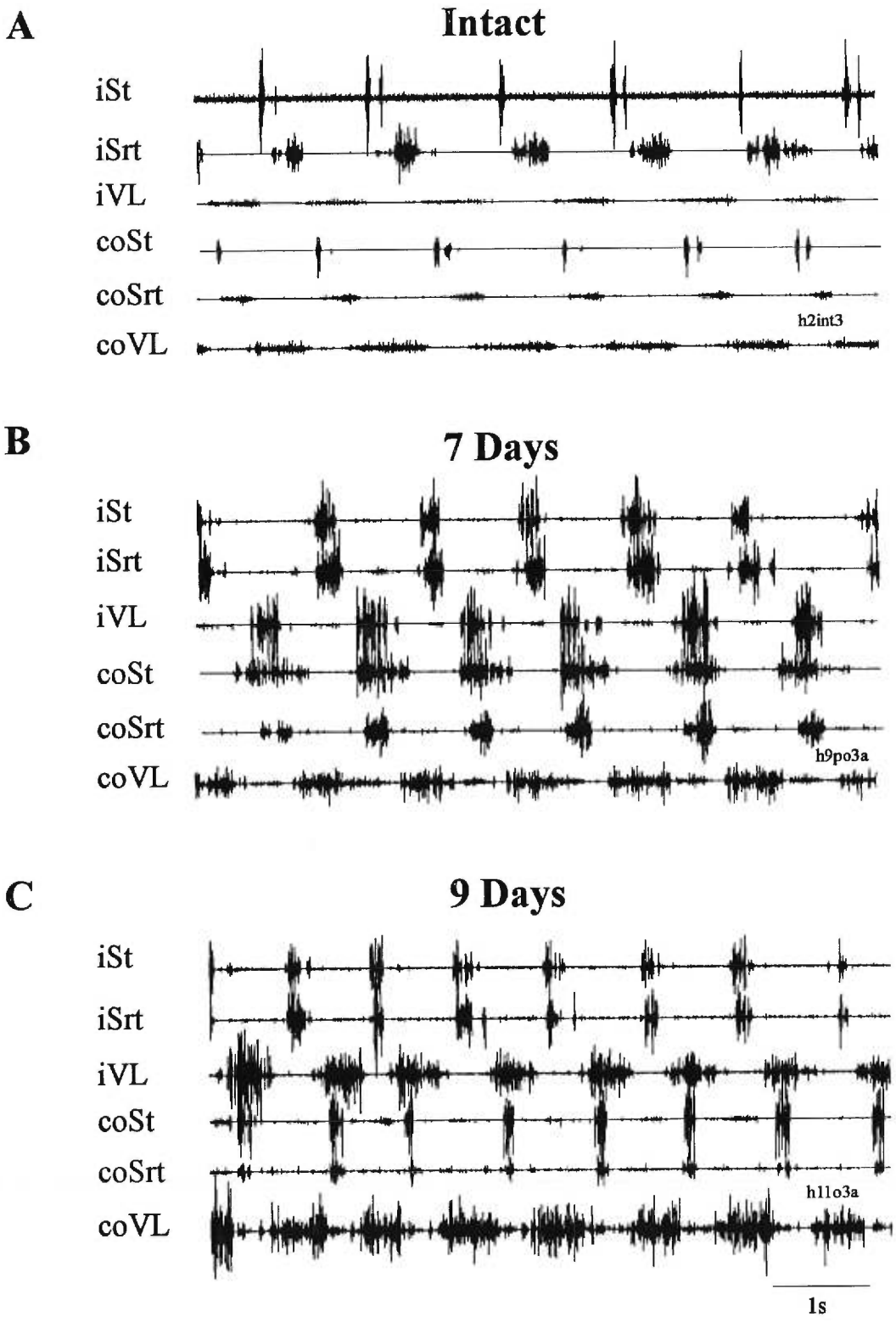
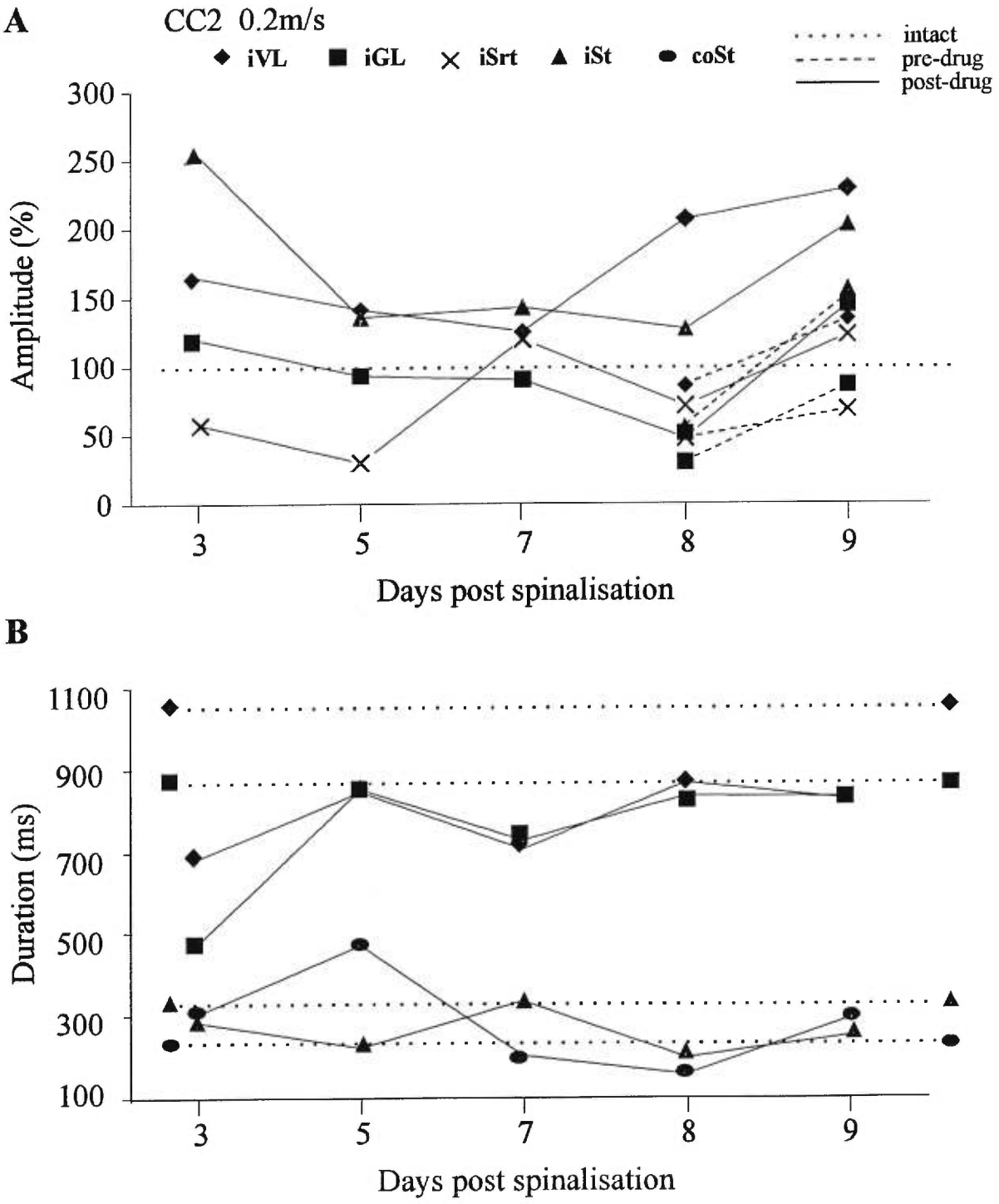

A
Intact
iSt
iSrt
iVL
coSt
coSrt
coVL
h2int3
B
7 Days
iSt
iSrt
iVL
coSt
coSrt
coVL
h9po3a
C
9 Days
iSt
iSrt
iVL
coSt
coSrt
coVL
h11o3a
1s

Figure 10.

A. Extensor (iVL, iGL) and flexor (iSt, coSt) EMG amplitude during locomotion (0.2m/s) as a function of days following spinalisation in cat CC2 before and following Clonidine injection. The horizontal dotted line represents the intact condition, the stippled lines represent the spinal pre-drug trials, and the solid lines represent the spinal-post-Clonidine trials. The amplitude was relative and was expressed as a percentage of the burst duration during intact condition (100%). Note that pre-drug values were available only on d8 and d9 as the cat was not walking before d8.

B. EMG burst duration of flexor and extensor muscles of the same cat following Clonidine injection from d3-d9. The values obtained during intact condition for each muscle are indicated by the horizontal dotted lines with corresponding symbols.


A
CC2 0.2m/s
iVL
iGL
iSrt
iSt
coSt
intact
pre-drug
post-drug
Amplitude (%)
300
250
200
150
100
50
0
3
5
7
8
9
Days post spinalisation
B
Duration (ms)
1100
900
700
500
300
100
3
5
7
8
9
Days post spinalisation

ARTICLE #2:

The effects of intrathecal α1- and α2-noradrenergic agonists and noradrenaline on locomotion in chronic spinal cats.

by

Connie Chau, Hugues Barbeau, Serge Rossignol

Journal of Neurophysiology, *in press*, 1998

Abstract

Noradrenergic drugs, acting on α adrenoceptors, have been found to play an important role in the initiation and modulation of locomotor pattern in adult cats following spinal cord transection. There are at least 2 subtypes of α adrenoceptors, α1- and α2-adrenoceptors. The aim of this study was to investigate the effects of selective α1- and α2-agonists in the initiation and modulation of locomotion in adult chronic cats in the early and late stages after complete transection at T13. Five cats, chronically implanted with an intrathecal cannula and EMG electrodes were used in this study. Noradrenergic drugs including α2-agonists (Clonidine, Tizanidine, and Oxymetazoline), and an antagonist, Yohimbine, one α1-agonist (Methoxamine) and a blocker, Prazosin, as well as noradrenaline were injected intrathecally. Electromyographic (EMG) activity synchronized to video images of the hindlimbs were recorded before and after each drug injection. The results show differential effects of α1- and α2-agonists in the initiation of locomotion in early-spinal cats (i.e. in the first week or so when there is no spontaneous locomotion), and in the modulation of locomotion and cutaneous reflexes in the late-spinal cats (i.e. when cats have recovered spontaneous locomotion). In early spinal cats, all three α2-agonists were found to initiate locomotion, although their action had a different time course. The α1-agonist, methoxamine, induced bouts of nice locomotor activity in 3 spinal cats some hours after injection, but only induced sustained locomotion in 1 cat in which the effects were blocked by the α1-

antagonist, Prazosin. In late spinal cats, while α2-agonists markedly increased the cycle duration and flexor muscle burst duration and decreased the weight support or extensor activity (effects blocked by an α2-antagonist, Yohimbine), α1-agonist increased the weight support and primarily the extensor activity of the hindlimbs without markedly changing the timing of the step cycle. While α2-agonists, especially Clonidine, markedly reduced the cutaneous excitability and augmented the foot drag, the α1-agonist was found to increase the cutaneous reflex excitability. This is in line with previously reported differential effects of activation of the two receptors on motoneuron excitability and reflex transmission. Noradrenaline, the neurotransmitter itself, increased the cycle duration and at the same time retained the cutaneous excitability, thus exerting both α1- and α2-effects. This work therefore suggests that different subclasses of noradrenergic drugs could be used to more specifically target aspects of locomotor deficits in patients after spinal injury or diseases.

Introduction

Different neurotransmitters such as noradrenaline, serotonin, excitatory amino acids (EAA) and acetylcholine have been identified to play a role in the initiation and modulation of locomotion in different animal preparations (for review, see Rossignol, 1996). For example, in *in vitro* neonatal rat preparation, locomotor activity have been found to be released by EAA (Kudo and Yamada 1987; Cazalets et al. 1990; Smith and Feldman 1987), serotonin (Cazalets et al. 1992; Cowley and Schmidt 1994; Kiehn and Kjaerulff 1996) and cholinergic drugs (Katakura and Chandler 1991). In chronic spinal cats, among the different pharmacological agents, noradrenergic drugs were found to be the most effective in initiating locomotion (Barbeau and Rossignol 1991; Barbeau et al. 1987). The importance of the noradrenergic system has been shown by early studies from Lundberg and colleagues (Anden et al. 1966a,b; Jankowska et al. 1967) who demonstrated the ability of noradrenergic agents to activate neuronal circuits that could be responsible for locomotor function. They showed that i.v. injection of the noradrenergic precursor, dihyroxyphenylalanine (DOPA) inhibited the transmission of short latency responses from the flexor reflex afferent (FRA), but released long latency and long duration discharges which not normally found in acute spinal cats. These late discharges often evolved as sequences of rhythmically alternating activity between flexors and extensors reminiscent of stepping. It was suggested indeed that the interneuronal circuitry generating the late discharges evoked after DOPA could be

responsible for generating locomotion. This was pursued by Grillner and Zangger (1979) who showed that a detailed locomotor rhythm can be generated by the neuronal circuitry within the spinal cord itself. Indeed, following the injection of the noradrenergic precursor (DOPA) and nialamide (a monoamine oxidase inhibitor), a pattern of rhythmic alternating discharges in antagonist hindlimb muscle nerves was observed in acute spinal and curarized cat. DOPA (i.v.) has been postulated to mediate its effects through the activation of noradrenergic receptors (Anden et al. 1966; Anden et al. 1966). Using a noradrenergic receptor agonist (Clonidine), Forssberg and Grillner (1973) demonstrated the ability of noradrenergic drugs to initiate locomotion. They showed in acute spinal cats (Th12) that following an i.v. injection of Clonidine, cats could walk with both hindlimbs when placed on a moving treadmill belt. They suggested that the descending noradrenergic system could «release» the spinal circuitry for stepping. These results were supported by work in our laboratory confirming that Clonidine (i.p.) can trigger hindlimb treadmill locomotion in adult chronic spinal cats (awake behaving animal) within the first week after spinalisation (Barbeau et al. 1987). In a recent paper (Chau et al. 1998) we reported the effects of early locomotor training with daily injection of Clonidine (intraperitoneally in 4 cats, and intrathecally in 1 cat) within the first week after spinal transection. In the present work, we have pursued these ideas with the aim of better identifying the potential of various noradrenergic drugs, in addition to Clonidine, acting on different receptors to initiate and modulate locomotion.

In contrast to our previous work where drugs were injected intraperitoneally,

the present study used an intrathecal cannula exclusively for drug delivery. This not only reduced some side effects encountered with i.p. injections but also greatly expanded our ability to explore a wider variety of drugs. Since the drugs were directly injected into the intrathecal space of the spinal cord, central effects of the drugs dominated over peripheral effects. It also made possible testing drugs that do not cross the blood brain barrier, such as Oxymetazoline and thus explore various types of α2-agonists. Thus adult spinal cats implanted with an intrathecal cannula may serve as a unique model where the effects of different pharmacological agents on locomotion can be studied.

Although the importance of noradrenergic system in inducing and modulating locomotion in spinal animal was established, relatively little information is available on the specificity of the receptors involved in mediating these locomotor effects. Although both β and α noradrenergic receptors are present in the spinal cord (Nicolas et al. 1993; Timmermans and van Zwieten 1982), α noradrenergic receptors have been shown in previous studies using DOPA or Clonidine to be involved in triggering locomotion, and thus would be the focus of this paper.

The α noradrenergic receptors are broadly subdivided into the α1- and α2-subtypes. The 2 subtypes of noradrenergic receptors have been reported to mediate different functions. For example, it has been found that activation of α1 receptors facilitate the flexor reflex whereas activation of α2 receptors appears to mediate inhibitory effects in acutely spinalised rats (Sakitama 1993). Clonidine acts primarily on the α2-adrenergic receptor (Timmermans and van Zwieten 1982;

Ruffolo and Hieble 1994; Marshall, 1983). It was shown that Clonidine was capable of stimulating central noradrenaline receptors in acute spinal rats suggesting the role of α2-receptor (Anden et al. 1970). So far, Clonidine remained the noradrenergic agonist most widely used to induce, ameliorate and modulate locomotion in acute or chronic spinalised cats (Forssberg and Grillner 1973; Barbeau and Rossignol 1991; Rossignol et al. 1995). Little is known about the effects of other α2-adrenergic receptors or effects mediated by α1-adrenoceptors.

The purpose of this study was to explore the functional role of α1- and α2-adrenoceptors in the initiation and modulation of locomotion and cutaneous reflexes following spinal cord transection in chronic spinal cats. As spinalisation removed all pre-synaptic receptors by removing all descending noradrenergic terminals, the receptors activated are presumed to be located post-synaptically. To compare with Clonidine, other selective α2-agonists, Tizanidine and Oxymetazoline were used and Yohimbine was injected in some cases to antagonize their effects. Methoxamine, a selective α1-adrenoceptor agonist (Marks et al. 1990) and Prazosin, a selective blocker were also studied. Finally, Noradrenaline itself was injected.

A more detailed understanding of the noradrenergic drugs, and their actions mediated by different receptors, is important to enhance our ability to optimize the therapeutic use of drugs in patients with spinal cord injury and potentially better target the pharmacotherapy to offset more specific locomotor deficits.

Methods

Five adult cats were used for this study. They were trained to walk on a motor driven treadmill belt and could walk continuously for at least 15 min at 0.3-0.4m/s. Following training, they were chronically implanted with EMG electrodes and an intrathecal catheter. In two cats, nerve cuffs electrodes were also chronically implanted on the superficial peroneal nerve. Once baseline recordings of the intact locomotion were made, cats were spinalised.

Surgical procedures

All surgeries were performed in aseptic conditions. Cats were anaesthetized with intravenous pentobarbital (Somnotol, 35mg/kg). Additional doses of barbiturates (3-5 mg/kg i.v.) were given as needed throughout the surgery to ensure that the animal remained deeply anesthetized. Lactate-Ringer solution was continuously given through an i.v. line during surgery. The body temperature was constantly monitored with a rectal thermometer and controlled by placing the cat on a heating pad. All procedures followed a protocol approved by the Ethics Committee of Université de Montréal.

1) Intrathecal catheterization. The intrathecal cannulation technique was adapted from the procedure of Espey and Downie (1995). A length of Teflon tubing (24LW) was connected to a cannula connector pedestal (Plastic One Inc.) covered with a dust cap. The tip of the catheter was perforated with a few holes on the side to ensure drug infusion. Prior to insertion, the catheter was filled with sterile saline and the dead space of the catheter was measured (~100 µl). With the cats's head secured in the stereotaxic frame, a midline incision was made from the cranium to C2-3 level. One end of the catheter was secured on the skull with acrylic cement as a port of entry while the other end was inserted through an opening in the cisterna magna down to approximately L4-L5 (Fig. 1). In one spinal cat (CC4), X-rays were taken at different times following the injection of an radio opaque dye into the cannula and showed that the tip of the cannula was located at L5, and that the diffusion of the radio opaque material was localized within the lumbosacral region.

Following the catheterization, the cannula was flushed daily with 100 µl sterile saline to prevent blocking. The location of the tip of the catheter was identified during post-mortem examination and is listed for all cats in table 1. Post-mortem examination also revealed that the cannula can leave an imprint on the cord. This compression did not, however, produce any apparent locomotor deficits in our cats since they all walk well following the implantation.

2) Implantation of EMG electrodes. Detailed description have been made elsewhere (Chau et al. 1998). Three cats were implanted with two 15 pin-head connectors (TRW Electronic Components Group) while 2 cats were implanted with only 1 connector secured to the cranium using acrylic cement. Seven or 14 pairs of the Teflon insulated stainless steel wires (previously soldered to the head connectors) were passed subcutaneously to small incisions made overlying the selected hindlimb muscles (Fig. 1). A pair of the stainless steel wire was sewn into the belly of each muscle. Prior to insertion, a small portion of the Teflon coating was removed from the Teflon-insulated stainless steel wires to be inserted in the muscle. Unpaired wires from the last pin of each connector, were placed under the skin of the neck to serve as an electrical ground. Bilaterally implanted muscles include Iliopsoas (Ip), a hip flexor; Sartorius (Srt), a hip flexor and knee extensor; Semitendinosus (St), a knee flexor and hip extensor; Vastus Lateralis (VL), a knee extensor; Gastrocnemius Lateralis (GL), an ankle extensor and knee flexor; Tibialis Anterior (TA), an ankle flexor. Electrodes were also inserted unilaterally into Gluteus medius (Glu), a hip abductor and extensor and in Gastrocnemius Medialis (GM), an ankle extensor and knee flexor.

3) Implantation of nerve cuff electrodes

In 2 cats (CC5, CC7), bipolar cuff electrodes (Julien and Rossignol 1982) (~1cm length) were used to stimulate the superficial peroneal nerve (~6 mm between electrodes leads). A 2-pin head connector, soldered with a pair of Teflon-

insulated stainless steel wires was used. The stainless steel wires were lead to the site of implantation subcutaneously. Using a custom-made apparatus, a u-shaped nerve cuff was made from polymer (Caulk Dentsply International Inc.). The wires were anchored to the nerve cuff and the small portion of stainless steel wires inside the cuff was cleared of the Teflon-insulation. The superficial peroneal nerve was placed in the cuff followed by absorbable gelatin sponge (Sterispon) soaked with saline solution to prevent damages related to secondary swelling and was completely sealed off using polymer.

4) Spinalisation. A laminectomy was performed at the Th13 vertebra. The dura was carefully removed, a few drops of Xylocaine (2%) was placed on the spinal cord and then a few injections (0.1-0.2cc each) directly into the spinal cord at the level of transection area were made. The exact location of the intrathecal cannula was first identified to avoid causing any damage to the cannula, then the spinal cord was completely severed progressively using micro scissors. The spinal canal could be clearly visualized and an absorbable hemostat (Surgicel, oxidized regenerated cellulose) was used to fill the space between the rostral and caudal ends of the spinal cord. The completeness of the spinal transection was later confirmed with histological analysis (10 micron sections using the Kluver-Barrera method).

Post-operative Cares

All animals were placed in an incubator immediately after surgery and monitored closely. Once the animals regained consciousness, they were placed in individual cages (104cm X 76cm X 94cm) with food and water. Torbugesic (Butorphenol tartrate, 0.05mg subcutaneously, every 6 hours) was given in the first post-op day to reduce discomfort. Spinal cats were placed in cages lined with foam mattresses and were attended to a few times daily to maintain the cleanliness of the head connectors, to flush the intrathecal cannula with sterile saline, to express the bladder manually, to inspect and clean the hindquarters. All our spinal cats remained very healthy and were kept for a period of 2-9 months (an averaged of 6 months) after spinalisation.

Recording procedures and protocol

A few days following the intrathecal catheterization and the implantation of EMG electrodes and/or nerve cuff electrodes, cats were placed on the treadmill to record locomotion. This served as the baseline controls (the *intact* trials). Following spinalisation, before drug injection (*pre-drug* trials) and at different intervals after each intrathecal drug injection (*post-drug* trials), locomotion and responses to mechanical and cutaneous stimulation were recorded.

Experiments were made at 2 stages after spinalisation. At the early stage (~1 week or so) after spinalisation when there was no spontaneous treadmill locomotion yet. These cats are referred to as *early-spinal cats*. With time and training, spinal cats are capable of attaining a well coordinated locomotor pattern with full weight support and plantar foot placement without drug injection (Barbeau and Rossignol 1987; Chau et al. 1998). These cats will be referred to as *late-spinal cats*.

Drug injections

The different noradrenergic drugs used in these experiments are the neurotransmitter noradrenaline (NE) (4-(2-amino-1-hydroxyethyl)-1,2-benzenediol) from RBI, α1- agonist methoxamine (α-(1-aminoethyl)-2,5-dimethoxybenzenemethanol) from RBI, α2-agonists including Clonidine (2,6,-dichloro-N-2-imidazolidinylid-enebenzenamine) from Sigma, Oxymetazoline (3-[(4,5-Dihydro-1H-imidazol-2-yl)methyl]-6-(1,1-dimthylethyl)-2,4-dimethylphenol) from Sigma, and Tizanidine (5-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-2,1,3-benzothiadiazol-4-amine) from Sandoz pharmaceuticals. In 2 cats (CC6 and CC8), an α2-antagonist, Yohimbine ((16α,17α)-17-Hydroxy yohimban-16-carboxylic acid methyl ester) from RBI and an α1-antagonist, Prazosin (1-(4-Amino-6,7,-dimethoxy-2-quinazo-linyl)-4-(2-furanylcarbonyl)piperazine) from Pfizer were used. The range of doses given during experiments was 4.9-12mM for NE, 2.0-8.0mM for Methoxamine, 0.4-4.0mM for Clonidine, 1.7-3.4 mM for Oxymetazoline, 1.0-3.9mM

for Tizanidine, and 2.6mM for both Yohimbine and Prazosin. All drugs were dissolved in sterile saline solution except Prazosin which was dissolved in 20% dimethyl sulfoxide (DMSO), 40% distilled water, and 40% saline. Drugs were injected as a bolus into the spinal cord through the intrathecal cannula. Most bolus injections were of 100 µl, but sometimes cumulative doses were given with injection volumes ranging from 25 µl to 200 µl per dose. Following each drug injection, a subsequent bolus injection of saline (~100 µl) was made to fill the dead space of the cannula and to ensure infusion of the drug into the intrathecal space of the spinal cord. The limit of volume given in one session was ~600 µl.

Locomotion

During the control period, locomotion at different speeds was recorded while the cats walked freely on the treadmill belt. Following spinalisation, the forelimbs of the spinal cat were placed on a platform (~2 cm above the treadmill) and locomotion of the hindlimbs was recorded (see Fig. 1). A Plexiglas separator (not shown) was put between the hindlimbs to prevent crossing of the hindlimbs resulting from increased adductor tonus often seen in spinal cats. In the early period post-spinalisation, the experimenter lift the tail of the cat to support the weight of the hindquarters of the cat and to provide equilibrium. With time, the cat was able to walk with complete weight support of the hindquarters and the experimenter only held the tail to provide equilibrium of the hindquarters.

The EMG signals were differentially amplified (Bandwidth of 100 Hz to 3

KHz). Twelve channels were recorded with a video recorder (Vetter Digital, model 4000A PCM recording adapter) with a frequency response of 1.2 KHz per channel.

Video images of the locomotor movements were captured by a digital camera (Panasonic 5100, shutter speed 1/1000s) and recorded on a video recorder (Panasonic AG 7300). For every recording session, reflective markers were placed on the bony landmarks of the left hindlimb facing the camera: the iliac crest, the femoral head, the knee joint, the lateral malleolus, the metatarsal phalangeal (MTP) joint and the tip of the 3rd toe (see Fig. 1). Additional markers were also placed either on the treadmill frame or on the trunk of the cat for calibration (10cm).

The kinematic and the EMG data were synchronized by means of a digital SMPTE (Society for Motion Picture and Television Engineers) time code. The time code was generated by a Skotel time code generator (model TCG-80N) and was simultaneously recorded on the EMG tape and on one audio channel of the VHS tape and was inserted as well into the video images.

Electrical Stimulation

Single pulse of 250 μs duration was delivered (Grass S88 stimulator) at 0.4-0.5 Hz through the cuff electrodes. The stimulation was given either at rest, standing or sitting. The stimulus signal was displayed on an oscilloscope together with selected EMGs. The threshold (T) of the stimulation was determined by observing a just detectable response in St at rest.

Mechanical stimulation

Mechanical stimuli were delivered by tapping the dorsum of the paw with a custom made hand-held tapper during the swing phase of locomotion. The tapper has a microswitch attached to indicate the moment of contact with the surface of the dorsum of the paw. The pulse generated by the switch was recorded on tape and also triggered a light emitting diode (LED) recorded on the video tape. The stimulus was applied randomly during the swing phase of locomotion but not exceeding once every three step cycles.

Fast Paw Shake (FPS)

To elicit a fast paw shake, the experimenter held the cat in the air and then dipped the paw into a bowl of lukewarm water. While both limb movements and EMG signals were recorded, only the EMG signal was analyzed for FPS.

Data Analysis

Video images were digitized using 2D PEAK Performance system (Peak Performance Technologies Inc., Englewood, CA). Displacement data, encoded by the X and Y coordinates of different joint markers were measured at 60 fields per second (i.e. a temporal resolution of 16.7ms). From these X-Y coordinates angular joint movements were calculated and could be displayed as continuous angular

displacements (running averages of 5 values) for a normalized step cycle or as stick diagrams. Each stick figure was also displaced from the previous one by the distance traveled by the foot so that the horizontal axis is twice that of the vertical axis. The distance between stick figures is also proportional to the velocity of the movement.

EMG data during locomotion were played back on an electrostatic polygraph (Gould, Model ES 1000) and a typical record of the animal's performance before and after drug injection was selected for analysis. The EMG signals were digitized at 1 KHz. Using a custom-made software, the onset and offset of bursts of activity were detected first automatically, and then corrected manually if needed. The EMGs were then rectified and, using St as the onset of the cycle (occasionally Srt), the EMGs were averaged over a number of cycles. The duration and amplitude of the muscle bursts were measured from individual records. The mean amplitude was calculated as the integral of the rectified EMG burst divided by its duration.

EMG responses to the electrical stimulation was digitized at 1 KHz, and computer averaged. Quantitative measures of the responses (amplitude and latency) were obtained using custom made software which integrate the region that was consistently greater or less than the mean prestimulus period by 2 standard deviations. For mechanical stimuli, the individual EMG responses to stimulation were shown before and after drug injection.

Results

The results reported here are from experiments in the 5 spinal cats in which different drugs were injected on different post-spinal days as summarized in table 1. All analyzed trials at different days are underlined. Even though some trials were not analyzed quantitatively, the videotapes and EMG data were always reviewed to verify the similarities or differences of drug effects in different spinal cats. While a range of doses has been tested, the analyses reported here refer mainly to trials where a dose of 3-4mM in a bolus of 100 μl was used for all α1- and α2-agonists which seems to produce optimal locomotor effects. For the antagonists, doses of 2.5-2.6mM were effective. In the case of NE, a higher dose, up to 12mM was sometimes required. The effects of a drug on locomotion were evaluated at 2 stages post-transection, at an early stage (early-spinal) when there was no spontaneous locomotion (i.e. <8 days), and at a later stage when the locomotor pattern was already established (late-spinal) prior to any drug injection. In all early-spinal cats, usually within the first week post-transection, no well-organized sustained locomotion can be elicited before drug injection. The ability of the different noradrenergic agonists to initiate locomotion could then be tested.

Initiation of locomotion in early-spinal cats

Effects of α2-noradrenergic agonist (Clonidine, Tizanidine, Oxymetazoline)

The ability of Clonidine, a well known α2-noradrenergic agonist, to trigger locomotion was consistently confirmed here in 4 spinal cats and is shown in Fig. 2. In this 8d-spinal cat (CC7), while there was no locomotion during the pre-drug trials (Fig. 2B), almost immediately (within 2 minutes) following Clonidine injection (3.8mM i.t. injected as a bolus of 100 µl) a well-organized locomotor pattern was observed (Fig. 2C). There was a marked increase in stance and swing duration comparable to the intact locomotion (Fig. 2A) as shown in the stick figures. This remarkable quasi instantaneous action of i.t. Clonidine was also seen in another spinal cat (CC4) in which locomotion was triggered within 3 minutes after injection. The Clonidine-elicited locomotion can be characterized as: 1) readily triggered requiring only a light touch to the perineum, 2) adaptable to treadmill speeds up to 1.0m/s, 3) sustained i.e. the cat could walk consistently 15-20 minutes at a time for a period of 2-3 hours, 4) the effects last for about 5 hours. While the Clonidine-elicited locomotion resembled the intact locomotion in many respects, there were also some distinct characteristics. For example, a knee sag was often observed towards the end of stance as noted in the stick figures (the iliac and hip markers are sloping downwards towards the end of the stance phase which is not normally seen before spinalisation (Fig. 2A)). Another distinct characteristic consistently observed after

clonidne was a pronounced foot drag during the initial swing phase (Fig. 2C) which was often followed by a greater elevation of the foot at the end of swing before putting down the foot.

Other α2-noradrenergic agonists, Tizanidine (n=2) and Oxymetazoline (n=2) were also capable of initiating locomotion in early spinal cats within the first week post transection. In Fig. 3, the stance, swing and cycle duration as well as stance length (measured from kinematic values) in a 3d, 4d and 8d spinal cat following injection of Clonidine, Oxymetazoline and Tizanidine, respectively, as well as a 8d spinal cat following injection of NE are shown. The values are expressed as a percentage of intact locomotion since the cats were not walking at this early stage post-transection. The three α2-agonists triggered locomotion similarly by increasing the step cycle duration, especially the swing duration. The locomotion initiated by NE was not as good as that triggered by the α2-agonists. For example, the stance length was 64% of the intact locomotion after NE as compared to the 99% following Oxymetazoline injection. As shown in table 2, while the cycle duration of the spinal locomotion was very small before Clonidine injection at 3, 5 and 8d post transection in CC4, CC5, and CC7 respectively, it was 113%, 92%, and 135% of intact values within minutes after Clonidine injection. Similarly, following Oxymetazoline injection in CC8 at 4d post-transection (3.4mM i.t.), the cycle duration was increased to 129% of intact locomotion. The ability of the cat to adapt its locomotion to increasing treadmill speed was also similar among the α2-agonists. The maximum speed the

early-spinal cats can achieve following injection of Tizanidine (n=2), Oxymetazoline (n=1) and Clonidine (n=2) injection was 0.6-0.7m/s, 0.8m/s, and 0.9-1.0m/s, respectively.

The concomitant EMG changes are also listed in table 2. Following all three α2-agonists, there was an increase in the knee flexor St activity, relatively more than that of the extensor activity which contribute to the marked increase in the swing duration. For example in CC4, following Clonidine injection, iSt burst duration was 247% of intact, while the extensors iGL, iVL, were 109 and 102% of intact. Similar results were observed in CC7 following Clonidine injection. Following Tizanidine injection in CC6, the burst duration of iSt was also much more augmented (230% of intact) than that of the extensors iGL, and iVL, which are 138% and 126% of intact. Following injection of Oxymetazoline, in CC8 the coSt burst duration (not shown) was also 319% of the intact locomotion as opposed to iGL and iVL which are 95 and 122% of intact, respectively. In conclusion, α2-agonists appeared to have a more potent effects on the hindlimb flexors muscles.

Although the three α2-agonists were similar in their ability to trigger locomotion in early-spinal cats, differences in the evoked locomotor pattern were seen. While Tizanidine resembled closely the effects of Clonidine, the kinematics of the locomotor pattern triggered by Oxymetazoline was different from that of Clonidine. For example, the increase in hip flexion was more marked after Oxymetazoline injection as compared to Clonidine, and the corresponding hip joint angular excursion was 144% and 87% of intact, respectively. The foot drag was

also much more exaggerated after Clonidine injection than Oxymetazoline. The ankle joint angular excursion of Clonidine- and Oxymetazoline-evoked locomotion were 197% and 137% of intact, respectively. The ability of the cat to support the weight of the hindquarter was good after Oxymetazoline injection as compared to Clonidine. For example, the knee sag, often observed after Clonidine was not observed with Oxymetazoline.

The time course of action among the three α2-agonists was also different as shown in Fig. 4. The locomotor effects were evaluated by measuring the stance duration at a speed of 0.6m/s. Within 5 minutes after Clonidine or Tizanidine injection, the cats was able to walk at 0.6m/s (Fig. 4A). Oxymetazoline, on the other hand, had a much slower onset, taking hours instead of minutes to reach the maximal locomotor effects (Fig. 4B, note that the time scale is different from Fig. 4A). The cat was unable to walk at 0.6m/s at 30 minutes or 2 hours after injection, however, when recording was made on the following day, a marked increase in the locomotor ability can be seen.

The duration of effects exerted by the three α2-agonists was also different. Following Tizanidine injection, the cat was unable to walk at 0.6m/s after 2.5 hours whereas following Clonidine injection, the cat was still able to walk at this speed even at 4.5 hours, an ability which only diminished at 6.5 hours after injection. The effects of both Clonidine and Tizanidine completely disappeared on the following day. With Oxymetazoline, however, locomotion at 0.6m/s could be maintained for at least 2 days after drug injection. A marked reduction in locomotion was seen by

the third day post-injection where the stance duration decreased by 40%. We do not, however, know the time it takes for the effects of Oxymetazoline to completely wear off.

The differences in the time course of action and locomotor pattern were consistently seen in all experiments, in all spinal cats both during early- and late-spinal period (see table 3).

Effects of an α1-noradrenergic agonist (Methoxamine)

The ability of Methoxamine to trigger locomotion in early spinal cats was much more inconsistent and different from the α2-noradrenergic agonists. We have tested the effects of Methoxamine in 3 early spinal cats, all of which had no locomotor activity prior to drug injection.

In 2 cats (CC5 and CC7), there was a significant increase in the ability of the cats to stand on a stationary surface, and to a varying degree, an increase in stepping movements following methoxamine injection. For example, in CC5, 90 minutes after Methoxamine injection (Fig. 5B), there was an attempt to increase stepping, as seen in the EMG traces. In another cat (CC7), 15 minutes after Methoxamine injection, while the increase in stepping ability was more pronounced (stance length was 88% of intact), it was never as convincing as that observed with α2-agonists. For example, the cats were unable to walk consistently (at least 10 consecutive step cycles) with weight support of the hindquarters or walk beyond

0.2m/s. In both spinal cats, however, transient bouts of nice locomotor activity (0.2m/s) with good weight support and an increase in the amplitude of EMG activity in flexors and extensors could be triggered with time (table 2). Figure 5C shows an example of bouts of locomotion (CC5) observed at 5 hour 40 mins after Methoxamine injection. With strong perineal stimulation, this cat was able walk with good weight support and plantar foot placement up to 0.2m/s. The cycle duration and stance length increased to 87% and 76% of the intact, respectively (see table 2). This was the maximal effects observed in this cat and is in sharp contrast with the effect of Clonidine given to this cat 2 days later (5d post-transection) (Fig. 5D). Sustained organized locomotion (0.4m/s) with large steps, weight support and foot placement was readily observed at 1 hour 30 mins post-injection of Clonidine requiring only minimal perineal stimulation.

Thus, from the observations of these 2 cats (CC5 and CC7), Methoxamine appeared not to be as effective as the α2-agonists in triggering locomotion. Despite an increase in stepping movements and bouts of organized locomotion with weight support, the cats were never able to walk beyond 0.2m/s.

However, contrary to the above observations, Methoxamine was found to be effective in triggering locomotion in another spinal cat (CC6) at 4 days post-transection as shown in Fig. 6. Before Methoxamine injection, no walking could be triggered on the moving treadmill belt even with strong perineal stimulation (Fig. 6B). Three hours after injection (Fig. 6C), the locomotor pattern significantly

improved and was robust requiring only minimal perineal stimulation. The cat was able to walk with plantar foot placement, support the weight of the hindquarters, take large steps and adapt to treadmill speed up to 0.4m/s. The step cycle duration and stance length at 0.4m/s were 101% and 89% of intact, respectively. This locomotor ability persisted till the following day (5 days post-transection). Thus, it appears that an α1-agonist, Methoxamine, was also capable of initiating locomotion at least in this cat at an early stage post-spinalisation.

In the same cat, 2 days later, injection of an α1-noradrenergic antagonist, Prazosin, was found to be effective in blocking the effects of Methoxamine on locomotion as shown in Fig. 7. Within 30 minutes following injection, there was no plantar foot placement, and instead, the cat continually struck the treadmill with the dorsum of the paw and was no longer capable of supporting its weight during locomotion. The step cycle duration and stance length decreased to 45% and 29% of intact, respectively, as compared to the corresponding values of 105% and 118% of intact before Prazosin injection. The effects of Prazosin appears to wear off by 1 hour 45 minutes following injection.

Thus, it suggests that the effects on locomotion seen in this cat (CC6) could be attributed to the effects mediated by noradrenergic α1-receptors.

Differences in locomotion induced by the α1- and α2-agonists

The locomotor pattern triggered by the α1-noradrenergic agonist, Methoxamine, differed from that evoked by α2-noradrenergic agonists such as Clonidine. In the α2-induced-locomotion, an exaggerated foot drag at the onset of swing was a consistent observation (Fig. 2C, Fig. 5D); in contrast, in the α1-induced locomotion, there was no foot drag at the onset of swing (Fig. 5C, Fig. 6C). In the Methoxamine-induced locomotion, the weight support of the hindquarters was also much better than the Clonidine-induced locomotion. This is partially reflected by the absence of knee sag in the Methoxamine-induced locomotion (Fig. 5C, Fig. 6C) as compared to the Clonidine-induced locomotion (Fig. 2C, Fig. 5D). As shown in the stick diagram (Fig. 6C), the iliac and hip trajectory remained leveled and no knee flexion was seen at the end of stance, both of which were often observed after Clonidine injection (Fig. 2C, Fig. 5C). The extensor activity of hindlimb muscle was also much increased after Methoxamine injection as compared to after Clonidine injection. Following Methoxamine injection in CC5 and CC6, at 3d and 4d post-spinalisation, the VL amplitude were 150% and 190% of intact, and the GL amplitude were 161% and 163% of intact, respectively. Following Clonidine injection in CC4 at 3d post spinalisation, the amplitude of VL and GL was 97% and 117% of intact respectively. Also, in the Clonidine-induced locomotion (Fig. 2C), the activity of proximal muscle such as the hip flexor, Srt, was well-organized (Fig. 2C, Fig. 5D). In the Methoxamine-induced locomotion, no organized Srt bursting activity

can be seen at this stage, but evolved with time (Fig. 6C, Fig. 8C).

The initial ability of the early-spinal cats to follow the maximal treadmill speed was also different. After Methoxamine injection, the maximum treadmill speed that the cat CC6 (4d) can follow was only at 0.4m/s as compared to Clonidine injection, where the maximum speed the cats CC4 (3d) and CC8 (3d), can follow was 0.8 and 0.6m/s, respectively. However, with time (6d post transection), CC6 was also able to adapt to 0.6m/s after Methoxamine injection. Finally, the time course of actions are also different. The effects of α1-agonist, Methoxamine, were much longer lasting as compared to the α2-agonist Clonidine and Tizanidine with the exception of Oxymetazoline which also produced long lasting effects.

Therefore, differential effects were observed with α1- and α2-agonist with respect to the locomotor pattern, EMG activity, the weight support ability and the time course of action.

Effects of noradrenaline

Noradrenaline was also found to be capable of triggering locomotion in the early spinal cat (CC8). Figure 8 shows the locomotor pattern of the 8d-spinal cat (CC5) cat during intact, pre-drug and post-drug period. There was no locomotion prior to drug injection (Fig. 8B). Locomotion with plantar foot placement began at 40 minutes following NE injection (not shown) (12mM i.t.). The pattern was

transient, and with time it became more robust, and by 1 hour, the cat was able to walk with plantar foot placement and weight support of the hindquarters (Fig. 8C). The raw EMG traces showed alternating bursting between the different hindlimb flexor and extensor muscles. The locomotion was characterized by steps shorter than in the intact. The step cycle duration and the stance length of the NE triggered locomotion were 58% and 72% of the intact locomotion, respectively. Thus, it appears that while NE readily triggered robust locomotion in early-spinal cats similar to α2-agonists, the effects were less potent than α2-agonists as shown in Fig. 3. In addition to exerting partial α2-effects, there was also no foot drag or knee sag observed in the NE-induced locomotion which also resembled the effects observed with α1-noradrenergic agonist. It appears then that mixed α1- and α2-effects could be evoked by NE as could be expected.

Modulation of locomotion parameters in late-spinal cats

In late-spinal cats, when the cat was capable of spontaneous locomotion, the ability of these drugs to modulate the already established locomotor pattern and their effect on cutaneous reflex excitability was assessed. The cutaneous reflex excitability of the hindlimbs was assessed by the response to mechanical and electrical stimulation as well as fast paw shake (FPS). The effects of the drugs on

locomotion and cutaneous reflex in all spinal cats and different experimental trials are summarized semi-quantitatively in table 3.

The modulatory effects of α2-agonists (Clonidine, Tizanidine, Oxymetazoline)

All 3 α2-agonists, Clonidine (12 injections), Tizanidine (7), and Oxymetazoline (7) were found to modulate the locomotor pattern in a similar fashion (table 3). Figure 9 shows an example of the effects of Tizanidine on locomotion in a 157d spinal cat (CC8). Before any drug injection, the locomotor pattern was well established with full weight support and plantar foot placement (Fig. 9A). Thirty minutes following the injection of Tizanidine (cumulative dose 4.8mM), there was a marked increase in the step length (117% of pre-drug) as shown in the stick figures (Fig. 9D), and an increase in the angular excursion in all joints, in particular the knee and ankle joint, as shown in the joint angle plots (Fig. 9E). An exaggerated foot drag at the onset of swing, resulting from an inadequacy to clear the ground during foot lift, was also observed as shown in the stick diagrams. The amplitude and duration of the flexor (Ip, Srt) muscles were increased which may contribute to the increase in swing duration. The duration of the extensor (GM and VL) bursts was also increased contributing to an increase in stance duration after Tizanidine injection, however, the amplitude of the ankle extensors GM was decreased (Fig. 9F) which might partially explained the decrease in weight support of the

hindquarters. The fact that these locomotor effects of Tizanidine were mediated by α2-adrenoceptors was further supported by the ability of Yohimbine, an α2-adrenoceptor antagonist, to block the effects (Fig. 9G). As soon as 15 minutes following Yohimbine (2.5mM i.t.) injection, there was a decrease in step length. The cycle duration decreased by 10% of the pre-drug trial, the swing duration decreased by 20% of the pre-drug trials, the weight support increased with a corresponding increase in the ankle extensor GM activities (26% of the pre-drug trials), and the exaggerated foot drag disappeared.

Although the three α2-agonists (Clonidine, Tizanidine, and Oxymetazoline) modulated the cycle duration and step length of locomotion of late-spinal cats similarly, some differences were noted (see table 3). For example, the weight support ability was more affected following Clonidine injection as compared to Oxymetazoline and Tizanidine injection. The decrease in weight support ability was seen in 75% of trials tested with Clonidine whereas the weight support ability was largely unchanged after Oxymetazoline injection. Three out of 7 trials (42.8%) after Tizanidine reported a decrease in weight support ability. Also, the degree of side effects produced by these α2-agonist in late-spinal cats were different. Both Clonidine (3.8mM i.t.) and Oxymetazoline (3.4mM i.t.) often produced some side effects (vomiting, dilated pupil, lethargy) but Tizanidine never did (4.7mM i.t.). These observations were consistently seen in 4 spinal cats.

The modulatory effects of Methoxamine

Figure 10 shows an example in CC6 of a Methoxamine injection alone (Fig. 10D,E,F) followed by a superimposed injection of Clonidine (Fig. 10G,H,I) allowing us to describe the modulatory effects of the combination of an α1-agonist and an α2-agonist.

Following Methoxamine injection (4mM i.t.), there was a marked increase in the extensor tonus of the hindlimbs and the joints appeared stiffer when the cat was standing (not shown). There was also some obvious spontaneous movements of the tail which were absent before. However, Methoxamine did not significantly modulate the well established locomotor pattern in late-spinal cats (11 injections) (table 3). As seen in the stick diagrams (Fig. 10D), there was no apparent increase in stance or swing duration as compared to the pre-drug trials. There was no foot drag nor knee sag at the end of stance (both are commonly seen with α2-agonist). Also, there was little differences in the angular excursion before and after Methoxamine injection (Fig. 10B, E). There was, however a marked increase in the EMG amplitude of the knee and ankle extensors, VL and GL (163% and 130% of the pre-drug values), respectively, and the proximal hip extensor, Glu (increased 5 fold). The burst duration of the Glu was also increased to 210% of the pre-drug value. The overall increase in the extensor muscle activity could contribute to the increased extensor tonus and resulted in a more rigid posture with extended hindlimbs.

Figure 10G,H,I show the combined effects of Methoxamine and Clonidine on locomotion in the same cat. Clonidine was injected to the same cat within 2 hour 47minutes of the Methoxamine injection, after marked effects of Methoxamine were obtained. Ten minutes following Clonidine injection, the stance and swing duration increased to 119% and 143%, respectively, of the pre-Clonidine values (Fig. 10G). While an exaggerated foot drag during the initial swing period was seen, there was no knee sag at the end of stance as often observed after Clonidine. This may be related to the increased extensor tonus. In addition, burst duration of flexors such as St and coSt were increased to 282% and 176% of the pre-drug value. The amplitude of the extensor muscle such as iVL and iGL although slightly decreased as compared to after Methoxamine injection, remained high at 131% and 100% of the pre-drug values, respectively. The proximal hip extensor, Glu burst amplitude and duration also remained high at 637% and 189% of the pre-drug value, respectively.

Thus, the resultant locomotor pattern showed a summation of effects mediated by both α1- and α2-agonists.

The modulatory effects of noradrenaline

The NE-modulated locomotor pattern also resembled (6 injections) the combined effects of α1- and the α2-agonists (Fig. 11J,K,L). Twenty three minutes after NE injection (4.9mM i.t.) there was a significant increase in the stance and

swing duration (Fig. 11J) similar to that seen with α2-agonist, Tizanidine (Fig. 11B) injected in the same spinal cat (CC7) at a different post-spinal day. This is in contrast with the α1-agonist, Methoxamine, where are no significant changes in the stance and swing duration was seen (Fig. 11C, Fig. 10D). An exaggerated foot drag during initial swing was also observed in both Tizanidine- and NE- induced locomotion but not in Methoxamine- induced locomotion. Also similar to Tizanidine-modulated locomotion (Fig. 9E, Fig. 11D), the angular excursions of all joints, in particular, the knee, ankle and MTP joint angular excursion were increased significantly following NE injection (Fig. 11K). These observations were consistently seen in 3 different spinal cats. Normalized EMG showed that following NE injection, the knee flexor St burst duration and amplitude increased to 139% and 143% of the pre-drug value, resembling the Tizanidine-induced locomotion. On the other hand, the NE-modulated locomotion also resembled the α1-modulated locomotion in some respects. For example, there was no knee sag at the end of stance (Fig. 10D, Fig. 11G). Also, the amplitude of the knee and ankle extensors increased to 287% and 229% of the pre-drug value, respectively, (Fig. 11L), which was similar to the Methoxamine-modulated locomotion (Fig. 10F).

Figure 12 summarizes the percentage change of the step cycle, stance and swing duration in all cats following injection of α2-agonists, one α1-agonist and noradrenaline. Similar effects were observed in the three α2-agonists (Fig. 12A,B,C). The increase in step cycle duration ranges from 20-40% of the pre-drug

trials, the increase in swing duration ranges from 30-80% of the pre-drug trials, whereas the increase in stance duration ranges from 10-40% of the pre-drug trials. Thus α2-agonists increased the swing duration more than the stance duration. Similar to the α2-agonist, the increase in swing duration following NE injection ranges from 116-166% of the pre-drug trials in 3 experiments done in spinal cats CC7 and CC8.

In contrast, following Methoxamine injection (Fig. 12D), there was very little changes in all 3 cats with respect to the step cycle duration, stance and swing duration as compared to the α2-agonists. However, the α1-agonist Methoxamine exerted marked effects on increasing the tonus and the weight support of the hindquarters of the cat, possibly by increasing the amplitude and duration of extensor muscles especially the proximal hip extensor such as Glu. The effects on locomotion after injection of NE resembled a combined effects of α1- and α2-agonists.

Modulation of cutaneous reflex excitability in late-spinal cats

In addition to changes observed in the locomotor pattern in cats following drug injection, there were also concurrent changes in the excitability of the cutaneous pathways as seen with mechanical or electrical stimulation, and fast paw shake (FPS). The results obtained from all spinal cats are also summarized in table 3.

Mechanical stimulation

The α2-agonists, Clonidine and Oxymetazoline, markedly reduced or abolished the response to tap in 100% and 67%, respectively, of all trials tested (table 3). Tizanidine, also decreased the response to tap but to a lesser extent; the reflex amplitude was decreased in 50% of the trials but remained unchanged in 50% of the trial.

An example of the response to tap is shown in Fig. 13. Before Clonidine (Fig. 13A), as soon as the tapper touched the paw there was a brisk response, i.e. a rapid knee, ankle, and MTP flexion, shown in the stick figures to clear the obstacle. Note that on the video records, the tapper was seen in contact with the dorsum of the paw in only one frame (2 fields) indicated by one arrow. After Clonidine, the brisk response to tap also disappeared as previously reported (Barbeau et al. 1987). Upon contact with the tapper, the knee failed to flex, instead the paw pushed continuously onto the tapper and eventually, by inertia, the limb continued its trajectory.

In contrast to the α2-agonists, α1-agonist (11 injections) and NE (5) did not reduced the response to tap in any of the tested trials. As shown in Fig. 13B, in the same cat CC4, following Methoxamine, there were no marked changes in the swing duration and the response to tap was still present. NE appeared to exert effects of both α1- and α2-types. As shown in Fig. 13C, there was both a marked increase in the swing duration (resembling the effects of Clonidine) and the cutaneous reflex

remained excitable (resembling the effects of Methoxamine).

Electrical stimulation

While α2-agonists consistently (7 injections) increased the threshold of stimulation or decreased the reflex response to electrical stimulation, α1-agonist (4) and noradrenaline (6) reduced the threshold and increased the reflex amplitude (table 3). Figure 14 shows an example of the activation of flexors and extensor muscles during the electrical stimulation of the superficial peroneal nerve before and after drug injection in the same cat CC7 at rest (standing). After Clonidine injection (Fig. 14A), there was also a marked decrease in the amplitude of the short latency response in the knee flexor St despite a much stronger stimulating current. Before Clonidine, a current of 0.75 mA was sufficient to activate St. Following Clonidine, St was not activated even with a current as high as 3mA, i.e. 4 times the strength used before Clonidine injection.

On the contrary, the short latency response in St was augmented following both NE and Methoxamine (Fig. 14B,C) with the stimulating parameters being kept constant before and after the drug injection.

The decrease in the short latency cutaneous response cannot be explained by changes in the motoneuronal excitability since following Clonidine injection, the hindlimb muscles were still active and the cats were able to walk on the treadmill. Thus, a decrease in the reflex transmission rather than a decrease in excitability

at the motoneuronal level is more likely to contribute to the attenuation of the short latency cutaneous response.

Fast Paw Shake (FPS)

With α2-agonists, FPS was abolished with Clonidine (12 injections), Oxymetazoline (5), and Tizanidine (3) (table 3). Figure 15 shows an example of the FPS before and after drug injection in 3 different spinal cats. In a 62d spinal cat, 15 minutes following Clonidine, the FPS disappeared (Fig. 15A). On the contrary, following Methoxamine (11 injections), the frequency of the FPS was enhanced (10Hz) (Fig. 15B). The frequency range of the FPS was within the range previously reported for spinal cats (Smith et al. 1985; Pearson and Rossignol 1991). Similarly, following NE (3 out of 5 trials), the FPS response was still present. Thus, with NE, while the locomotor effects seemed to resembled the α2-agonist as mentioned before, the cutaneous effects resembled the α1-agonist.

Summary of modulation of the excitability of cutaneous pathways in late-spinal cats

Methoxamine (α1-agonist) and NE consistently increased the cutaneous excitability in 4 spinal cats (see table 3). The three α2-agonists (Clonidine, Tizanidine, and Oxymetazoline) reduced the excitability of cutaneous reflex of late-spinal cats to a different extent. Among the 3 noradrenergic agonists, Clonidine

was the most potent one in reducing the cutaneous excitability. It consistently and markedly increased the threshold of electrical stimulation and it abolished both FPS and responses to tap. Oxymetazoline and Tizanidine, on the other hand, did not reduce the excitability of the cutaneous reflex as markedly as Clonidine (table 3). For example, Tizanidine only reduced the response to tap in 50% of trials tested. It slightly increased the threshold of electrical stimulation in 67% of the trials and did not change the threshold in the remaining 33% of the trials.

Discussion

Summary of the results

α2-noradrenergic agonists (Clonidine, Tizanidine, and Oxymetazoline) and α1-noradrenergic agonist (Methoxamine) appear to have different effects on spinal locomotion. Firstly, α2-noradrenergic agonists were all capable of initiating locomotion in cats within minutes during the first week following spinalisation whereas the α1-noradrenergic agonist was not as effective. In the one case in which it clearly triggered locomotion (CC6), it took at least 2-3 hours before the effects could be seen. Secondly, while all α2-noradrenergic agonists modulated the already well established locomotor pattern in late spinal cats similarly, such as increasing markedly the angular excursion, step cycle duration, in particular, the swing duration, α1-noradrenergic agonist Methoxamine did not. Thirdly, α2-noradrenergic agonists decreased the cutaneous excitability as opposed to α1-agonist which increased the cutaneous excitability. Fourthly, in contrast to the α1-noradrenergic agonist which increased the weight support of the hindquarters, the α2-noradrenergic agonists did not, or sometimes decreased the weight support of the hindquarters. Fifthly, while α2-noradrenergic agonists consistently exaggerated the foot drag seen at the onset of swing and α1-noradrenergic agonists did not. Lastly, some degree of differences in weight support ability and cutaneous reflex modulation were observed among the three α2-agonists. NE, as expected, exerted

both α2 and α1 effects, that is, on one hand, it initiated locomotion in the early stage and prolonged the step cycle (α2 effects) while preserving cutaneous reflex excitability (α1 effect). While NE increased the hindlimb flexor activity (α2 effect), it also increased the extensor activity of the hindlimbs (α1 effect).

Effects of noradrenergic agonists on locomotion

Effects of the Clonidine reported here agree with earlier findings (Barbeau et al. 1987; Barbeau and Rossignol 1991). The ability of NE to initiate locomotion in our study was also consistent with study of others (Kiehn et al. 1992). Our findings also show that other α2 agonists such as Tizanidine and Oxymetazoline can initiate locomotion. Their action is possibly mediated through post-synaptic α2-adrenoceptors as spinalisation resulted in the degeneration of pre-synaptic terminals and receptors, and Yohimbine could block the effects. Although less consistent than the α2-agonist, α1-agonist has been shown to be capable of initiating locomotion in one early spinal cats possibly through the actions on the α1-adrenoceptor since the effect was blocked by Prazosin, an α1-blocker.

The mechanisms on how the specific noradrenergic agonists initiate locomotion is unclear. Our findings suggest that while activation of both α1- and α2-receptor can initiate locomotion, the actions of α1-appeared to be more involved with increasing the output amplitude of the muscle whereas the α2-receptors are more involved with control of the rhythm of the locomotor pattern.

The differential effects of α1- and α2-agonist are more evident in late spinal cats when they were already walking prior to any drug injection. α2-adrenoceptors activation modulated significantly the timing of the muscles, especially the flexors, whereas activation of α1-adrenoceptors modulated the timing of muscle activation to a much lesser extent (Fig. 12). For example, a relatively small dose of Clonidine (0.4mM i.t.) increased the duration of the St by 30% in a cat CC7. In contrast, α1-agonist increased the output amplitude of the extensor muscles to a much greater extent than the α2-agonist. Thus, it is possible that while α2-agonists exert effects primarily on interneurons which coordinate the timing between the flexor and extensor muscles, α1-agonists may act also on motoneurons. The effects of α1-agonist may be similar, to some extent, to our previous work on the modulatory effect of 5-HT agonists or precursor, 5-hydroxytryptophan (5-HTP), which significantly increased the output amplitude of pre-existing muscle activity but failed to initiate locomotion (Barbeau and Rossignol 1991).

Plateau potentials causing long-lasting excitability increase has been reported in motoneurons of cats and turtle (Hounsgaard et al. 1988; Conway et al. 1988; Kiehn 1991). They are induced by L-DOPA, Clonidine, or 5-HTP, and in interneurons in rats (Kiehn et al. 1996) induced by *N*-methyl-D-aspartate (NMDA) and 5-HTP. Plateau potentials are suggested to be of major importance in providing an increase in the gain of motoneuronal activity. These unique active membrane properties have been implicated to be important in generating and shaping motor rhythm. In addition to serotonergic drugs, L-Dopa, and Clonidine have also been

found to induce plateau potential in flexor and extensor motoneurons in spinal cats (Conway et al. 1988). The plateau potentials in motoneurons was reported to contribute to the late long-lasting reflexes observed in spinal cats after L-DOPA injection. While Clonidine was shown to induce plateau potential in motoneurons suggesting the activation of $\alpha 2$ receptors, we cannot exclude the possibility of activation of $\alpha 1$ receptors with higher doses of Clonidine. Till now, there is no information regarding the effects of specific $\alpha 1$-agonist on spinal motoneuron. $\alpha 1$-adrenoceptors, however, have been found to mediate plateau potential in smooth muscles in periphery (Venkova and Krier 1995).

Noradrenergic drugs were also found to exert excitatory effects on spinal motoneuron (White et al. 1991; Ault and Evans 1978) and interneurons (Weight and Salmoiraghi 1966). The facilitation was found to be mediated by $\alpha 1$-receptor as the effects can be abolished by $\alpha 1$-antagonist, Prazosin. Furthermore, in the presence of Prazosin, Clonidine reduced the motoneuronal discharges, which can be antagonized by Yohimbine (Hirayama et al. 1988). Thus, it is suggested that the facilitation and suppression exerted by NE was mediated by $\alpha 1$- and $\alpha 2$-receptor, respectively (for review, see Ono and Fukuda 1995).

In our study, following Methoxamine injection in late-spinal cats, there was an increase in the extensor tonus of the hindlimb, an increase in the stiffness of the joint, and an increase in the amplitude and sometimes the duration of the extensors muscles and a marked increase in weight support of the hindquarters. It is possible that these effects were partially due to the increased level of motoneuronal

excitability mediated by α1-adrenoceptors. Rawlow and Gorka (1986) also reported an increase in the anterior tibialis muscle tonus after the injection of a selective α1-receptor agonist, St 587 in spinal rats. Clonidine, mediated by α2-adrenoceptors, was also found to reduce the excitability of motoneuron and the tonic activity of the hindlimb muscles (Tremblay and Bedard 1986). The depressant effects of Clonidine on motoneurons may partially explain the reduced weight support in our late-spinal cats following Clonidine injection.

In addition to differential effects on locomotion, α1- and α2-agonist was found to affect other spontaneous movements differently. For example, the spontaneous tail movements we observed after Methoxamine injection might be analogous to the "spontaneous tail-flicks" observed in rats by others (Bervoets and Millan 1994) induced by 5-HT1A receptors. They reported that α1-adrenoceptors and α2-adrenoceptors were also found to mediate and inhibit, respectively, the 5-HT1A receptor-induced "spontaneous tail-flick" response in rat lumbar spinal cord.

Localization of α1- and α2-noradrenergic receptors in the spinal cord

The differences in localization of these 2 receptor subtypes within the spinal cord could reflect their different functional roles. α2-receptors are predominantly found at the dorsal horn, substantia gelatinosa, and the intermediate zone, close to the central canal, and α1-receptors are found also in high density in the motoneuron area in the ventral horn (Pascual et al. 1992; Roudet et al. 1994; Roudet et al. 1993;

Giroux et al. 1995). Spinal α1-receptors were also found to become supersensitive after deafferentation suggesting that the receptors are located post-synaptically to NE fibres or terminals (Roudet et al. 1993). The presence of α1-receptors in the hindlimb motoneuronal area may indicate the importance of α1-receptors in modulating the motoneuronal excitability whereas the predominant localization of α2-receptor in the dorsal horn may explain their role in reducing the excitability of the cutaneous reflex.

Effects of noradrenergic agonists on cutaneous excitability

In this study, while Clonidine reduced the excitability of the cutaneous pathways, Methoxamine and NE enhanced it (increase in the response to tap, FPS and a decrease in threshold for electrical stimulation). The FPS response, a high frequency synchronous activity of flexor and extensor muscles was also reported in fictive preparation (Pearson and Rossignol 1991) was reduced by α2-agonists and frequency augmented by α1-agonist, or NE. These facilitatory effects of Methoxamine and NE were likely to be mediated by the α1 excitatory actions (Sakitama 1993).

On the other hand, following Clonidine, cutaneous reflex excitability was markedly decreased. This is in accordance with earlier studies which also showed that Clonidine (i.p.) reduced significantly the cutaneous excitability in chronic spinal cat (Barbeau et al. 1987). Also, early studies by Lundberg and colleagues showed that DOPA depressed the short latency reflex evoked by the flexor reflex afferent

(FRA)(Anden et al. 1966b). Other α2-agonist, Tizanidine, has also been reported to dose-dependently reduce the EMG response of the flexor reflex induced by stimulation of cutaneous afferents in awake, non-anaesthetized monkeys (Corboz et al. 1991). The inhibitory effects can be prevented by pre-treatment of Yohimbine confirming that the depressant effects were mediated by α2-receptors.

α1- and α2-receptors has also been reported to facilitate and inhibit flexor reflex evoked by the group II muscle afferent, respectively (Sakitama 1993). Their differential effects may contribute to the differential effects they exert on the ability to support the weight of the hindquarters. In anesthetized spinal rats, it has been shown that while a low dose of NE inhibited the flexor reflex evoked by group II afferents, a higher dose of NE facilitated the reflex. In spinal rats pre-treated with Yohimbine, the effects of low dose of NE shifted from inhibition to facilitation. Also in these rats, Prazosin injection dose-dependently antagonized the facilitation effects. Thus, NE exerts a facilitation and an inhibition on the flexor reflex evoked by group II afferents through the activation of α1- and α2-receptors, respectively (Sakitama 1993). Other studies have shown that Clonidine depressed the transmission of group II muscle afferents to either the motoneurons or the first order interneurons in cats (Bras et al. 1990; Schomburg and Steffens 1988). It is thus possible that the decrease in the transmission of group II afferents, via the activation of α2-receptors, will decrease the tonic activity of the stretch receptors thus reducing the weight support ability and spasticity (Eriksson et al. 1996) of the spinal cat.

Foot drag

The marked foot drag during initial swing consistently observed after Clonidine injection might in part due to the decrease in the transmission of cutaneous afferents. The decrease in cutaneous excitability may decrease the efficacy of the 'compensatory mechanism' whereby tactile stimulation of the dorsum of the foot, an additional flexion is normally evoked in the spinal cat during swing (Forssberg 1979). Other contributing factors may be related to the changes in the timing of the recruitment of muscles. For example, following spinalisation, the ankle flexor TA was found to be activated sooner than the knee flexor St, and the hip and knee flexors were activated almost synchronously (Barbeau and Rossignol 1987; Chau et al. 1998). Collectively, these results in the paw moving forward before being adequately lifted which results in the foot drag during swing (Belanger et al. 1996).

Possible changes in receptor-mediated function after spinalisation

It is known that following spinalisation which removes all descending monoaminergic terminals, changes of the postsynaptic receptors will occur. One of these changes is the development of receptor supersensitivity (Barbeau and Bedard 1981; Roudet et al. 1993; Roudet et al. 1994). Noradrenergic receptors have been reported to become supersensitive and that the functional

supersensitivity was attributed to an increase in the number of receptors (Roudet et al. 1994; Nygren and Olson 1976; Roudet et al. 1993; Roudet et al. 1994) and a loss of an uptake system (removal of the descending terminals)(Hirayama et al. 1991). The specificity of these supersensitive receptors are likely to remain unchanged (Hirayama et al. 1991). However, it is possible that the physiological response that they mediate may be different from the intact state depending on the changes in the receptor sensitivity or efficacy. For example, it has been reported that the depressant effects of Clonidine and Tizanidine on the long latency polysynaptic flexor reflex (latency of at least 30 ms) can change to facilitation after spinalisation in rats (Kehne et al. 1985; Chen et al. 1987). Here, we measured only the short latency reflex (latency of about 10ms) evoked by stimulating the low threshold cutaneous afferent and thus, a direct comparison of the fore-mentioned studies (more than 30ms) cannot be made. However, it is possible that α1-agonist, given at a high dose may also exert its action on α2-adrenoceptor, and vice-versa. Finally, among the α2-adrenoceptors, there exists at least 4 different subtypes, $\alpha2_A$, $\alpha2_B$, $\alpha2_C$, $\alpha2_D$, and the α2-agonist have different affinity to these different subclass (for review, see Ruffolo et al. 1993).

Therefore, in light of these complex receptors interactions and properties, it is difficult to understand in simple terms the possible functions mediated by the α1- and α2-agonists on locomotion. However, our results clearly indicated almost opposite effects between the α1- and α2-agonists. For example, we observed a consistent finding that Clonidine inhibited the cutaneous reflex, but Methoxamine

and NE enhanced it (Fig. 15, 16, 17). Therefore, it was unlikely that Clonidine was acting on the α1-receptor.

Recently, α2-agonists with imidazoline structures have been shown also to act on the noradrenergic, imidazoline receptors (Ruggiero et al. 1995; Nicholas et al. 1995). It was reported in rats that the imidazoline receptors did not mediate the antinociceptive action of Clonidine (Monroe et al. 1995). However since there is little known about the role of imadazoline receptors, we cannot rule out the possibility that α2-agonists may exert some of its effects through their activation.

Long term effects

Oxymetazoline was the slowest acting drug among the three α2-agonists tested but also the longest lasting one (Fig. 3). The delayed onset of Oxymetazoline may be related to its the low lipid solubility (Sherman et al. 1987). Oxymetazoline is also resistant to catabolism by monoamine oxidase and does not cross the blood brain barrier which collectively might contribute to the prolonged effects.

The long latency and duration of effects of Methoxamine was suggested to be related to its pharmacological property (Marks et al. 1990). For example, Methoxamine has a high affinity but low activity at the α1-adrenoceptor; it is also removed slowly from the site of action due to the low affinity to the amine uptake system.

Other mechanisms contributing to the long terms effects include the possible changes in some early immediate gene (IEG) expression mediated in part by noradrenaline which may mediate some long-term CNS changes such as learning. Activation of α2-receptors was shown to mediate an inhibitory influence on the immediate early gene (IEG) expression in rat forebrain whereas α1-agonist did not inhibit or increase the expression c-*fos* gene in the both the cortex and pineal gland (Shen et al. 1995; Bing et al. 1991; Carter 1992).

NE has also been shown to enhance the long-term potentiation (LTP) in rat hippocampal slices (via β-adrenoceptor) suggesting the possible role of NE in memory acquisition (Hopkins and Johnston 1988). A combined partial block of α1-adrenoceptors and NMDA receptors also decreased learning (Riekkinen et al. 1996). It has also been suggested that α1-adrenoceptors may interact with the NMDA receptors activity (Klarica et al. 1996). Therefore, we cannot exclude the possibility that the long term effects described here with intrathecal injections of noradrenergic agonists may be exerted through changes in gene expression or complex interactions with other membrane receptors.

Significance of an Intrathecal delivery of drug

The use of an intrathecal catheter for drug delivery is advantageous for obvious reasons. Firstly, it reduced markedly the side effects of the α1- and α2-noradrenergic agonists. In our study, although some side effects (such as gagging

or vomiting) were occasionally still observed with Clonidine (3.8mM) and Oxymetazoline (3.4mM) especially in late-spinal cats, the side effects were less severe than after intraperitoneal Clonidine. In the case of Methoxamine (4.0mM i.t.) no side effect was observed. This is in sharp contrast with Methoxamine given intraperitoneally (5mg/kg) in normal cats, where severe side effects including restlessness and hyperventilation were observed in one case. In addition, for the first time, an immediate action of α2-agonist such as Clonidine and Tizanidine directly on the spinal cord neurons was demonstrated with the use of an intrathecal cannula. While in the previous paper (Chau et al. 1998) the effects of Clonidine were not recorded until 30 minutes after the intrathecal injection, in the present paper, the remarkable locomotor effects elicited by Clonidine in early-spinal cats (n=2) was observed as early as 1-2 minutes following injection, suggesting its potent effects on the neural circuitry for the generation of locomotion. Such immediate effects can never be ascertained by i.p. injection due to inherent delays.

Clinical significance

The present study may serve as a basis for future clinical studies aiming at enhancing locomotor performance in subjects following injury to the CNS such as spinal cord injury or cerebrovascular accidents. Clinical studies using different pharmacological agents, such as Clonidine and Cyproheptadine (a serotonergic antagonist) were done in attempt to decrease spasticity and/or improve locomotion

in subjects with partial spinal cord injuries (Fung et al. 1990; Nance et al. 1985; Norman and Barbeau, 1993; Wainberg et al. 1990; Nance et al. 1994).

The reported therapeutic effects of Clonidine, however, have been conflicting. Clonidine was found to reduce the spasticity but did not initiate or improve locomotion in patients with clinically complete spinal cord injury (Stewart et al. 1991). In another study, Clonidine was found to improve locomotion of subjects with more spasticity and functional deficits but not in patients with less impaired locomotion (Norman and Barbeau, 1993). Another study using intrathecal Clonidine (15-90 μg i.t.) reported that while 3 out of 8 spinal cord injured subjects showed an improvement in walking speed and reduced spasticity, the remaining patients showed no change or a deterioration of the locomotor pattern and sometimes a decrease in weight support abilities with higher dosage (Barbeau et al. 1998). It was shown (Dietz et al. 1995) that in 2 patients with complete paraplegia, while the overall locomotor pattern improved with intrathecal NE, it deteriorated 25 minutes after Clonidine injection (40 μg i.t.) with flaccid paresis of the legs. Recently, in our laboratory, while cats with partial spinal lesion that had already recovered voluntary quadrupedal locomotion showed a deterioration in the locomotor performance after Clonidine (25-150 μg/100 μl) and improvement after Methoxamine (50-150 μg/100 μl) (Brustein et al. 1996), cats with a complete spinal lesion which had residual locomotor deficits showed a marked improvement in the locomotor pattern after Clonidine (10-100 μg/100 μl)(Rossignol et al. 1995).

In light of these conflicting and limited therapeutic effects of Clonidine, it is

essential to better understand the mechanism of Clonidine and the receptors involved in mediating the specific effects. It is also necessary to explore different α2-agonists as each of the α2-agonists differ in their physiochemical properties (Timmermans and Van Zwieten 1981; Ruffolo and Hieble 1994). By testing different α2-agonists, it is possible to select a drug that will offer greatest therapeutic potential while minimizing other undesirable effects. As shown in our findings, while all 3 α2-agonists were capable in initiating locomotion in early-spinal cats, differences with respect to modulation of weight support ability and cutaneous excitability were seen among them (see table 3).

It is also important to better understand the specific effects of the noradrenergic agonist to better target the functional deficits. For example, while clondine may exert important effects on the generation of the locomotor rhythm and increase the cycle duration, it may not be as useful to improve postural deficits as it decreased the weight support and cutaneous excitability. The use of another α2-agonist which has less depressant effects on cutaneous reflex and weight support such as Tizanidine, may be a better choice. In addition, while patients with neurological deficits manifesting spasticity may benefit from Clonidine in reducing the spasticity, it is possible that the patients may at the same time rely on this "spasticity" to some extent to maintain the tone and weight support ability.

Thus, it is imperative to achieve a balance between reducing spasticity but maintaining the necessary muscle tone for postural and locomotor function. The results in this study, showing differential effects mediated by α1- and α2-

noradrenergic agonist, indicate that one way to achieve this balance may be through a combination of drugs. For example, it is plausible that a combination of both α2-agonist (such as Clonidine) and α1-agonist (Methoxamine) may be beneficial to patients with spinal cord injury. The effects of α1-agonist which increased markedly the weight support ability, combined with the effects of the α2-agonists which increased the cycle duration and step length would likely produce the optimal locomotor effects in patients. The efficacy of such a therapeutic intervention awaits further investigation in the clinical trials. Finally, the long term effects exerted by single injections of Oxymetazoline and Methoxamine should not be overlooked as it may also present interesting therapeutic advantages.

In conclusion, the present study confirms that the descending noradrenergic system plays a crucial role in the initiation and modulation of locomotion in chronic spinal cat. However, the fact that spinal animal can recover locomotion suggests that the descending noradrenergic pathways are not absolutely required for the control of locomotion. This study also suggests that NE can exert its action at different levels of control of locomotion, the sensory transmission, the interneuronal mechanism and the motoneuronal level, through the activation of α1- and α2-noradrenergic receptors. The role of the other receptors such as β receptors and imidazoline receptors in the control of locomotion still remains unknown and should be further investigated.

Acknowledgments

C.C. was supported by the following fellowships: the Fonds pour la formation de Chercheurs et l'Aide à la Recherche (F.C.A.R.), the Canadian Neuroscience Network the network of centres of excellence and the Groupe de recherche sur le système nerveux central (GRSNC). H.B. is a scholar of the Fonds de la recherche en Santé du Quebec (FRSQ). This work was supported by the Canadian Neuroscience Network and a Group grant from the Medical Research of Canada. We are grateful to Janyne Provencher and France Lebel for their competent help during surgeries, experiments, analyses and preparation of illustrations. We also acknowledge the late R. Bouchoux for mechanical equipment; P. Drapeau and G.Messier for help with computer programming; C. Gagner for maintenance and help with the electronic equipment; J. Faubert for help during surgery; and C. Gauthier for designing Fig. 1. Many thanks to Dr. Tomas Reader for his helpful suggestions on the manuscript

References

Anden, N. E., Corrodi, H., Fuxe, K., Hökfelt, B., Rydin, C., and Svensson, T. Evidence for a central noradrenaline receptor stimulation by Clonidine. *Life Sciences* 9: 513-523, 1970.

Anden, N. E., Jukes, M. G., and Lundberg, A. The effect of DOPA on the spinal cord. 2. A pharmacological analysis. *Acta physiol. scand.* 67: 387-397, 1966.

Anden, N. E., Jukes, M. G., Lundberg, A., and Vyklicky, L. The effect of DOPA on the spinal cord. 1. Influence on transmission from primary afferents. *Acta physiol. scand.* 67: 373-386, 1966.

Ault, B. and Evans, R. H. The action of catecholamines on the isolated hemisected spinal cord of the immature rat. *J. Physiol. (lond)* 278: 41P-42P,1978.

Barbeau, H. and Bedard, P. Denervation supersensitivity to 5-HT in rats following spinal transection and 5,7 dihydroxytryptamine injection. *Neuropharmacology* 20: 611-616, 1981.

Barbeau, H., Julien, C., and Rossignol, S. The effects of clonidine and yohimbine on locomotion and cutaneous reflexes in the adult chronic spinal cat. *Brain Res.*

437: 83-96, 1987.

Barbeau, H., Pepin, A., Norman, K., Ladouceur, M., and Leroux, A. Walking following spinal cord injury:control and recovery. *Neuroscientist* 4:14-24, 1998.

Barbeau, H. and Rossignol, S. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res.* 412: 84-95, 1987.

Barbeau, H. and Rossignol, S. Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. *Brain Res.* 546: 250-260, 1991.

Barbeau, H. and Rossignol, S. Enhancement of locomotor recovery following spinal cord injury. *Current Opinion in neurology* 7: 517-524, 1994.

Belanger, M., Drew, T., Provencher, J., and Rossignol, S. A comparison of treadmill locomotion in adult cats before and after spinalization. *J. Neurophysiol.* 76: 471-491, 1996.

Bervoets, K. and Millan, M. J. 5-HT1A receptor-induced spontaneous tail-flicks response. V. Opposite modulation of 5-HT1A receptor-induced spontaneous tail-flicks by alpha 1A- as compared with alpha 2D-adrenoreceptors in rat lumbar

spinal cord. *J. Pharmacol. Exper. Therap.* 269: 110-120, 1994.

Bing, G., Filer, D., Miller, J., and Stone, E. Noradrenergic activation of immediate early genes in rat cerebral cortex. *Mol. Brain Res.* 11: 43-46, 1991.

Bras, H., Jankowska, E., Noga, B., and Skoog, B. Comparison of effects of various types of NA and 5-HT agonists on transmission from group II muscle afferents in the cat. *Eur. J. Neurosci.* 211: 1029-1039, 1990.

Brustein, E., Lebel, F., Provencher, J., and Rossignol, S. Effects of noradrenegic (NE) and Serotoninergic (5-HT) agonists on the locomotion of adults cats after bilateral ventral and ventrolateral spinal lesions. *Soc. Neurosci. Abstr.* 22:1843, 1996.(Abstract)

Carter, D. A. Neurotransmitter-stimulated immediate-early gene responses are organized through differential post-synaptic receptor mechanism. *Mol. Brain Res.* 16: 111-118, 1992.

Cazalets, J.R., Grillner, P., Menard, I., Cremieux, J., and Clarac, F. Two types of motor rhythm induced by NMDA and amines in an in vitro spinal cord preparation of neonatal rat. *Neurosci.Lett.* 111:116-121, 1990.

Cazalets, J.R., Sqalli-Houssaini, Y., and Clarac, F. Activation of the central pattern generators for locomotion by serotonin and excitatory amino acids in neonatal rat. *J.Physiol.* 455:187-204, 1992.

Chau, C., Barbeau, H., and Rossignol, S. Early locomotor training with clonidine in spinal cats. *J. Neurophysiol.* 79 (1): 392-409, 1998.

Chen, D. F., Bianchetti, M., and Wiesendanger, M. The adrenergic agonist tizandine has differential effects on flexor reflexes of intact and spinalized rat. *Neurosci.* 23: 641-647, 1987.

Conway, B. A., Hultborn, H., Kiehn, O., and Mintz, I. Plateau potentials in alpha-motoneurones induced by intravenous injection of L-Dopa and clonidine in the spinal cat. *J. Physiol.* 405: 369-384, 1988.

Corboz, M., Palmer, C. I., Palmeri, A., and Wiesendanger, M. Tizanidine-induced depression of polysynaptic cutaneous reflexes in nonanesthetized monkeys is mediated by an alpha 2-adrenergic mechanism. *Experimental. Neurology* 111: 210-216, 1991.

Cowley, K.C. and Schmidt, B.J. A comparison of motor patterns induced by N-methyl-D-aspartate, acetylcholine and serotonin in the in vitro neonatal rat spinal

cord. *Neurosci.Lett.* 171:147-150, 1994.

Dietz, V., Colombo, G., Jensen, L., and Baumgartner, L. Locomotor capacity of spinal cord in paraplegic patients. *Ann. Neurol.* 37: 574-582, 1995.

Eriksson, J., Olausson, B., and Jankowska, E. Antispastic effects of L-dopa. *Exp.Brain Res.* 111: 296-304, 1996.

Espey, M. J. and Downie, J. W. Serotonergic modulation of cat bladder function before and after spinal transection. *Eur. J. Pharmacol.* 287: 173-177, 1995.

Forssberg, H. Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. *J. Neurophysiol.* 42: 936-953, 1979.

Forssberg, H. and Grillner, S. The locomotion of the acute spinal cat injected with clonidine i.v. *Brain Res.* 50: 184-186, 1973.

Fung, J., Stewart, J. E., and Barbeau, H. The combined effects of clonidine and cyproheptadine with interactive training on the modulation of locomotion in spinal cord injured subjects. *J. Neurol. Sci.* 100: 85-93, 1990.

Giroux, N., Aloyz, R.S., Rossignol, S., and Reader, T. Serotonin 1a and α_1-,

α_2-noradrenergic receptors in the spinal cord of spinalizes cats. *Soc. Neurosci.Abstr.* 21:926, 1995.

Grillner, S. and Zangger, P. On the central generation of locomotion in the low spinal cat. *Exp. Brain. Res.* 34: 241-261, 1979.

Hirayama, T., Ono, H., and Fukuda, H. Functional supersensitivity of a1-adrenergic system in spinal ventral horn is due to absence of an uptake system and not to postsynaptic change. *Brain Res.* 539: 320-323, 1991.

Hirayama, T., Ono, H., and Fukuda, H. Effects of adrenergic agents on ventral horn cells in rat spinal cord slices. *Biomed. Res.* 9: 343-351, 1988.

Hopkins, W. F. and Johnston, D. Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. *J. Neurophysiol.* 59: 667-687, 1988.

Hounsgaard, J., Hultborn, H., Jespersen, J., and Kiehn, O. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J. Physiol.* 405: 345-367, 1988.

Jankowska, E., Jukes, M. G., Lund, S., and Lundberg, A. The effect of DOPA on the

spinal cord. 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors. *Acta physiol. scand.* 70: 369-388, 1967.

Julien, C. and Rossignol, S. Electroneurographic recordings with polymer cuff electrodes in paralyzed cats. *J. Neurosci. Meth.* 5: 267-272, 1982.

Katakura, N. and Chandler, S.C. Iontophoretic analysis of the pharmacologic mechanisms responsible for initiation and modulation of trigemnical motoneuronal discharge evoked by intra-oral afferent stimulation. *Brain Res.* 549:66-77, 1991.

Kehne, J. H., Gallager, D. W., and Davis, M. Spinalization unmasks clonidine's alpha1-adrenergic mediated excitation of the flexor reflex in rats. *J. Neurosci.* 5: 1583-1590, 1985.

Kiehn, O. Plateau potentials and active integration in the 'final common pathway' for motor behaviour. *TINS* 14: 68-73, 1991.

Kiehn, O., Hultborn, H., and Conway, B. A. Spinal locomotor activity in acutely spinalized cats induced by intrathecal application of noradrenaline. *Neurosci. Lett.* 143: 243-246, 1992.

Kiehn, O., Johnson, B. R., and Raastad, M. Plateau properties in mammalian spinal interneurons during transmitter-induced locomotor activity. *Neurosci.* 75: 263-273, 1996.

Kiehn, O. and Kjaerulff, O. Spatiotemporal characteristics of 5-HT and dopamin-induced rhythmic hindlimb activity in the in vitro neonatal rat. *J.Neurophysiol.* 75(4):1472-1482, 1996.

Klarica, M., Fage, D., and Carter, C. Pharmacology of N-methyl-D-aspartate-evoked [3H]noradrenaline release in adult rat spinal cord. *Eur. J. Pharmacol.* 308: 135-144, 1996.

Kudo, N. and Yamada, T. N-Methyl-D,L-aspartate-induced locomotor activity in a spinal cord -hindlimb muscles preparation of the newborn rat studied in vitro. *Neurosci.Lett.* 75:43-48, 1987.

Marks, S. A., Stein, R. D., Dashwood, M. R., and Gilbey, M. P. [^{3}H]Prazosin binding in the intermediolateral cell column and the effects of iontophoresed methoxamine on sympathetic preganglionic neuronal activity in the anaesthetized cat and rat. *Brain Res.* 530: 321-324, 1990.

Marshall, K. C. Catecholamines and their actions in the spinal cord. In: *Handbook*

of the spinal cord: Pharmacology, edited by R. A. Davidoff. New York: Marcel Dekker Inc. 1983, p. 275-328.

Monroe, P. J., Smith, D. L., and Smith, D. J. Spinal imidazoline receptors do not mediate the antinociceptive action of intrathecal clondine in rat. *Ann. NY. Acad. Sci.* 763: 497-500, 1995.

Nance, P. W., Bugaresti, J., Shellenberger, K., Shermata, W., and Martinez-Arizala, A. North American tizanidine study group. Efficacy and saftey of tizanidine in the treatment of spasticity in patients with spinal cord injury. *Neurology* 44: S44-S52, 1994.

Nance, P. W., Shears, A. H., and Nance, D. M. Clonidine in spinal cord injury. *J. Can. Med. Assoc.* 133: 41-42, 1985.

Nicholas, A. P., Pieribone, V., Dagerlind, A., Meister, B., Elde, R., and Hokfelt, T. In situ Hybridization: A complementary method to radioligand-mediated autoradiography for localizing adrenergic, alpha-2 receptor-producing cells. *Ann. N. Y. Acad. Sci.* 763: 222-242, 1995.

Nicholas, A.P., Pieribone, V.A., and Hokfelt, T. Cellular localization of messenger RNA for beta-1 and beta-2 adrenergic receptors in rat brain: an in situ hybridization

study. *Neuroscience* 56(4):1023-1039, 1993.

Norman, K. E. and Barbeau, H. Comparison of cyproheptadine,clonidine and baclofen on the modulation of gait pattern in subjects with spinal cord injury. In: *Spasticity:mechanisms and management*, edited by A. Thilmann, D. Burke and Z. Rymer. New York: Springer-Verlag, 1993, p. 410-425.

Nygren, L.-G. and Olson, L. On spinal noradrenaline receptor supersensitivity: correlation between nerve terminals densities and flexor reflexes various times after intracisternal 6-hydroxydopamine. *Brain Res.* 116: 455-470, 1976.

Ono, H. and Fukuda, H. Pharmacology of descending noradrenergic systems in relation to motor function. *Pharmac. Ther.* 68: 105-112, 1995.

Pascual, J., del Arco, C., Gonzalez, A. M., and Pazos, A. Quantative light microscopic autoradiographic localizatioin of α_2-adrenoceptors in the human brain. *Brain Res.* 585: 116-127, 1992.

Pearson, K. G. and Rossignol, S. Fictive motor patterns in chronic spinal cats. *J. Neurophysiol.* 66: 1874-1887, 1991.

Rawlow, A. and Gorka, Z. Involvement of postsynaptic α-$_1$ and α-$_2$ adrenoceptors

im the flexor reflex activity in the spinal rats. *J. Neurol. Transm.* 93-105, 1986.

Riekkinen, M., Stefannski, R., Kuitunen, J., and Riekkinen, P. Effects of combined block of a1-arenoceptor and NMDA receptors on spatial and passive avoidance behaviors in rats. *Eur. J. Pharmacol.* 300: 9-16, 1996.

Rossignol, S. Neural control of stereotypic limb movements. In: *Handbook of Physiology , section 12. Exercise: regulation and integration of multiple systems.* edited by L. B. Rowell and J. T. Sheperd. Bethesda: American Physiological Society, 1996, p. 173-216.

Rossignol, S. and Barbeau, H. Pharmacology of locomotion: an account of studies in spinal cats and spinal cord injured subjects. *The journal of the american paraplegia society* 16: 190-196, 1993.

Rossignol, S., Barbeau, H., and Chau, C. Pharmacology of locomotion in chronic spinal cat. In: *Alpha and gamma motor systems*, edited by A. Taylor, M. H. Gladden and R. Durbaba. New York, London: Plenum Press, 1995, p. 449-455.

Rossignol, S., Barbeau, H., and Julien, C. Locomotion of the adult chronic spinal cat and its modification by monoaminergic agonists and antagonists. In: *Development and plasticity of the mammalian spinal cord*, edited by M. Goldberger, A. Gorio and

M. Murray. Padova: Fidia Research Series III, Liviana Press, 1986, p. 323-345.

Roudet, C., Mouchet, P., Feuerstein, C., and Savasta, M. Normal distribution of alpha2-adrenoreceptors in the rat spinal cord and its modification after noradrenergic dernervation: a quantitative autoradiographic study. *J. Neurosci. Res.* 39: 319-329, 1994.

Roudet, C., Savasta, M., and Feuerstein, C. Normal distribution of alpha-1-adrenoceptors in the rat spinal cord and its modification after noradrenergic denervation: A quantitative autoradiographic study. *J. Neurosci. Res.* 34: 44-53, 1993.

Ruffolo, R. R. and Hieble, J. P. α-Adrenoreceptors. *Pharmac. Ther.* 61: 1-64, 1994.

Ruffolo, R. R., Nichols, A. J., Stadel, J. M., and Hieble, J. P. Pharmacologic and therapeutic applications of α_2-adrenoceptors subtypes. *Annu. Rev. Pharmacol. Toxicol.* 32: 243-279, 1993.

Ruggiero, D. A., Regunathan, S., Wang, H., Milner, T., and Reis, D. Distribution of imidazoline receptor binding protein in the central nervous system. *Ann. New. York. Acad. Sci.* 763: 201-221, 1995.

Sakitama, K. Intrathecal noradrenaline facilitates and inhibits the flesor reflex

mediated by group II afferents fibres via α1- and α2-receptors, respectively. *Japan. J. Pharmacol* 62: 131-136, 1993.

Schomburg, E. D. and Steffens, H. The effect of DOPA and clonidine on reflex pathways from group II muscle afferents to alpha-motoneurones in the cat. *Exp.Brain Res.* 71: 442-446, 1988.

Shen, P.-J., Burazin, T. C. D., and Gundlach, A. L. Noradrenergic regulation of immediated early gene expression in rat forebrain: differential effects of α_1- and α_2-adrenoceptor drugs. *Mol. Brain Res.* 28: 222-230, 1995.

Sherman, S., Loomis, C., Milne, B., and Cervenko, F. Prolonged spinal analgesia in the rat with the α-adrenoceptor agonist oxymetazoline. *Eur. J. Pharmacol.* 140: 25-32, 1987.

Smith, J.C. and Feldman, J.L. In vitro brainstem-spinal cord preparations for study of motor systems for mammalian respiration and locomotion. *J.Neurosci.Meth.* 21:321-333, 1987.

Smith, J. L., Hoy, M. G., Koshland, G. F., Phillips, D. M., and Zernicke, R. F. Intralimb coordination of the paw-shake response: a novel mixed synergy. *J. Neurophysiol.* 54: 1271-1281, 1985.

Stewart, J. E., Barbeau, H., and Gauthier, S. Modulation of locomotor patterns and spasticity with clonidine in spinal cord injured patients. *J. Can. Sci. Neurol.* 18: 321-332, 1991.

Timmermans, P. B. M. W. M. and Van Zwieten, P. A. Mini-Review: The postsynaptic α2-adrenoreceptor. *J. Auton. Pharmac.* 1: 171-183, 1981.

Timmermans, P. B. M. W. M. and van Zwieten, P. A. α2 Adrenoceptors: Classification, Localization, Mechanisms, and Targets for Drugs. *J. Med. Chem* 25: 1389-1401, 1982.

Tremblay, L. E. and Bedard, P. J. Effects of clonidine on motoneuron excitability in spinalized rats. *Neuropharmacology* 25: 41-46, 1986.

Venkova, K. and Krier, J. Postjunctional alpha 1- and beta-adrenoceptor effects of noradrenaline on electrical slow waves and phasic contractions of cat colon circular muscle. *Brit. J. Pharmacol.* 116: 3265-3273, 1995.

Wainberg, M., Barbeau, H., and Gauthier, S. The effects of cyproheptadine on locomotion and on spasticity in patients with spinal cord injuries. *J. Neurol. Neurosurg. Psychiat.* 53: 754-763, 1990.

Weight, F. F. and Salmoiraghi, G. C. Responses of spinal cord interneurons to acetylcholine, norepinephrine and serotonin administered by microelectrophoresis. *J. Pharmacol. Exper. Therap.* 153: 420-427, 1966.

White, S. R., Fung, S. J., and Barnes, C. D. Norepinephrine effects on spinal motorneurons. *Prog. Brain Res.* 88: 343-350, 1991.

Table I. This table shows all experimental trials done in this study. The numbers shown indicate the post-spinal experimental days. Underlined numbers indicate that data obtained during the experiment have been used for more detailed and quantitative kinematics and EMG analyses. The location of the tip of the catheter is identified during post-mortem examination.

Cat (Location of the tip of the i.t. catheter)	Clonidine	Oxymetazoline	Tizanidine	Methoxamine	Noradrenaline
CC4 (L5, left, dorsolateral)	3, 4, 5, 6, 7, 8 ,9, 10, 38, 62	49, 54	n/a	42, 46	n/a
CC5 (L3, left, dorsolateral)	5, 10, 128	138, 165	136	3, 4, 6, 132	7, 8, 9
CC6 (L5, right ventrolateral)	11, 54, 56, 103, 195	132	8, 105, 106, 188	4, 6, 9, 11, 27, 74, 200	119
CC7 (L4-L5 ventrolateral)	8, 28, 29, 30, 32, 100, 148, 276, 277	10, 15, 177, 243	9, 149, 151, 240	7, 72, 119, 154	164
CC8 (L4, central dorsal)	3, 11, 70	4, 8, 73	157	7, 66, 98	27, 131

Table II.

The step cycle and muscle burst durations after the injection of noradrenergic drug in early spinal cats when no locomotion could be elicited prior to drug injection. The step cycle and the burst durations are expressed as percentages of the intact values obtained before spinalisation. D: days, min: minutes, h: hour

Table II. The step cycle and muscle burst durations after the injection of noradrenergic drug in early spinal cats

	Clonidine (3.8mM/100 µl)			**Oxymetazoline** (3.4mM/100 µl)			**Tizanidine** (3.9mM/100 µl)			**Methoxamine** (4.0mM/100 µl)			**Noradrenaline** (12.0mM/100 µl)		
Cat	**CC4 (3d)** 30min			**CC8 (4d)** 24h			**CC6 (8d)** 30min			**CC5 (3d)** 5.5h			**CC5 (8d)** 1h		
	n	duration (ms)	percent of intact (%)	n	Duration (ms)	percent of intact (%)	n	duration (ms)	percent of intact (%)	n	duration (ms)	percent of intact (%)	N	duration (ms)	percent of intact (%)
step cycle	7	1050±35	113	9	935±41	129	8	1048±43	93	6	789±63	47	9	952±101	58
iSrt	10	275±48	101		n/a	n/a	6	309±46	123		n/a	n/a	11	203±48	53
iSt	10	179±51	247	10	128±32	110	8	153±54	230	8	252±65	58	11	156±27	36
iVL	10	588±27	102	10	550±49	122	8	682±45	126	9	820±91	85	11	588±120	61
iGL	10	544±39	109	10	370±55	95	6	666±87	138	9	788±74	85		n/a	n/a
Cat	**CC5 (5d)** 90 min			**CC8 (4d)** 48h			**CC7 (9d)** 5 min			**CC6 (4d)** 3h					
	n	duration (ms)	percent of intact (%)	n	Duration (ms)	percent of intact (%)	n	duration (ms)	percent of intact (%)	n	duration (ms)	percent of intact (%)			
step cycle	4	922±112	92	8	804±50	111	9	1122±43	121	9	996±54	100			
iSrt	15	262±96	98		n/a	n/a	12	401±34	130	4	225±42	96			
iSt	15	209±44	122	16	81±23	70	11	192±21	264	11	180±11	205			
iVL	15	571±102	97	16	461±57	102	13	561±25	108	12	564±34	94			
iGL	15	590±82	105		329±42	85	13	446±42	113	13	452±51	84			
Cat	**CC7 (8d)** 2 min									**CC7 (7d)** 90min					
	n	duration (ms)	Percent of intact (%)							n	duration (ms)	percent of intact (%)			
step cycle	9	1448±177	135							5	1190±239	89			
iSrt	8	506±181	138							17	591±99	140			
iSt	8	207±47	385							17	278±67	236			
iVL	7	873±103	101							15	527±105	74			

Table III.

A summary of the primary effects of the Noradrenaline, α1- and α2-noradrenergic agonists on locomotion and cutaneous excitability in all experimental trials. The total number of spinal cats used for the different drug trials and the total number of experiments made are indicated. Only cats that were capable of walking spontaneously without drugs were included in this table. The doses and the range of post-spinalisation days are shown. The frequency of observation of different effects is also shown as a ratio indicated on the left hand side of each block. For example, in the case of Clonidine, all drug trials (12/12) showed an increase in step duration, step length and marked toe drag, and in 7 out of 7 experiments where responses to electrical stimulation were tested, there was either an increase in the threshold (T) of stimulation at rest (standing) or a decrease in the reflex response with the same stimulation intensity. All stimulating parameters (frequency, 0.4 Hz, pulse duration, 250 μs) were kept constant before and after drug injection.

Table III. A summary of the primary effects of the drugs on locomotion and cutaneous excitability in all experimental trials.

Drugs (type of agonist) **number of experiments**	**dose (mM)**	**days**	**Effects on Locomotion on 4 spinal-cats**	**Effects on Cutaneous Excitability on 4 spinal-cats**		
				response to Tap	**FPS**	**Electrical Stimulation**
Clonidine (α2-agonist) **12**	0.9-3.8	11-277	9/12 ↓weight support and knee sag 12/12 ↑↑ step duration ↑↑ step length ↑↑ foot drag	12/12 abolished	12/12 abolished	7/7 ↑↑ T pre: 0.75-1.0mA post:3.0-5.3mA ↓ reflex amplitude
Oxymetazoline (α2-agonist) **7**	0.3-3.4	10-132	5/7 weight support similar 7/7 ↑↑step duration prolonged effects 6/7 ↑ foot drag	4/6 abolished 2/6 ↓, but present	5/6 abolished	2/2 ↑↑ T pre: 1.0-2.6mA post:1.5-5.0mA ↓ reflex amplitude
Tizanidine (α2-agonist) **7**	1.0-4.7	105-240	4/7 weight support similar 3/7 ↓ weight support and knee sag 7/7 ↑↑step length ↑↑cycle duration ↑↑ foot drag	3/6 ↓, but present 3/6 brisk response	3/6 abolished	4/6 ↑ T pre: 0.3-0.45mA post:0.45-0.7mA ↓ reflex amplitude 2/6 no change in T
Methoxamine (α1-agonist) **11**	4.0	11-200	9/11 ↑weight support ↑extensor amplitude cycle duration similar 7/11 ↑tonus of tail (spontaneous tail movement)	11/11 ↑reflex amplitude	11/11 ↑ response frequency and amplitude	4/4 ↓ T pre: 0.3-0.4mA post: 0.1-0.2mA ↑ reflex amplitude
Noradrenaline (neurotransmitter) **6**	4.7-10.7	27-164	weight support variable 6/6 ↑step length ↑cycle duration 5/6 ↑ foot drag	5/5 ↑reflex amplitude	3/5 ↑ response frequency and amplitude	5/6 ↓ T pre: 1.0mA post: 0.7mA ↑ reflex amplitude

Figure 1.

Scheme showing the experimental set-up during locomotion. The hindlimbs of the cat were placed on the moving treadmill belt while the forelimbs stood on a stationary platform (≈2 cm above). Both the head connector and the cannula inlet port are fixed on the head as shown. The details of the recording procedures and synchronization procedures are described in the Method. The four joint angles are measured so that flexion will result in a decrease of angular values. MTP: metatarso-phalangeal joint.

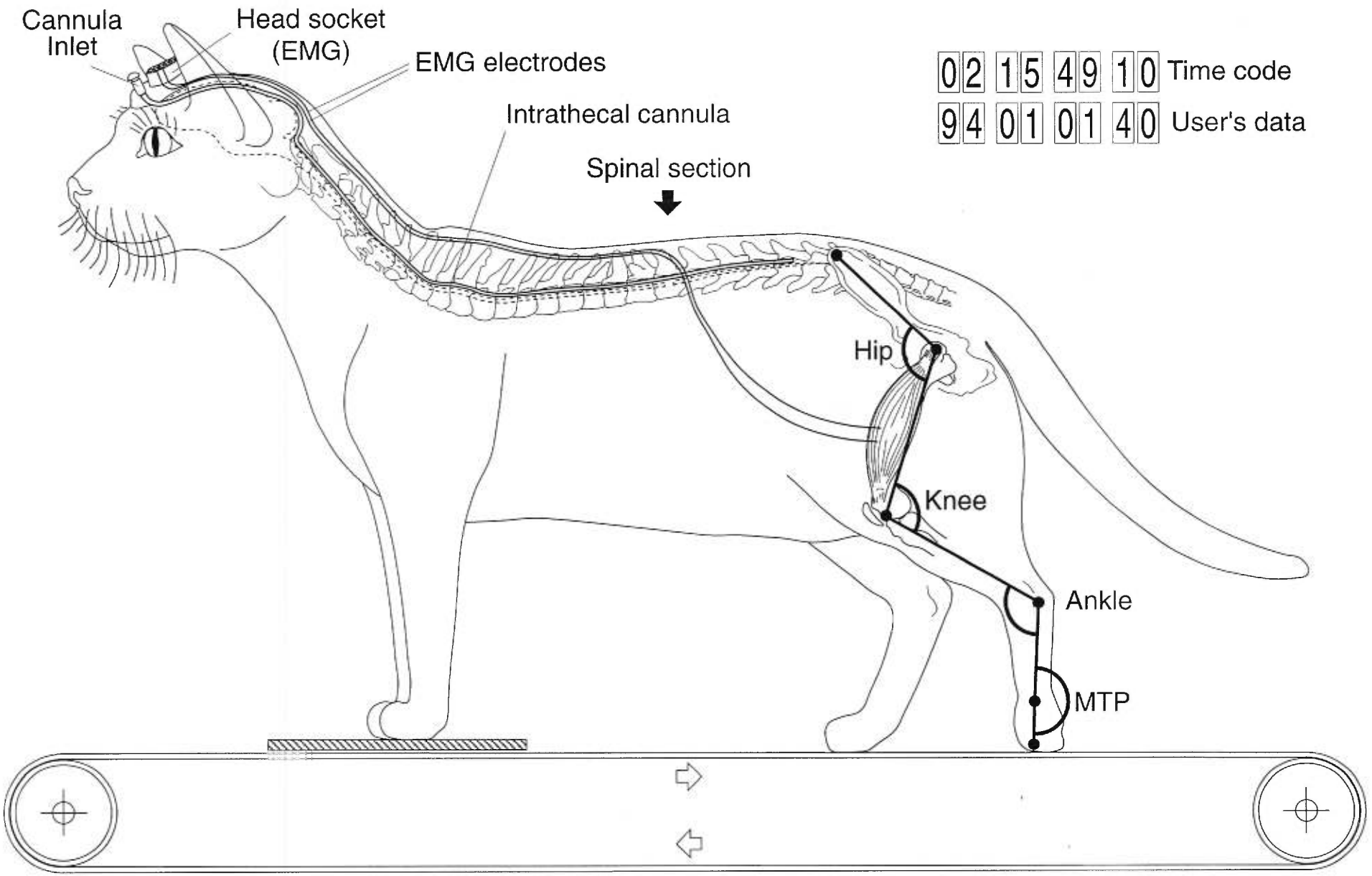

Cannula
Inlet
Head socket
(EMG)
EMG electrodes
Intrathecal cannula
Spinal section
02 15 49 10 Time code
94 01 01 40 User's data
Hip
Knee
Ankle
MTP

Figure 2.

The effect of an α2-agonist, Clonidine, on the initiation of locomotion in an 8d spinal cat (CC7).

A. Stick diagram (1 step cycle) and raw EMG traces of hindlimb flexor and extensor muscles during intact locomotion, before spinalisation. Treadmill speed at 0.3m/s.

B. Locomotion at 8d post spinalisation before any drug injection.

C. Locomotion recorded at 2 minutes following Clonidine injection (4mM i.t.). The muscle gains of iSt and coSt EMG were decreased to 0.4 and 0.5 times the gain of recording in intact cat. i: ipsilateral; co: contralateral.

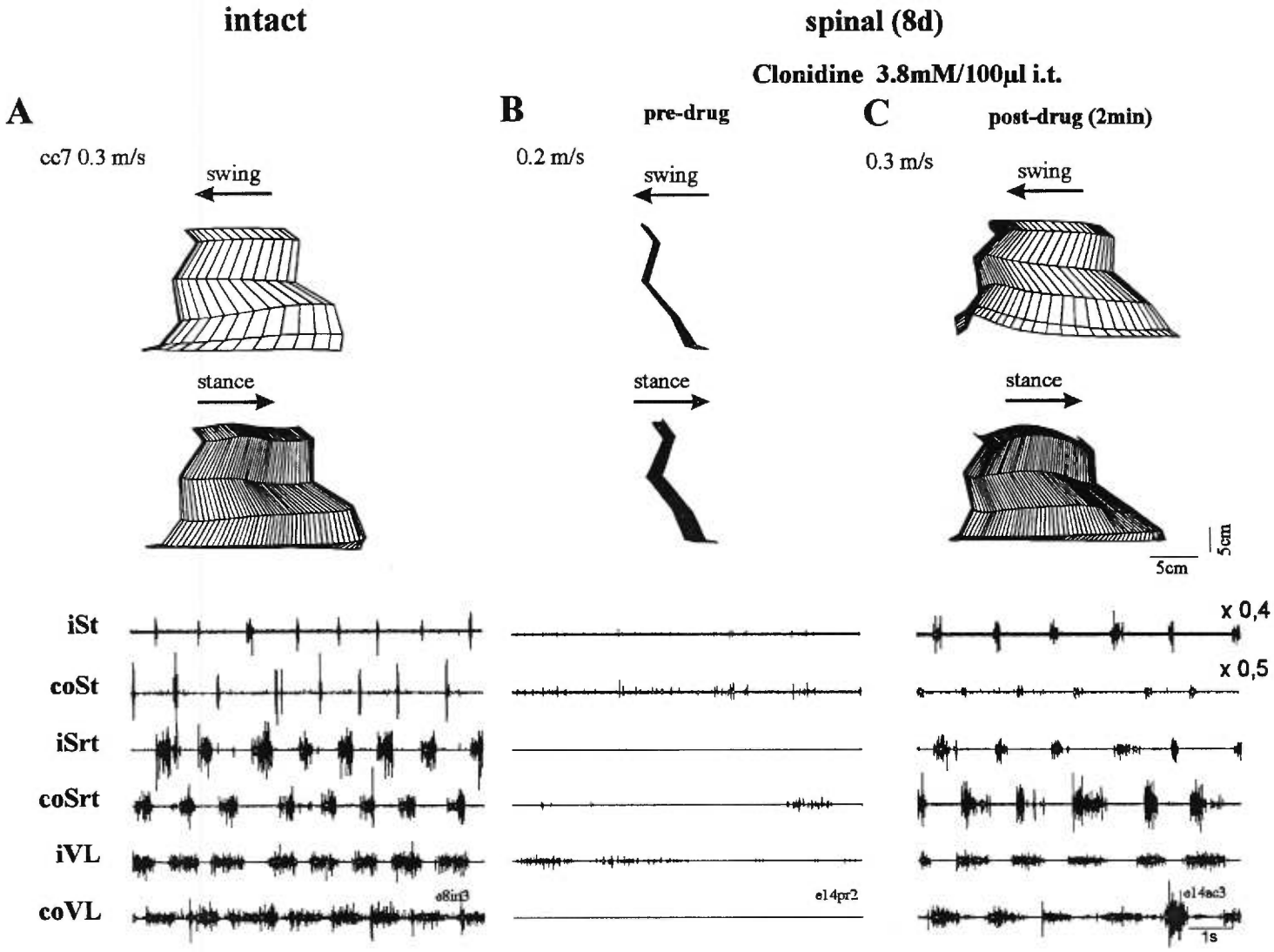

intact
spinal (8d)
Clonidine 3.8mM/100µl i.t.
A
B
C
pre-drug
post-drug (2min)
cc7 0.3 m/s
0.2 m/s
0.3 m/s
swing
stance
5cm
5cm
x 0,4
x 0,5
iSt
coSt
iSrt
coSrt
iVL
coVL
e8in3
e14pr2
e14ac3
1s

Figure 3.

Histograms of cycle duration, stance duration, swing duration, and stance length (obtained form kinematic data) expressed as percentages of intact locomotion in 4 spinal cats CC4 (3d), CC8 (4d), and CC6 (8d), and CC5 (8d) following i.t. injection of Clonidine (3.8mM), Oxymetazoline (3.4mM) and Tizanidine (3.9mM), and noradrenaline (12.0mM), respectively. A horizontal broken line at 100% refers to values obtained from the cats during intact locomotion, prior to spinalisation. Note that the cats were not walking prior to the drug injection.

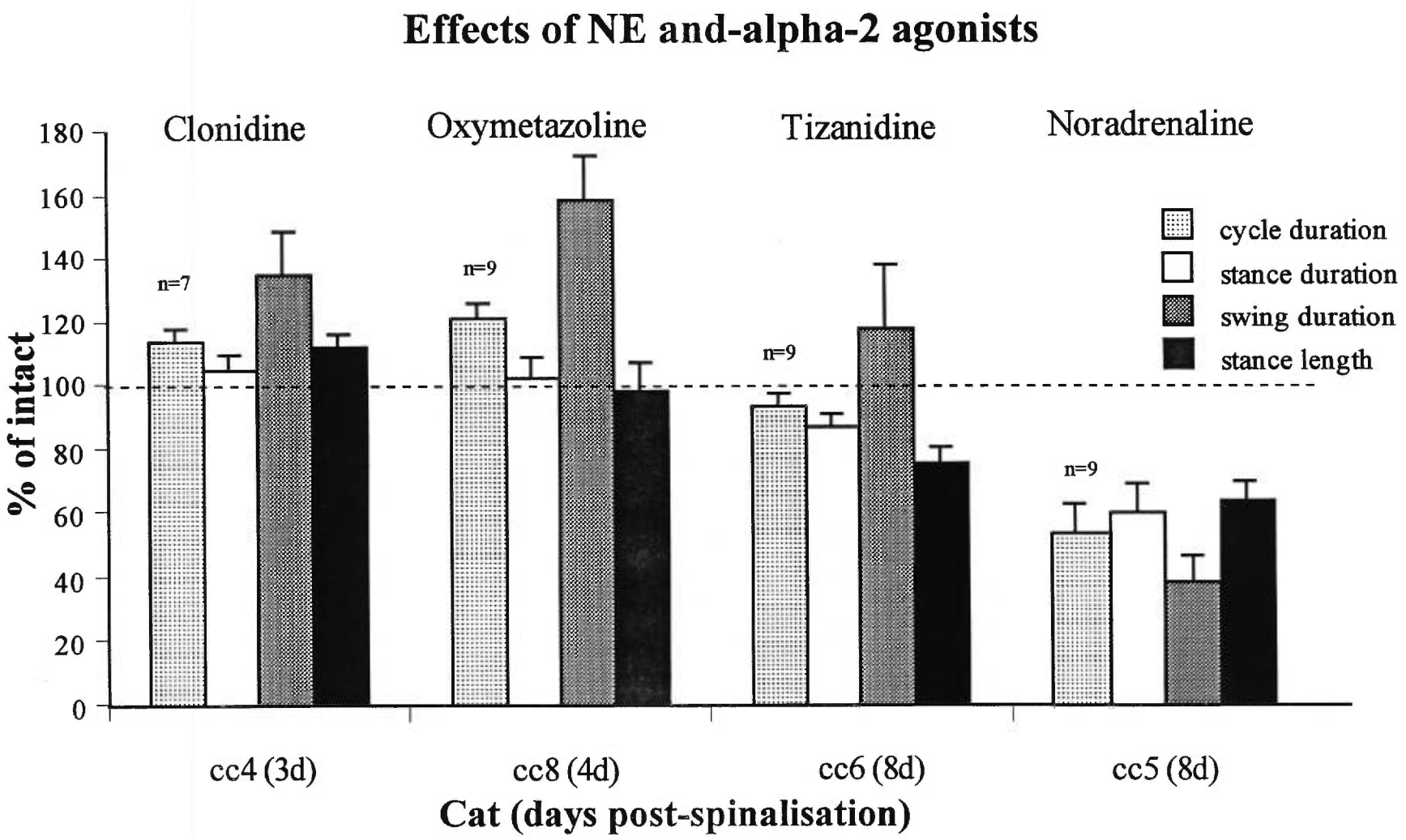

Effects of NE and-alpha-2 agonists
Clonidine
Oxymetazoline
Tizanidine
Noradrenaline
cycle duration
stance duration
swing duration
stance length
% of intact
180
160
140
120
100
80
60
40
20
0
n=7
n=9
n=9
n=9
cc4 (3d)
cc8 (4d)
cc6 (8d)
cc5 (8d)
Cat (days post-spinalisation)

Figure 4.

Time course of action of the 3 α2-noradrenergic agonists. The changes in the stance duration (during locomotion at 0.6m/s) as a function of time after the injection of Clonidine, Tizanidine, and Oxymetazoline in CC4 C(3d), CC7 (9d), and CC8 (4d) respectively, was measured to evaluate the effects of the drugs. Note the different time scale between Fig. 4A (Clonidine and Tizanidine) and Fig. 4B (Oxymetazoline).

In **A**, At 2.5 and 6.5 hours after Tizanidine and clondine injections respectively, the stance duration was at zero as the locomotion returned to the pre-drug non walking status. The effects of Clonidine and Tizanidine completely dissipated on the following day. In **B**, Oxymetazoline takes some 2 hours to have an effect and lasts for several days.

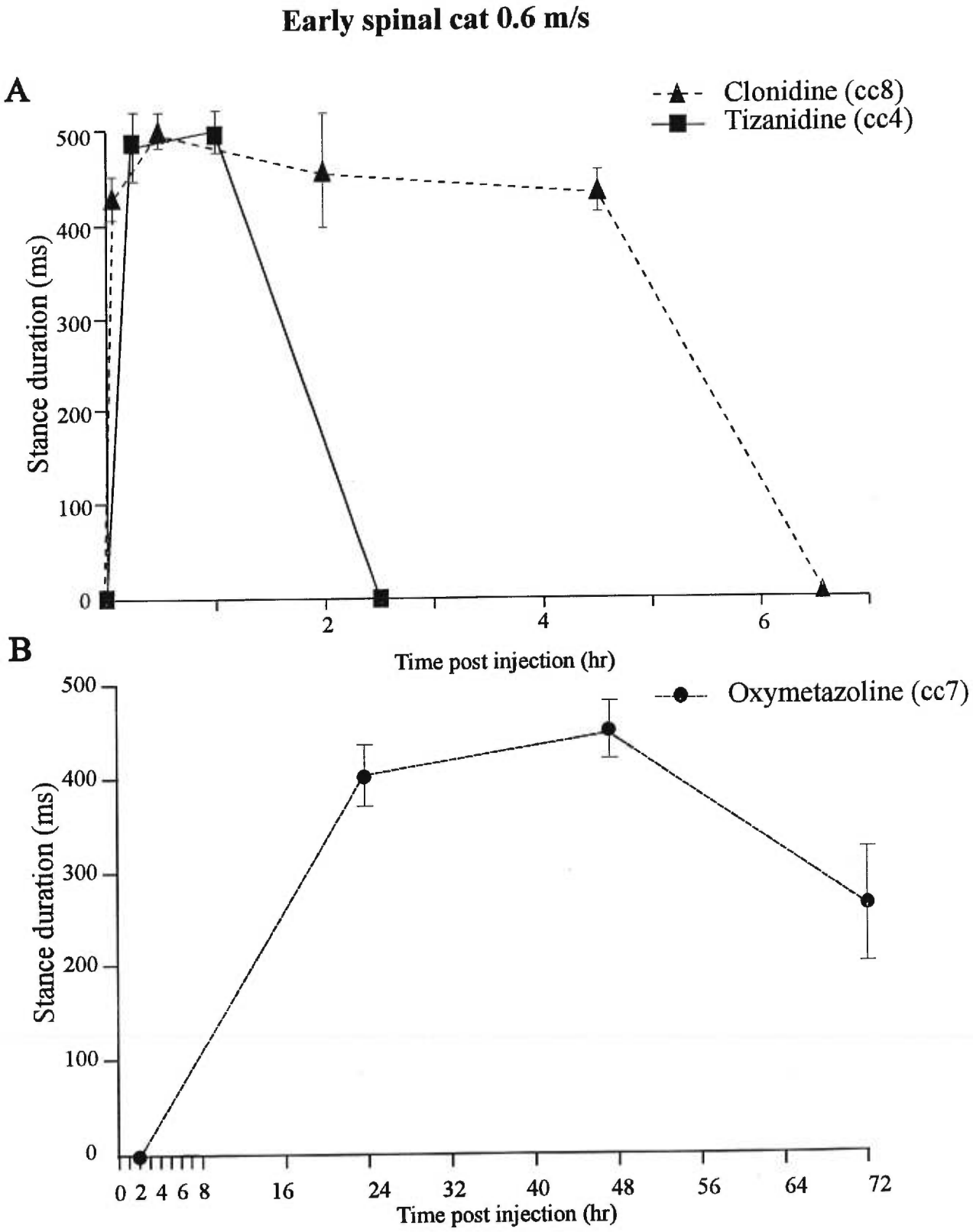

Early spinal cat 0.6 m/s
A
Clonidine (cc8)
Tizanidine (cc4)
Stance duration (ms)
500
400
300
200
100
0
2
4
6
Time post injection (hr)
B
Oxymetazoline (cc7)
Stance duration (ms)
500
400
300
200
100
0
0 2 4 6 8
16
24
32
40
48
56
64
72
Time post injection (hr)

Figure 5.

The effects of an α1-agonist, Methoxamine, on a spinal cat (CC5) at 3d post transection as compared to the effects of Clonidine (α2-agonist) on the same cat at 5d post-transection. A. Locomotion before Methoxamine injection. B. Ninety minutes following Methoxamine injection, the cat had rhythmic movements of the knee but very little movements of the hip. The hindlimb was being dragged on the treadmill with the paw behind the hip joint. C. One bout of locomotor activity at 0.2m/s could be observed at a longer time interval after injection of Methoxamine (5 hours 40 minutes). Note that the EMG activity in the proximal muscles is still not well organized, at least on the contralateral side. D. In contrast to the effects of Methoxamine, 90 minutes following Clonidine injection in the same spinal cat 2 days later (5d post-transection), there was a well-organized locomotion at a treadmill speed of 0.4m/s characterized by large alternating steps and well developed EMG activities of the hindlimbs even in the more proximal muscles such as Srt on both sides.

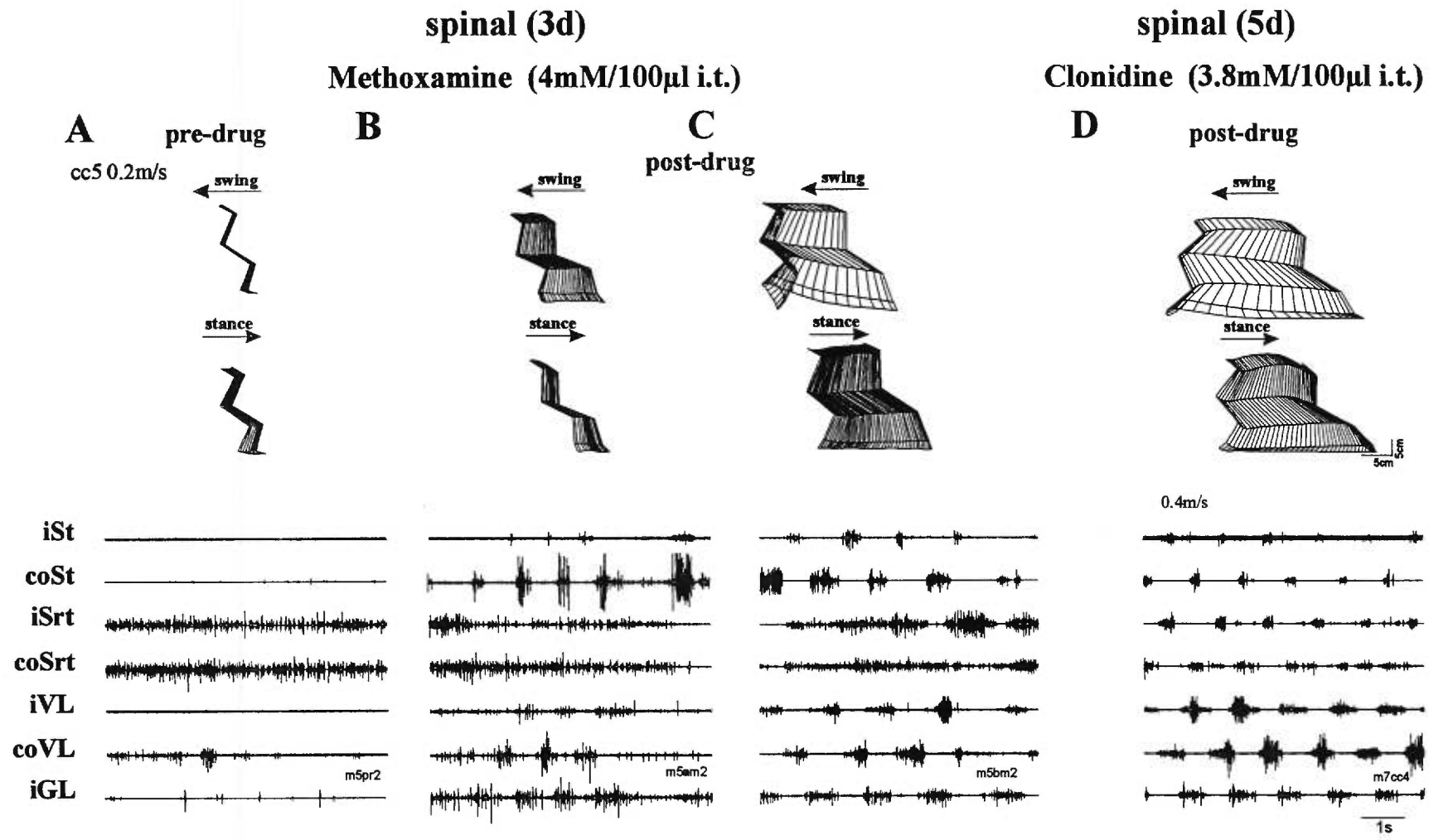

spinal (3d)
Methoxamine (4mM/100µl i.t.)
spinal (5d)
Clonidine (3.8mM/100µl i.t.)
A
pre-drug
cc5 0.2m/s
B
C
post-drug
D
post-drug
swing
stance
5cm
0.4m/s
iSt
coSt
iSrt
coSrt
iVL
coVL
iGL
m5pr2
m5am2
m5bm2
m7cc4
1s

Figure 6.

An α1-agonist, Methoxamine, was found to initiate locomotion in a spinal cat CC6 at 4d post-transection.

A. Locomotor pattern during intact condition at 0.4m/s.

B. No locomotion was seen before Methoxamine injection.

C. 3 hours after Methoxamine injection (4mM i.t.), organized locomotor pattern was recorded at the same treadmill speed as the intact locomotion. Alternating EMG bursts of activity were observed in the hindlimb muscles, whereas the hip flexor Srt of both hindlimbs showed more or less tonic activity.

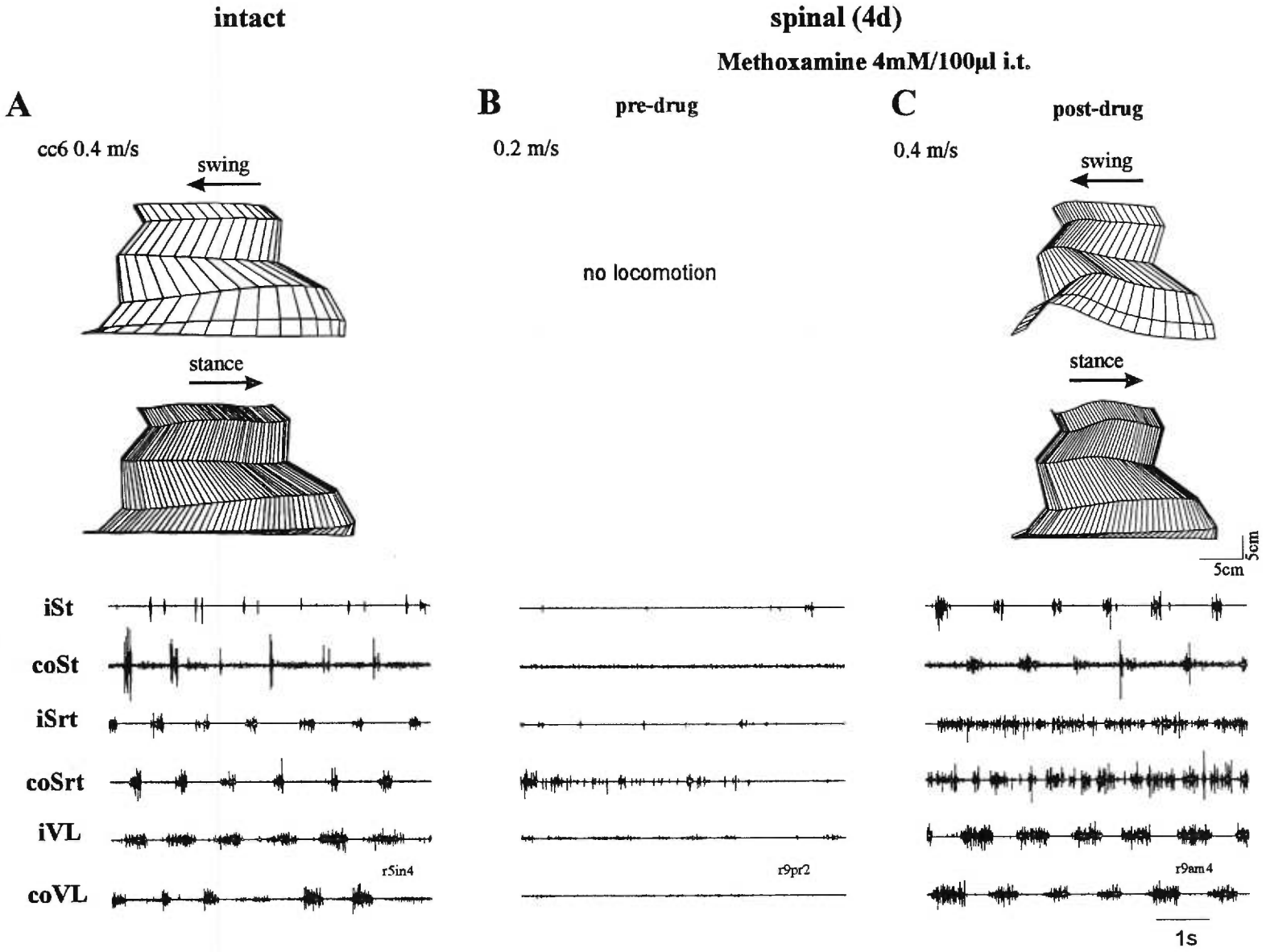

intact
spinal (4d)
Methoxamine 4mM/100µl i.t.
A
B
pre-drug
C
post-drug
cc6 0.4 m/s
0.2 m/s
0.4 m/s
swing
stance
no locomotion
5cm
5cm
iSt
coSt
iSrt
coSrt
iVL
coVL
r5in4
r9pr2
r9am4
1s

Figure 7.

The locomotor effects of the α1-agonist, Methoxamine, as shown in the previous figure was blocked by an α1-antagonist, Prazosin.

A. In spinal cat CC6, at 6d post-transection, no locomotion was seen before drug injection.

B. 3 hours after Methoxamine injection (4mM i.t.), the cat was able to walk with weight support and plantar foot placement at a treadmill speed of 0.4m/s.

C. Injection of Prazosin markedly reduced the step amplitude 34 minutes after and the rhythmic movements were confined to the knee and the ankle. The gain of coSt shown in 7B and 7C was increased 2.5 times relative to the pre-drug trials.

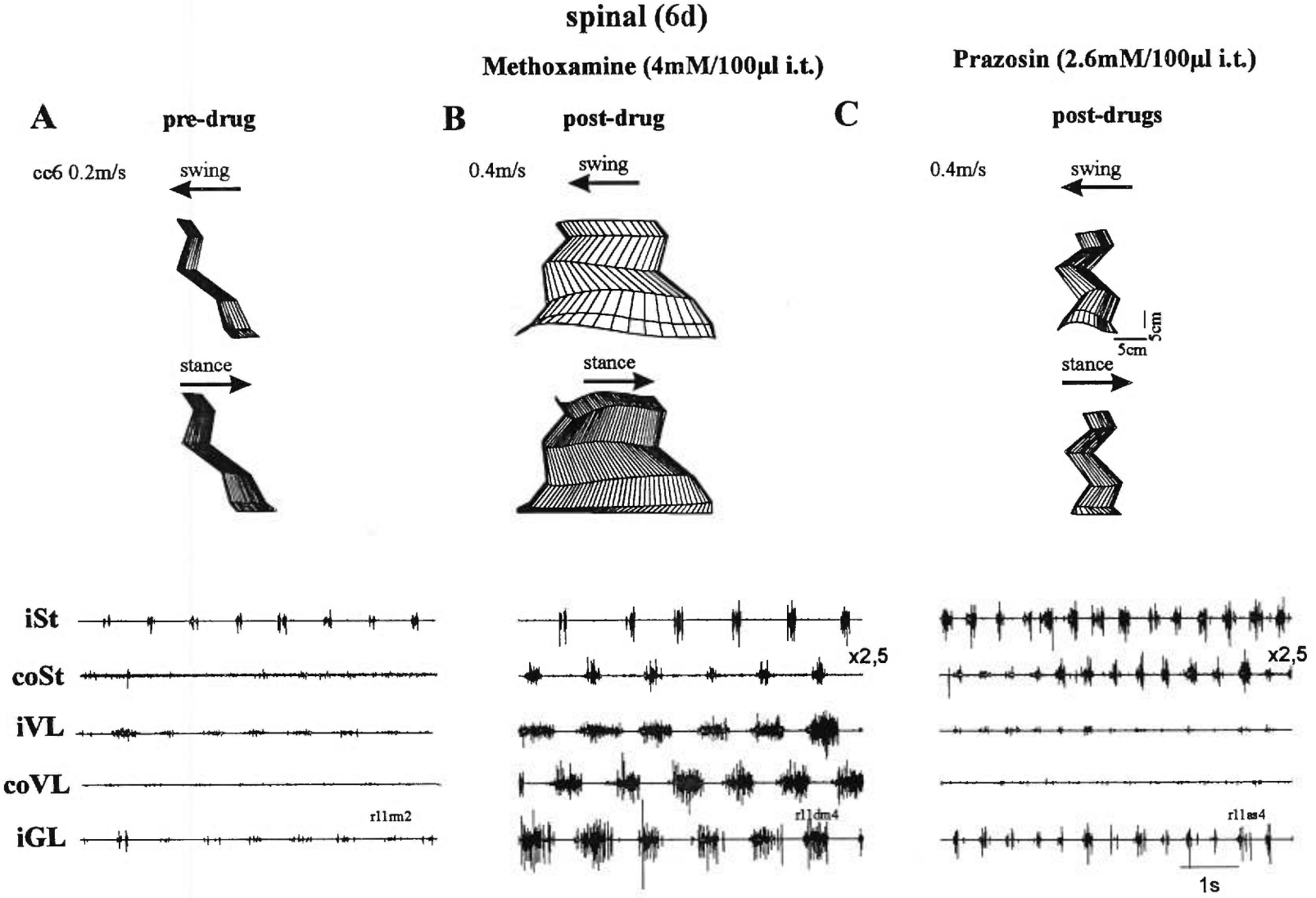

spinal (6d)
Methoxamine (4mM/100µl i.t.)
Prazosin (2.6mM/100µl i.t.)
A
pre-drug
B
post-drug
C
post-drugs
cc6 0.2m/s
swing
0.4m/s
swing
0.4m/s
swing
stance
stance
stance
5cm
5cm
iSt
coSt
iVL
coVL
iGL
rl1m2
x2,5
rl1dm4
x2,5
rl1as4
1s

Figure 8.

The effect of NE on initiating locomotion in an 8d spinal cat.

A. Locomotion during intact condition.

B. No locomotion was observed before drug injection.

C. Locomotor pattern at 1 hour following the injection of NE (12mM i.t.) At a treadmill speed of 0.2m/s, organized hindlimb EMG activity was seen. Note that the EMG activity of the hip flexor Srt is not well organized compared to knee flexors St, or knee extensor VL.

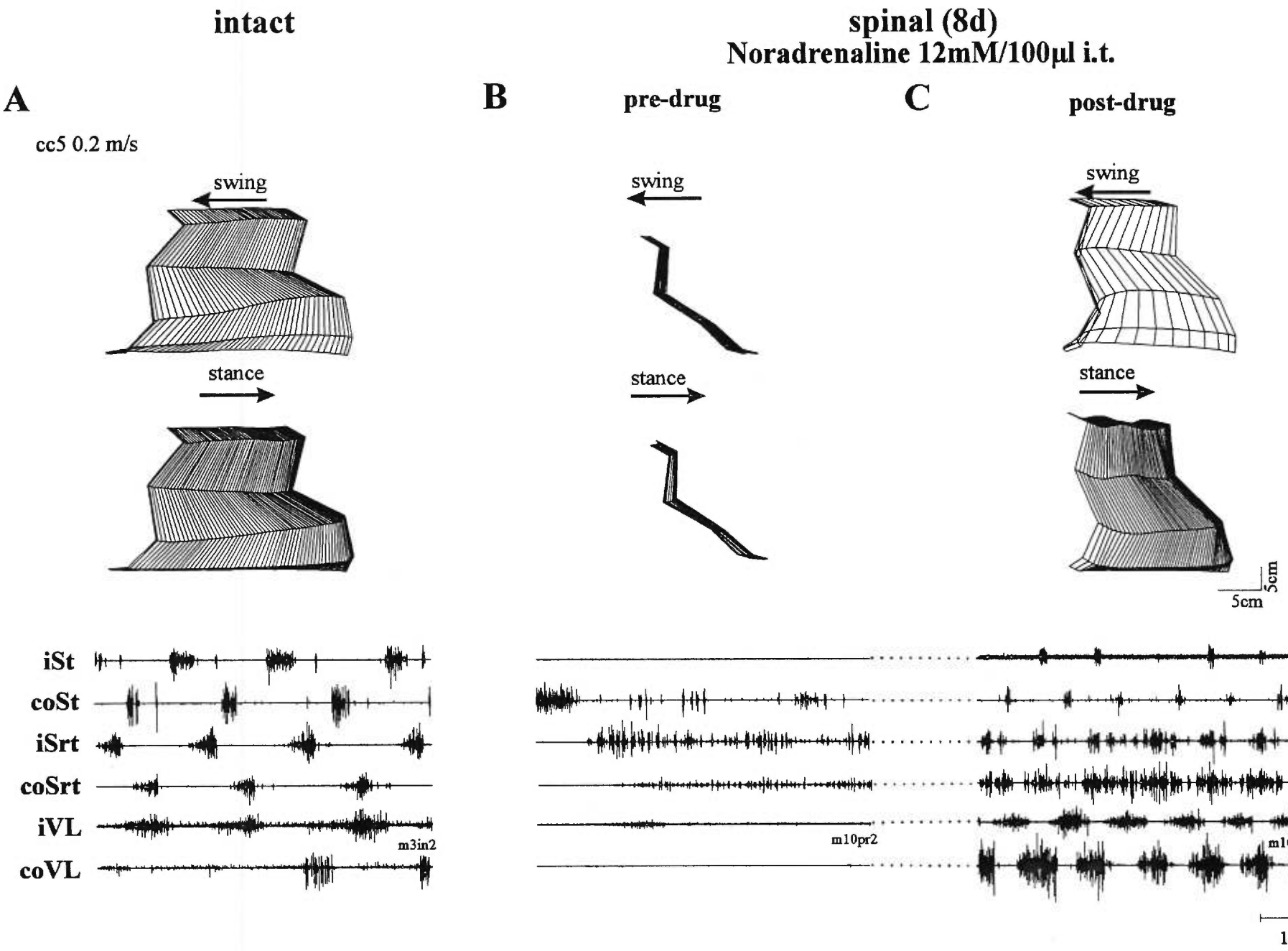

intact
spinal (8d)
Noradrenaline 12mM/100µl i.t.
A
B
pre-drug
C
post-drug
cc5 0.2 m/s
swing
stance
swing
stance
swing
stance
5cm
5cm
iSt
coSt
iSrt
coSrt
iVL
coVL
m3in2
m10pr2
m10na2
1s

Figure 9.

The effects of an α2-agonist, Tizanidine and an α2-antagonist, Yohimbine, on the locomotion of a spinal cat (CC8) at 157d post-transection.

A.B.C. Locomotion at treadmill speed of 0.4m/s prior to receiving any drug. The duty cycles are represented by horizontal lines with downward arrows indicating foot contacts and upward arrows indicating foot lifts.

D.E.F. Locomotor pattern recorded 30 minutes after injection of a 3mM dose of Tizanidine following a first dose of 2mM given 1hour 55 minutes before.

G.H.I. Locomotion recorded 15 minutes after Yohimbine injected 27 minutes after the records in D.E.F.

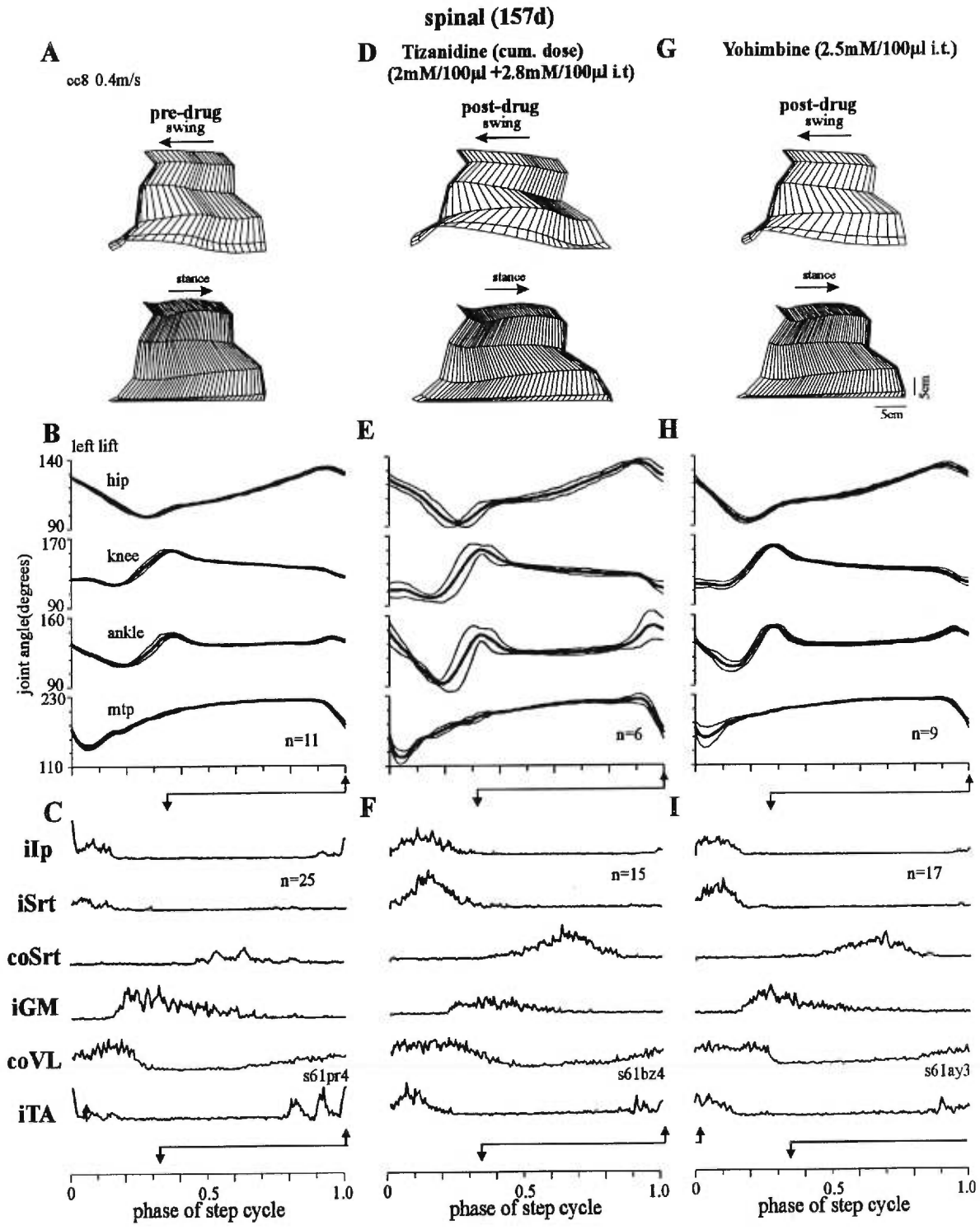

spinal (157d)
A
cc8 0.4m/s
pre-drug
swing
stance
D
Tizanidine (cum. dose)
(2mM/100µl +2.8mM/100µl i.t)
post-drug
swing
stance
G
Yohimbine (2.5mM/100µl i.t.)
post-drug
swing
stance
3cm
5cm
B
left lift
140
hip
90
170
knee
90
160
ankle
90
230
mtp
110
joint angle(degrees)
n=11
E
n=6
H
n=9
C
iIp
n=25
iSrt
coSrt
iGM
coVL
s61pr4
iTA
0
0.5
1.0
phase of step cycle
F
n=15
s61bz4
0
0.5
1.0
phase of step cycle
I
n=17
s61ay3
0
0.5
1.0
phase of step cycle

Figure 10.

The combined effects of α1-agonist, Methoxamine and α2-agonist, Clonidine, on a 11d spinal cat.

A. B. C. Stick diagrams, averaged angular plot, and averaged normalized EMG data during locomotion before injection of any drug.

D,E,F. Locomotor pattern recorded 2h30 after Methoxamine injection alone.

G.H.I. Clonidine was injected 17 minutes after the previous recording, that is 2h47 minutes after Methoxamine injection. Locomotion recorded 10 minutes following Clonidine (3.8mM i.t.) in the same cat during the same experiment.

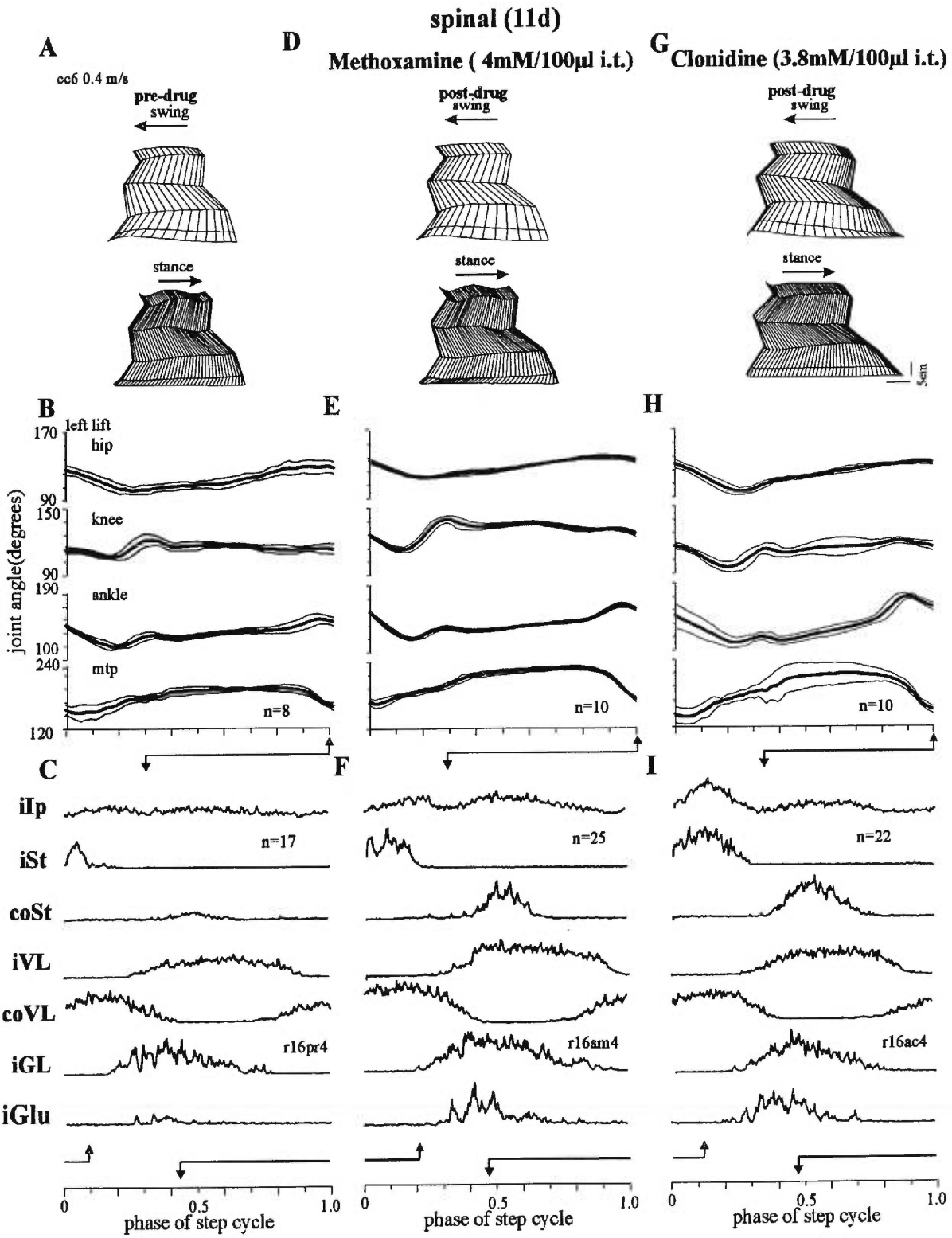

spinal (11d)
A
D
Methoxamine (4mM/100µl i.t.)
G
Clonidine (3.8mM/100µl i.t.)
cc6 0.4 m/s
pre-drug swing
post-drug swing
post-drug swing
stance
stance
stance
5cm
B
E
H
left lift hip
knee
ankle
mtp
joint angle(degrees)
170
90
150
90
190
100
240
120
n=8
n=10
n=10
C
F
I
iIp
iSt
coSt
iVL
coVL
iGL
iGlu
n=17
n=25
n=22
r16pr4
r16am4
r16ac4
0
0.5
1.0
phase of step cycle
phase of step cycle
phase of step cycle

Figure 11.

A comparison of the effect of an α2-, α1-agonist, and NE in the same cat. The effects of α2-agonist, Tizanidine, α1-agonist, Methoxamine, and noradrenaline on a spinal cat CC7 at different post-transection days, respectively.

A.B.C. Locomotor pattern at 151d post-transection before any drug injection.

D.E.F. On the same day (151d), locomotion recorded at 30 minutes following Tizanidine injection.

G.H.I. On 154d post-transection, locomotion recorded at 3 hours following Methoxamine injection.

J.K.L. On the 164d post-transection, locomotor pattern recorded at 23 minutes after noradrenaline injection.

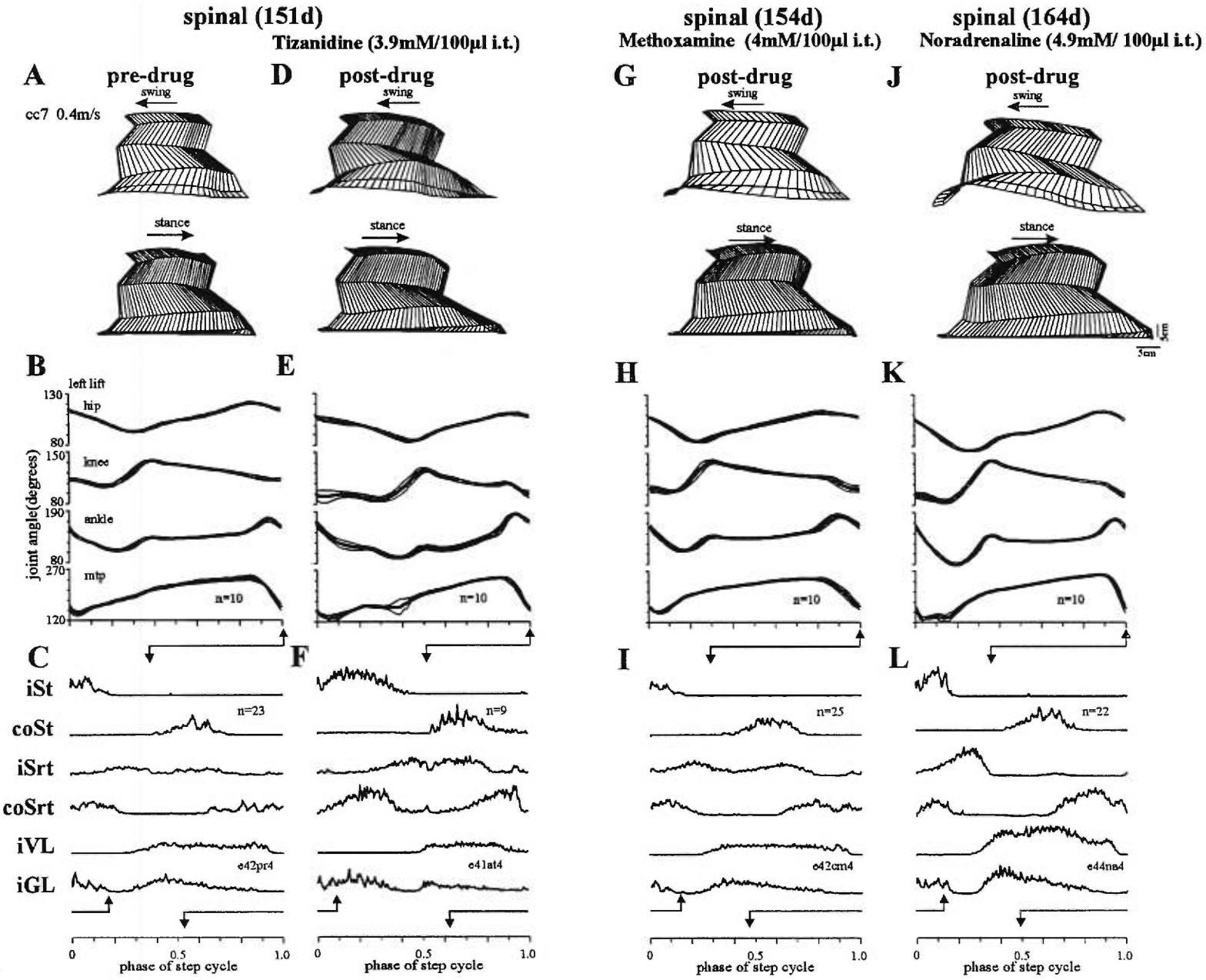

spinal (151d)
Tizanidine (3.9mM/100µl i.t.)
spinal (154d)
Methoxamine (4mM/100µl i.t.)
spinal (164d)
Noradrenaline (4.9mM/ 100µl i.t.)
A
pre-drug
D
post-drug
G
post-drug
J
post-drug
cc7 0.4m/s
swing
stance
B
E
H
K
left lift
hip
knee
ankle
mtp
joint angle(degrees)
n=10
C
F
I
L
iSt
coSt
iSrt
coSrt
iVL
iGL
n=23
n=9
n=25
n=22
e42pr4
e41at4
e42cm4
e44ns4
phase of step cycle
5cm

Figure 12.

Histograms showing the modulatory effects of three α2-agonists (Clonidine, Oxymetazoline, and Tizanidine), the α1-agonist, Methoxamine, and NE on the cycle, stance and swing duration in different late spinal cats. The cycle, stance and swing duration were expressed as percentages of the pre-drug trials.

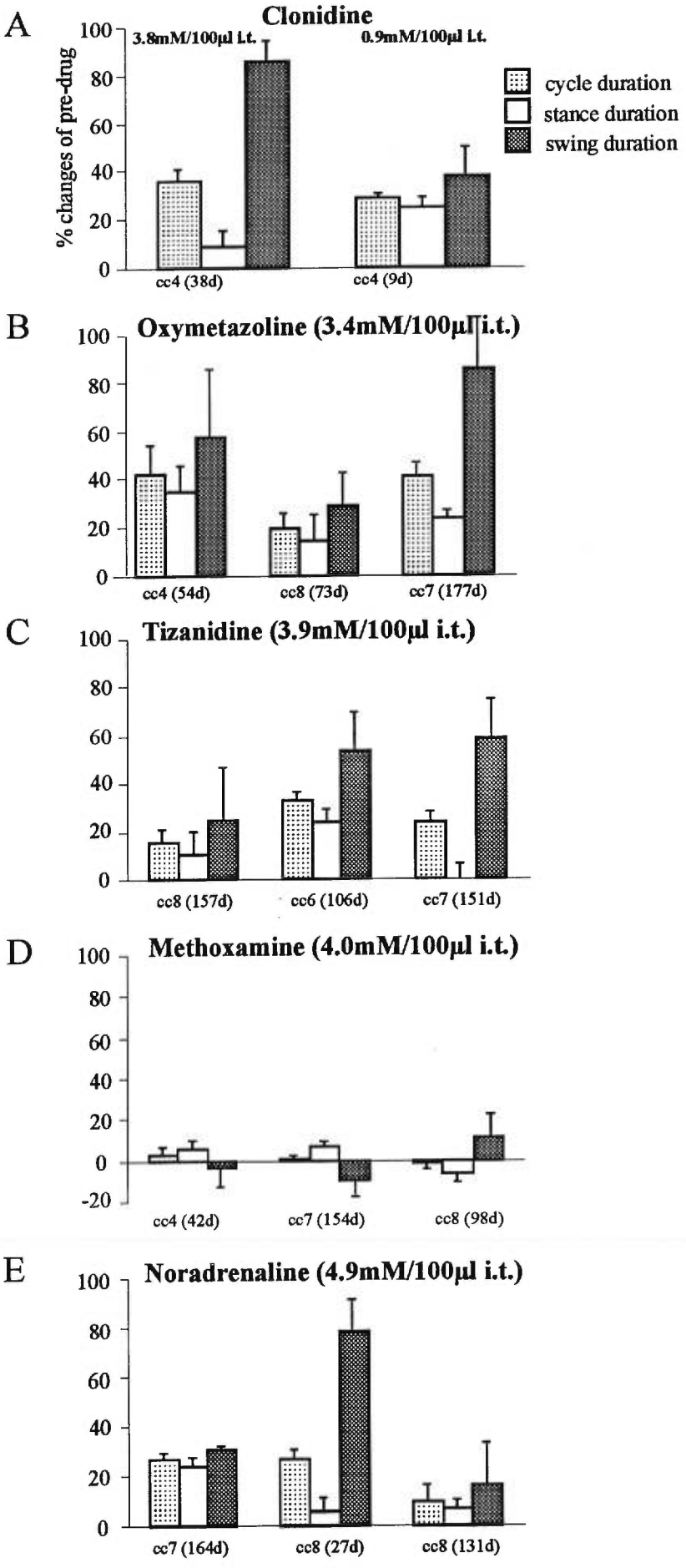

A
Clonidine
3.8mM/100µl i.t.
0.9mM/100µl i.t.
% changes of pre-drug
cycle duration
stance duration
swing duration
cc4 (38d)
cc4 (9d)
B
Oxymetazoline (3.4mM/100µl i.t.)
cc4 (54d)
cc8 (73d)
cc7 (177d)
C
Tizanidine (3.9mM/100µl i.t.)
cc8 (157d)
cc6 (106d)
cc7 (151d)
D
Methoxamine (4.0mM/100µl i.t.)
cc4 (42d)
cc7 (154d)
cc8 (98d)
E
Noradrenaline (4.9mM/100µl i.t.)
cc7 (164d)
cc8 (27d)
cc8 (131d)

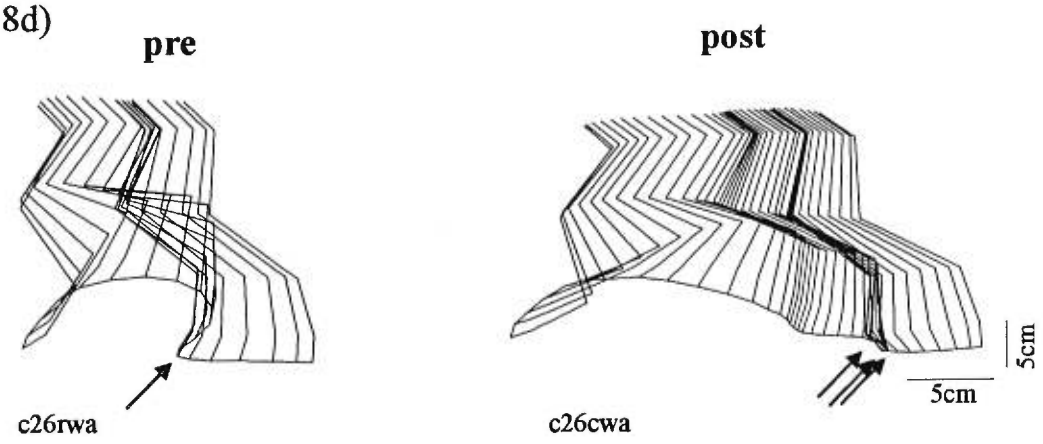
Figure 13.

Stick diagrams showing response to mechanical stimulation (tapper) applied to the dorsum of the paw during swing of CC4 (38d), CC4 (46d), and CC8 (27d) pre- and post-Clonidine, Methoxamine, and NE injection, respectively. Arrows underneath the stick figures indicate the video frames during which where the dorsum of the paw was contacted with the tapper.

Mechanical Stimulation

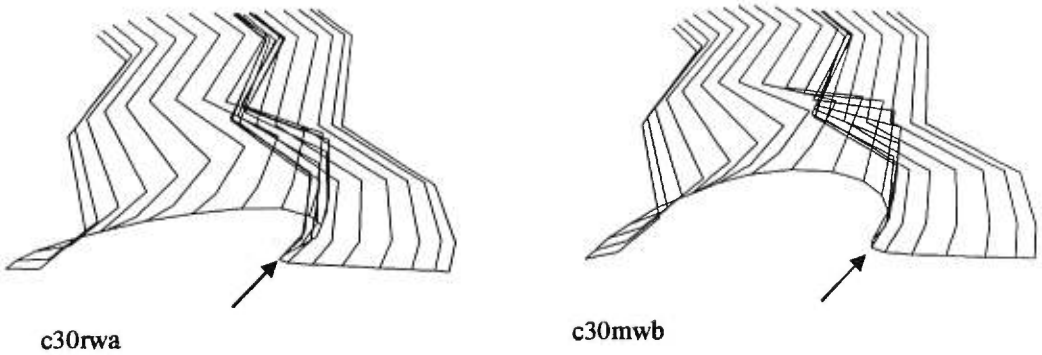
A **Clonidine (3.8mM/100µl i.t.)**

cc4 (38d)



B **Methoxamine (4mM/100µl i.t.)**

cc4 (46d)



C **Noradrenaline (10mM/100µl i.t.)**

cc8 (27d)

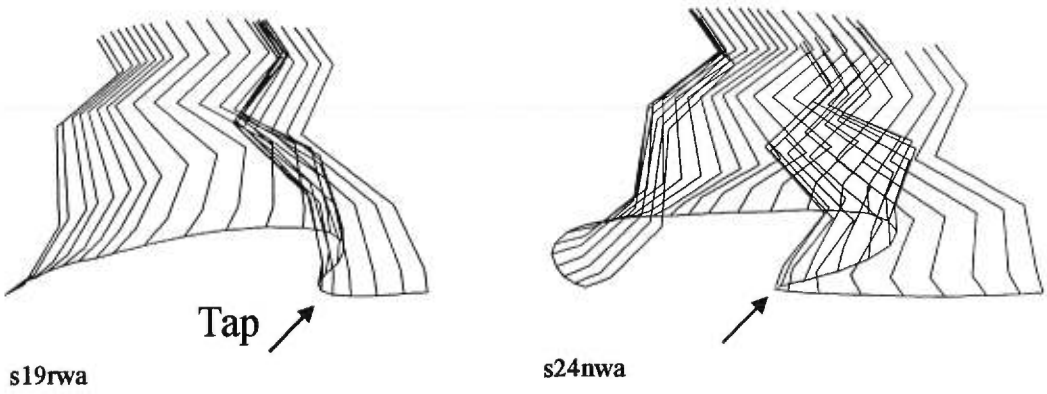


Figure 14.

A comparison of responses to electrical stimulation of the superficial peroneal nerve of CC7 at rest (standing) after different drugs.

A. The averaged response of 20 and 10 stimuli before and after Clonidine injection, respectively. The current delivered before Clonidine injection was 0.75mA and was 3.0mA following injection. No response can be seen even at this current.

B. Averaged response of 9 and 10 stimuli before and after Methoxamine, respectively. The current of the stimulation stayed the same (0.6mA) before and after Methoxamine injection.

C. Averaged response to 10 and 15 stimuli before and after NE injection, respectively. The current of stimulation before and after NE injection was 0.5mA.

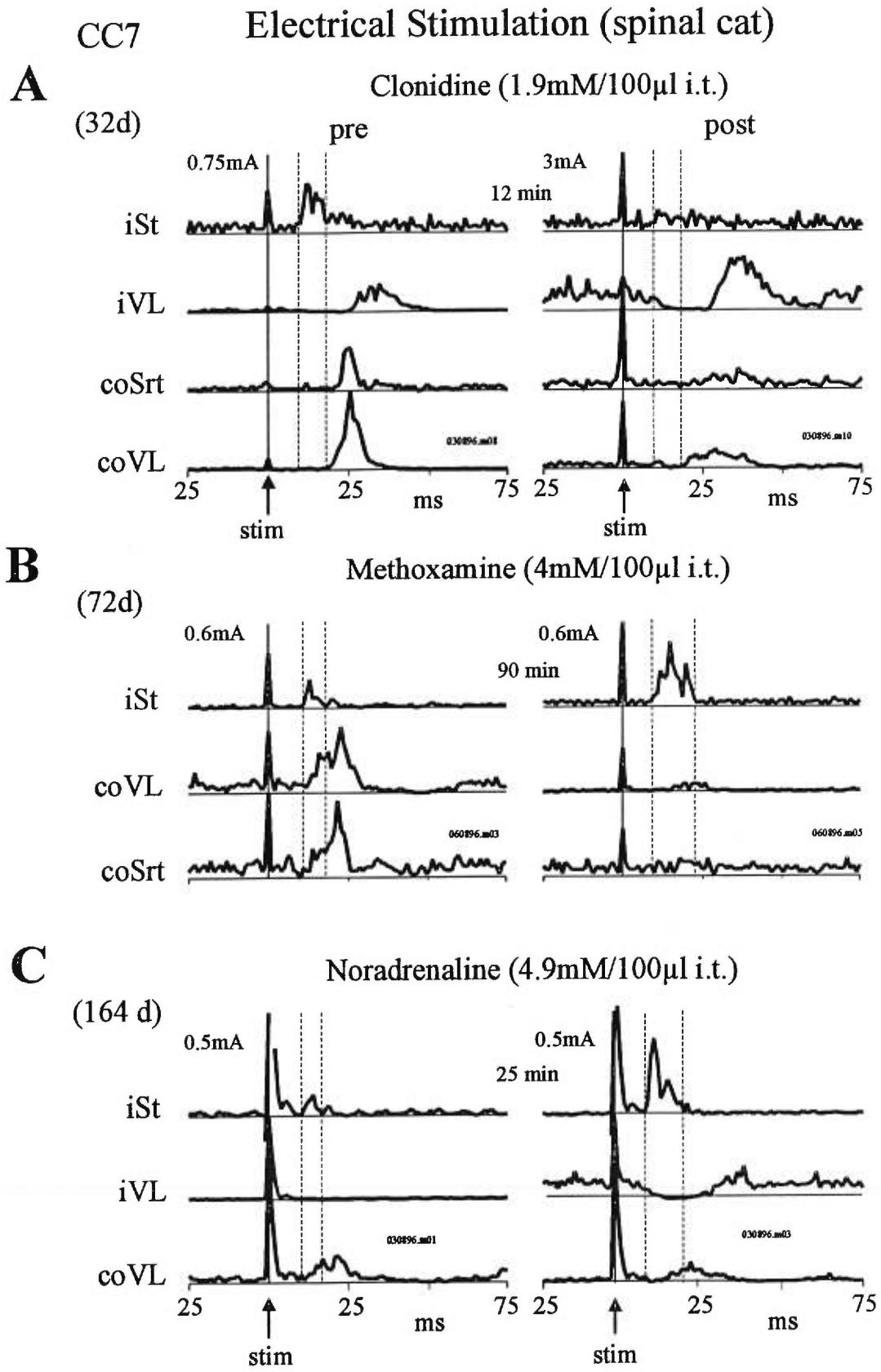
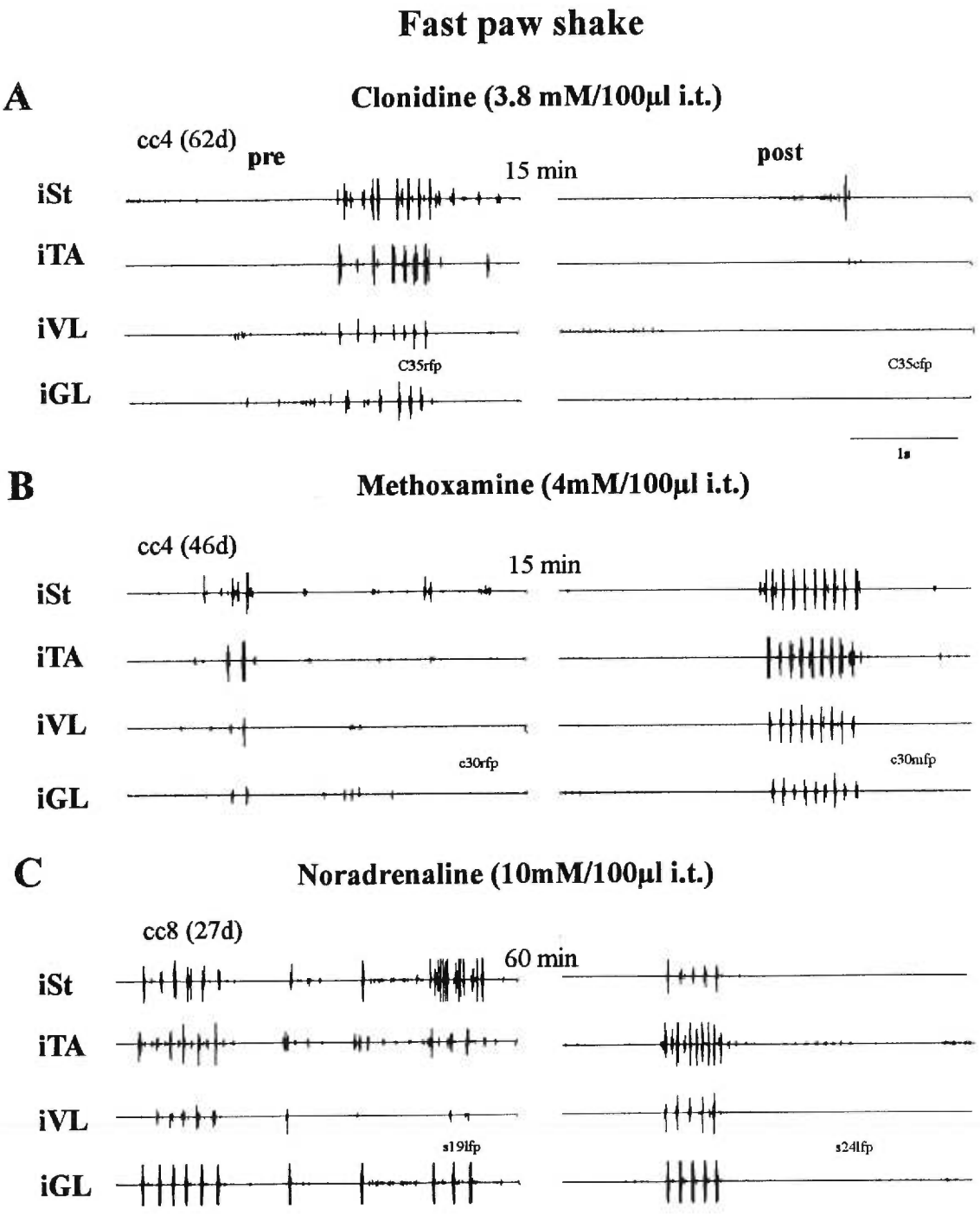

CC7
Electrical Stimulation (spinal cat)
A
Clonidine (1.9mM/100µl i.t.)
(32d)
pre
post
0.75mA
3mA
12 min
iSt
iVL
coSrt
coVL
25
25
ms
75
stim
B
Methoxamine (4mM/100µl i.t.)
(72d)
0.6mA
0.6mA
90 min
iSt
coVL
coSrt
C
Noradrenaline (4.9mM/100µl i.t.)
(164 d)
0.5mA
0.5mA
25 min
iSt
iVL
coVL

Figure 15.

Fast paw shake (FPS) responses before and after Clonidine, Methoxamine and NE injection in CC4 (62d), CC4(46d), and CC8(27d), respectively. FPS was evoked by dipping the paw in lukewarm water, and is indicated by raw EMG traces of hindlimb flexors and extensors in spinal cats before and after different drug injections.


Fast paw shake
A
Clonidine (3.8 mM/100µl i.t.)
cc4 (62d)
pre
15 min
post
iSt
iTA
iVL
iGL
C35rfp
C35cfp
1s
B
Methoxamine (4mM/100µl i.t.)
cc4 (46d)
15 min
iSt
iTA
iVL
iGL
c30rfp
c30mfp
C
Noradrenaline (10mM/100µl i.t.)
cc8 (27d)
60 min
iSt
iTA
iVL
iGL
s191fp
s241fp

GENERAL DISCUSSION

Two questions were addressed in this project: 1) will intensive interactive locomotor training given soon after spinal cord transection enhanced locomotor recovery?; 2) what are the roles of different α2- and α1- noradrenergic agonists on locomotion and cutaneous reflex transmission soon after transection and at a later stage after transection? We found that early intensive interactive locomotor training with Clonidine enhanced locomotor recovery, and different α2- and α1- noradrenergic agonists can mediate selective functions on locomotion and reflexes. The detailed results of these questions were presented in the first and second article, respectively.

In the general discussion, the findings and interpretations will first be summarized. The detailed discussion points made in each article will not be repeated here. Then we will discuss the clinical implications of these results and the use of pharmacological tools in the rehabilitation of locomotion in spinal cord injured patients. Finally, some questions for future research are proposed in the hope of further advancing the use of pharmacotherapy in rehabilitation.

Summary of results and discussion

Daily Clonidine with early locomotor training enhances the recovery of locomotion (article 1).

In the present study, spinal cats were injected with Clonidine on d3 post-transection which enabled them to walk on the treadmill with weight support, thus allowing efficient locomotor training. It was shown that the 5 spinal cats that received daily injections of Clonidine to allow early locomotor training, recovery of locomotion could be seen as early as 6-11d post-transection at which time they were able to walk with weight support and proper foot contact. The ability of the cats to walk on the treadmill with weight support and plantar foot placement consecutively for at least 8 step cycles without any drug was taken as an indication of locomotor recovery. The recovery period was shorter than that reported in the literature (3-4 weeks) (Barbeau and Rossignol 1987; Bélanger et al. 1996). However, in those studies, early training could not have been as effective since the cats were not walking with any weight support during the first week post-transection.

Locomotor recovery is a progressive process and is revealed by Clonidine (article 1).

The day to day improvement (d3-d4-d7) of locomotion was apparent only after Clonidine injection as the cats were not walking before Clonidine injection during that period. Thus, Clonidine revealed an ongoing recovery process starting

as early as the first day after training suggesting that there is a marked plasticity of the spinal cord. It is possible that training might have induced or interacted with the ongoing plastic changes of the spinal cord following transection such as neurochemical (eg. up-regulation of noradrenergic receptors), anatomical (eg. collateral sprouting) or physiological changes in a form of "spinal learning".

While Clonidine can initiate locomotion which allows training, little is known about other noradrenergic agonists. It is important to explore the roles of other noradrenergic agonists in initiating and modulating locomotion as this will allow us to better understand the receptors involved in mediating the locomotor functions and also to possibly identify the most beneficial noradrenergic drug for clinical testing.

Differential effects of α1- and α2-noradrenergic agonists on locomotion and cutaneous excitability, noradrenaline exerted both α1- and α2- effects (article 2).

In early-spinal cats, α2-agonists such as Clonidine, Tizanidine and Oxymetazoline were all capable to initiate sustained and robust locomotion in early-spinal cats consistently (within 8 days post-transection). Methoxamine (α1-agonist), on the other hand, only triggered sustained organized locomotion in 1 cat, and in 2 cats, triggered only transient bouts of organized locomotion.

In late-spinal cats, all α2 agonists modulated the locomotor pattern similarly by increasing the step cycle duration, especially, the swing duration, accompanied

by a marked increase in the flexor muscle activity. Clonidine decreased the extensor muscle activity in a dose-dependent fashion. Methoxamine, on the other hand, did not modulate the cycle duration, but increased the extensor tonus. Also while a paw drag and knee sag was frequently observed after α2-agonist injections, it was not observed in Methoxamine-modulated locomotion.

All α2 agonists, especially Clonidine, also markedly reduced the cutaneous excitability in late-spinal cats whereas methoxamine increased the cutaneous excitability.

Noradrenaline was found to also initiate and modulate locomotion similarly as the α2-agonists. However, it was also found to increase cutaneous excitability similarly to Methoxamine (α1-agonist). The mixed effects exerted by noradrenaline is not surprising as being the neurotransmitter, it would act on both the α1- and α2-adrenoceptors.

These results support the existing literature which shows that α1- and α2-agonist mediate different effects. The depressant effects of α2- agonists on reflex transmission were also in agreement with the existing reports (see introduction). Our findings also suggest that while α2-agonists are more involved in controlling the timing of the muscle activation, α1-agonist, Methoxamine is involved in increasing the output of the muscle activity, especially that of extensors.

The Time course of actions of the different noradrenergic agonists is different (article 2).

Clonidine and Tizanidine (α2 agonists) exert effects almost immediately following their injection (within minutes), which lasted for a few hours and completely disappeared the next day. Oxymetazoline (α2-agonist) and Methoxamine (α1-agonist) had slower onset of actions (hours) but much longer lasting effects (2-3 days and 1 day, respectively).

The different time course may be related to pharmacological properties of the drugs as well as noradrenergic involvement in mechanisms such as immediate early gene (IEG) expression and long-term potentiation (LTP).

In summary, our studies showed that in chronic spinal cats, 1) early intensive locomotor training and daily Clonidine injections accelerated and enhanced hindlimb locomotor recovery, 2) selective α2- and α1- noradrenergic agonists play a different role in initiating and modulating locomotion as well as the cutaneous excitability. While all α2 agonists consistently initiated robust organized locomotion within the first week post-transection, the α1 agonist (methoxamine) tested did not. α2- and α1- agonists also attenuated or augmented, respectively, cutaneous reflexes. Noradrenaline appears to exert mixed α1- and α2- effects.

Clinical implications

Conventional therapeutic techniques used in rehabilitation clinics for patients with spinal cord injury usually emphasize muscle strengthening, reduction of spasticity, maintenance of joint mobility through active and passive range of motion exercises, balance and gait training. These are important as they help prevent the development of muscles contractures, and help maintaining muscle strength and help restoring some balance control which are crucial in function such as transfer ability or activities of daily living (ADL). However, conventional therapy especially with respect to gait training has been unsatisfactory and limited. Most gait training was done in the parallel bar where a considerable amount of the body weight (depending on the level of spinal lesion) was supported by the forearms of the patients. The posture of the patient is usually not upright but with hip and knee in a flexed position.

Advances in the field of rehabilitation of spinal cord injured patients have been made. For example, based on previous animal findings, clinical trials have supported that interactive locomotor training enhanced the recovery of locomotor functions in complete and incomplete paraplegic patients (Visintin and Barbeau 1989; Barbeau et al. 1992; Fung et al. 1990; Wernig and Muller 1992; Dietz et al. 1994; Dietz et al. 1995; Visintin and Barbeau 1994; Wernig et al. 1995; Stewart et al. 1991). Interactive training usually involves locomotion on a motor driven

treadmill with the body weight supported by a parachute harness as well as assisted by a therapist who manually helps when necessary to initiate and maintain stepping. In the literature, improvements in locomotion were usually measured by the reduction in the body weight support needed, the increase in distance covered on the treadmill or the ability to walk at an increased treadmill speed (Wernig and Muller 1992; Dietz et al. 1995; Dietz et al. 1994; Fung et al. 1990). However, in patients with functionally complete paraplegia, sometimes no significant improvement was found despite daily training (Wernig et al. 1995). Other forms of rehabilitation include the use of gait orthosis (Bernardi et al. 1995) and functional electrical stimulation (FES)(Wieler et al. 1995; Stein et al. 1990). Results showed that some functional improvement can be achieved with FES such as increase in walking speeds. A recent study showed that FES, however, did not increase the muscle bulk nor did it modify the muscle fibers in paraplegic patients (Greve et al. 1993). The use of reciprocating gait orthosis does not seem efficient as it requires high energy exertion by the patient where they have to stop every few steps otherwise the exercise became anaerobic (Bernardi et al. 1995).

To date, the use of pharmacotherapy (mostly Clonidine) as part of the rehabilitation strategy has been in patients with locomotor deficits from spinal cord injury or stroke, and has been limited and conflicting. For example, in patients with clinically complete spinal cord injury, Clonidine did not markedly improve locomotion (Stewart et al. 1991; Norman and Barbeau, 1993) or deteriorate locomotion (Dietz

et al. 1995) whereas NE enhanced it (Dietz et al. 1995). Barbeau and colleagues reported that in 2 patients with chronic incomplete spinal cord injuries, locomotion was enhanced with a treatment regimen which incorporated the combined effects of Clonidine and cyproheptadine (a serotonergic antagonist) together with a training program of the treadmill while the patient was supported by a body weight support harness system (Fung et al. 1990). Other studies showed that Clonidine improved the walking speed and reduced spasticity in 3 out of 8 spinal cord injured subjects, but did not change or deteriorate (with higher dose) locomotion in the other ones (Remy-Neris et al. 1996; Barbeau et al. 1997). Thus, despite advances made in the rehabilitation field to improve locomotion in patients with spinal cord injury, much improvement is still needed.

Our first study shows that locomotor training with Clonidine could be beneficial to patients if started early after injury. At an early stage post-injury, Clonidine may be useful in eliciting locomotion upon which training can be performed. A possible mechanism is the ability of Clonidine and daily training during this early post-spinalisation period to interact maximally with the segmental plastic changes occurring in the cord. In the literature, locomotor training was often delayed. Locomotor training usually did not start before 1 year after injury. A recent study separated 2 groups of patients into acute (6-13 months) and chronic groups (>1year) depending on the time lapse between the commencement and training and time of injury (Wernig et al. 1995). The acute group was found to achieve greater

functional improvements than the chronic group. As compared to the chronic group, the acute group comprised a larger number of patients that became non-wheelchair-bound and who were originally wheelchair-bound (Wernig et al. 1995). Thus, It is possible that *earlier* training may have a more potent effect on the outcome of the locomotor recovery.

Our second study emphasized the use of various pharmacological agents, which can be powerful tools as they can affect different parameters of locomotion such as rhythm generation, output of the locomotor pattern, cutaneous transmission, postural control and tonus. We showed that selective activation of α2- and α1-noradrenergic receptors can mediate different functions, and that among the α2 noradrenergic agonist, some differences can also be seen. This further suggests the complexity of the use of drugs in attempts to enhance motor control. However, a better understanding of the basic pharmacology of the drug, such as the receptors involved, the time course of action, as well as the physiological effects it mediates, is essential and fundamental and will enhance our ability to use pharmacotherapy as a rehabilitation tool. Our study suggests that different noradrenergic agonists could be used to target specific deficits. For example, α1 agonist may be more important in improving posture or the output of the pattern as it increases the sensory transmission and amplitude of the hindlimb muscle activity. On the other hand, α2 agonists may have a more important role in releasing the spinal locomotor rhythm and reducing spasticity. In the later post-transection stage,

however, due to the marked depressive effects of Clonidine on sensory transmission, its use for improving locomotor performance could be less desirable.

To summarize, our study showed that early training with clonidne soon after transection can enhance recovery of locomotion. The use of selective drugs can also target specific locomotor deficits. These are important concepts that underlie the development of new rehabilitation strategies for patients with spinal cord injury and may further advance our ability to enhance locomotor recovery.

Future research

A better understanding of the use of pharmacology will no doubt be essential to further our ability to use pharmacological tools more effectively in the field of rehabilitation. In this study, we have studied the specific effects of different noradrenergic agonists on locomotion and cutaneous reflexes. However, in the continual pursuit of a pharmacological approach for enhancement of locomotor function, a lot of questions remain to be answered.

First, most pharmacological work has been done in acute preparations, or in isolated spinal cord preparations, with obvious advantages in exploring the effects of different drugs (Kerkut and Bagust 1995). However, it is also important to transfer this knowledge and study the effect of drugs in a awake behaving chronic animal especially if we hope to incorporate pharmacotherapy as a treatment strategy in rehabilitation of patients. For example, while there are accumulating evidences that EAA activate the spinal locomotor centers and induce locomotion in a range of species (see introduction), our recent work show that EAA was unable to trigger locomotion in awake chronic spinal cats (Chau et al. 1994). In late-spinal cat, however, an NMDA receptor antagonist blocked the locomotion, which was restored by NMDA thus suggesting the importance of NMDA receptors in locomotor functions (Chau et al. 1994).

Second, other drugs such as inhibitory amino acids (GABA or glycine) have been shown to be important in modulating different aspects of the locomotion and should be investigated in more details.

A better understanding of the inhibitory amino acids is also of particular interest as GABAergic agonist, Baclofen, has been used routinely in the clinic. While its effects on reducing spasticity is established, its effects on locomotor function seems less promising. Following spinal cord transection, it has been shown that the level of GABA and GAD67 mRNA increased, suggesting an increase in inhibition in segments below the lesion (Edgerton et al. 1997). The extent of

recovery has been suggested to be limited by an increase in inhibitory processes at the segmental level (Goldberger, 1974). It has been shown that a GABAergic antagonist, Bicucculine enhanced locomotor recovery in chronic kittens with spinal cord transection (Robinson and Goldberger 1986). The activation of GABAergic or glycinergic receptors has been shown to abolish locomotor rhythm in isolated neonatal rats spinal cord (Cazalets et al. 1994; Cowley and Schmidt 1995). Preliminary work has also been done in this laboratory and shows that Baclofen deteriorated the established locomotor pattern in chronic spinal cats (Chau et al. 1995). It is therefore important to further examine the role of GABAergic agonists on locomotion.

CONCLUSION

A better understanding of the complexity of the changes occurring in the spinal cord after an injury in animals is indeed essential to understand how to help in the rehabilitation of sensory-motor functions in man. Two major contributions of this work were the use of locomotor training during the early recovery period using a noradrenergic agent, and the possibility to use more specific noradrenergic agents to enhance locomotor recovery or the expression of the locomotor pattern. We also showed that a great deal of spinal segmental plasticity is possible soon following spinal cord transection.

Overall, this work has helped to develop a preclinical model in which various approaches (physiological and pharmacological) can be evaluated before further investigations are made in humans.

REFERENCES

(General introduction and discussion)

Amos, A., Armstrong, D. M., and Marple-Horvat, D. E. Responses of motor cortical neurones in the cat to unexpected perturbations of locomotion. *Neurosci. Lett.* 104: 147-151, 1989.

Anden, N. E., Jukes, M. G., and Lundberg, A. The effect of DOPA on the spinal cord. 2. A pharmacological analysis. *Acta physiol. scand.* 67: 387-397, 1966a.

Anden, N. E., Jukes, M. G., Lundberg, A., and Vyklicky, L. The effect of DOPA on the spinal cord. 3. Depolarization evoked in the central terminals of ipsilateral Ia afferents by volleys in the flexor reflex afferents. *Acta physiol. scand.* 68: 322-336, 1966b.

Anden, N. E., Jukes, M. G., Lundberg, A., and Vyklicky, L. The effect of DOPA on the spinal cord. 1. Influence on transmission from primary afferents. *Acta physiol. scand.* 67: 373-386, 1966c.

Andersson, O., Forssberg, H., Grillner, S., and Lindquist, M. Phasic gain control of the transmission in cutaneous reflex pathways in motoneurones during "fictive" locomotion. *Brain Res.* 149: 503-507, 1978a.

Andersson, O., Forssberg, H., and Lindquist, M. Phase dependent modulation of the transmission in reflex pathways during "fictive locomotion". *Acta physiol. scand.* 102: 8A, 1978b.

Armstrong, D. M. Supraspinal contributions to the initiation and control of locomotion in the cat. *Prog. Neurobiol.* 26: 273-361, 1986.

Armstrong, D. M. and Marple-Horvat, D. E. Role of the cerebellum and motor cortex in the regulation of visually controlled locomotion. [Review] [55 refs]. *Can. J. Physiol. Pharmacol.* 74: 443-455, 1996.

Arshavsky, Y. I., Gelfand, I. M., and Orlovsky, G. N. The cerebellum and control of rhythmical movements. *TINS* 6: 417-422, 1983.

Barbeau, H. and Bedard, P. Denervation supersensitivity to 5-HT in rats following spinal transection and 5,7 dihydroxytryptamine injection. *Neuropharmacol.* 20: 611-616, 1981.

Barbeau, H., Chau, C., and Rossignol, S. Noradrenergic agonists and locomotor training affect locomotor recovery after cord transection in adult cats. *Brain Res. Bull.* 30: 387-393, 1993.(see appendix A)

Barbeau, H., Dannakas, M., and Arsenault, B. The effects of locomotor training in spinal cord injured subjects: a preliminary study. *Restorative Neurol. and Neurosci.* 12: 93-96, 1992.

Barbeau, H., Julien, C., and Rossignol, S. The effects of clonidine and yohimbine on locomotion and cutaneous reflexes in the adult chronic spinal cat. *Brain Res.* 437: 83-96, 1987.

Barbeau, H., Pepin, A., Norman, K., Ladoucer, M., and Leroux, A. Walking following spinal cord injury: control and recovery. *The Neuroscientist* 1997. (in press)

Barbeau, H. and Rossignol, S. Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res.* 412: 84-95, 1987.

Barbeau, H. and Rossignol, S. Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. *Brain Res.* 546: 250-260, 1991.

Barthe, J. Y. and Grillner, S. Neurotensin-induced modulation of spinal neurons and fictive locomotion in the lamprey. *J. Neurophysiol.* 73: 1308-1312, 1995.

Bedard, P., Barbeau, H., Barbeau, B., and Filion, M. Progressive increase of motor activity induced by 5-HTP in the rat below a complete section of the spinal cord. *Brain Res.* 169: 393-397, 1979.

Bélanger, M., Drew, T., Provencher, J., and Rossignol, S. A comparison of treadmill locomotion in adult cats before and after spinalization. *J. Neurophysiol.* 76: 471-491, 1996.

Bernardi, M., Canale, I., Castellano, V., Di Filippo, L., Felici, F., and Marchetti, M. The efficiency of walking of paraplegic patients using a reciprocating gait orthosis. *Paraplegia* 33: 409-415, 1995.

Bjorklund, A. and Lindvall, O. Catecholaminergic brain stem regulatory systems. In: *Handbook of physiology. The nervous system IV.* edited by V. B. Mountcastle, F. E. Bloom and S. R. Geiger. Bethesda: American Physiological society, 1986, p. 155-235.

Bjorklund, A. and Skagerberg, G. Descending monoaminergic projections to the spinal cord. In: *Brain stem control of spinal mechanisms*, edited by B. Sjolund and

A. Bjorklund. North-Holland: Elsevier Biomedical Press, 1982, p. 55-88.

Bradley, N. S. and Smith, J. L. Neuromuscular patterns of stereotypic hindlimb behaviors in the first postnatal months. II. Stepping in spinal kittens. *Develop.Brain Res.* 38: 53-67, 1988.

Bras, H., Cavallari, P., Jankowska, E., and McCrea, D. A. Comparison of effects of monoamines on transmission in spinal pathways from group I and II muscle afferents in the cat. *Exp.Brain Res.* 76: 27-37, 1989.

Bras, H., Jankowska, E., Noga, B., and Skoog, B. Comparison of effects of various types of NA and 5-HT agonists on transmission from group II muscle afferents in the cat. *Eur. J. Neurosci.* 211: 1029-1039, 1990.

Brodin, L. and Grillner, S. The role of putative excitatory amino acid neurotransmitters in the initiation of locomotion in the lamprey spinal cord. I. The effects of excitatory amino acid antagonists. *Brain Res.* 360: 139-148, 1985a.

Brodin, L. and Grillner, S. The role of putative excitatory amino acid neurotransmitters in the initiation of locomotion in the lamprey spinal cord. II. The effects of amino acid uptake inhibitors. *Brain Res.* 360: 149-158, 1985b.

Brodin, L., Grillner, S., Dubuc, R., Ohta, Y., Kasicki, S., and Hökfelt Reticulospinal neurons in lamprey: transmitters, synaptic interactions and their role during locomotion. *Arch. ital. Biol.* 126: 317-345, 1988.

Brodin, L., Grillner, S., and Rovainen, C. M. N-methyl-D-aspartate (NMDA), kainate and quisqualate receptors and the generation of fictive locomotion in the lamprey spinal cord. *Brain Res.* 325: 302-306, 1985.

Brodin, L., Theodorsson, E., Christenson, J., Cullheim, S., Hokfelt, T., Brown, J. C., Buchan, A., Panula, P., Verhofstad, A., and Goldstein, M. Neurotensine-like peptides in the CNS of lampreys: chromatographic characterization and immunohistochemical localizatoin with reference to aminergic markers. *Eur. J. Neurosci.* 2: 1095-1109, 1990.

Brown, T. G. The intrinsic factors in the act of progression in the mammal. *Proc. Roy. Soc. London B.* 84: 308-319, 1911.

Brown, T. G. On the nature of the fundamental activity of the nervous centres together with an analysis of the conditioning of rhythmic activity in progression and a theory of the evolution of function in the nervous system. *J. Physiol.* 48: 18-46, 1914.

Brustein, E. and Rossignol, S. Recovery of treadmill locomotion after bilateral chronic ventral and ventrolateral spinal lesion in the adult cat: deficits and adaptative mechanisms. *J. Neurophysiol.* submitted1997.

Calancie, B., Needham-Shropshire, B., Jacobs, P., Willer, K., Zych, G., and Green, B. A. Involuntary stepping after chronic spinal cord injury.Evidence for a central rhythm generator for locomotion in man. *Brain* 117: 1143-1159, 1994.

Carlsson, A., Falck, B., Fuxe, K., and Hillarp, N. A. Cellular localization of monoamines in the spinal cord. *Acta physiol. scand.* 60: 112-119, 1964.

Carp, J. S. and Wolpaw, J. R. Motorneuron plasticity underlying operantly conditioned decrease in primate H-reflex. *J. Neurophysiol.* 72: 431-442, 1994.

Carp, J. S. and Wolpaw, J. R. Motorneuron propertities after operantly conditioned increase in primate H-reflex. *J. Neurophysiol.* 73: 1365-1373, 1995.

Carr, P. A., Huang, A., Noga, B. R., and Jordan, L. M. Cytochemical characteristics of cat spinal neurons activated during fictive locomotion. *Brain Res. Bull.* 37: 213-218, 1995.

Carrier, L., Brustein, E., and Rossignol, S. Locomotion of the hindlimbs following a neurectomy of the ankle flexors in intact and spinal cats: a model for study of plasticity. *J. Neurophysiol.* 77: 1979-1993, 1997.

Cattaert, D., Pearlstein, E., and Clarac, F. Cholinergic control of the walking network in the crayfish Procambarus clarkii. *J.Physiol.(Paris)* 89: 209-220, 1995.

Cazalets, J. R., Grillner, P., Menard, I., Cremieux, J., and Clarac, F. Two types of motor rhythm induced by NMDA and amines in an in vitro spinal cord preparation of neonatal rat. *Neurosci. Lett.* 111: 116-121, 1990.

Cazalets, J. R., Sqalli-Houssaini, Y., and Clarac, F. Activation of the central pattern generators for locomotion by serotonin and excitatory amino acids in neonatal rat. *J. Physiol.* 455: 187-204, 1992.

Cazalets, J. R., Sqalli-Houssaini, Y., and Clarac, F. GABAergic inactivation of the central pattern generators for locomotion in isolated neonatal rat spinal cord. *J. Physiol.* 474: 173-181, 1994.

Chau, C., Provencher, J., Lebel, F., Barbeau, H., and Rossignol, S. Effects of GABAergic drugs on locomotion in adult chronic spinal cats. *Soc. Neurosci. Abstr.* 21, no 173.12, p.420, 1995.

Chau, C., Provencher, J., Lebel, F., Jordan, L., Barbeau, H., and Rossignol, S. Effects of intrathecal injection of NMDA receptor agonist and antagonist on locomotion of adult chronic spinal cats. *Soc. Neurosci. Abstr.* 20 no.241.14: 573, 1994.

Chen, D. F., Bianchetti, M., and Wiesendanger, M. The adrenergic agonist tizandine has differential effects on flexor reflexes of intact and spinalized rat. *Neurosci.* 23: 641-647, 1987.

Cohen, A. H. and Wallen, P. The neuronal correlate of locomotion in fish. *Exp. Brain. Res.* 41: 11-18, 1980.

Conway, B. A., Hultborn, H., and Kiehn, O. Proprioceptive input resets central locomotor rhythm in the spinal cat. *Exp.Brain Res.* 68: 643-656, 1987.

Conway, B. A., Hultborn, H., Kiehn, O., and Mintz, I. Plateau potentials in alpha-motoneurones induced by intravenous injection of L-Dopa and clonidine in the spinal cat. *J. Physiol.* 405: 369-384, 1988.

Cooper, J. R., Bloom, F. E., and Roth, R. H. The biochemical basis of neuropharmacology. New York: Oxford University Press, 1991. p.220.

Corboz, M., Palmer, C. I., Palmeri, A., and Wiesendanger, M. Tizanidine-induced depression of polysynaptic cutaneous reflexes in nonanesthetized monkeys is mediated by an alpha 2-adrenergic mechanism. *Experimental. Neurology* 111: 210-216, 1991.

Cowley, K. C. and Schmidt, B. J. A comparison of motor patterns induced by N-methyl-D-aspartate, acetylcholine and serotonin in the in vitro neonatal rat spinal cord. *Neurosci. Lett.* 171: 147-150, 1994.

Cowley, K. C. and Schmidt, B. J. Effects of inhibitory amino acid antagonists on reciprocal inhibitory interactions during rhythmic motor activity in the in vitro neonatal rat spinal cord. *J. Neurophysiol.* 74: 1109-1117, 1995.

Dahlstrom, A. and Fuxe, K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta physiol. scand.* 62: 1-79, 1964.

Dai, X., Douglas, J.R., Nagy, J.I., Noga, B.R., and Jordan, L.M. Localization of spinal neurons activated during treadmill locomotion using the c-fos immunohistochemical method. *Soc.Neurosci.Abstr.* 16:889 no.368.4, 1990.

Dale, N. and Roberts, A. Excitatory amino acid receptors in *Xenopus* embryo spinal cord and their role in the activation of swimming. *J. Physiol.* 348: 527-543, 1984.

Davies, J. and Quinlan, J. E. Selective inhibition of responses of feline dorsal horn neurons to noxious cutaneous stimuli by tizandine (DS103-282) and noradrenaline: involvement of α2-adrenoceptors. *Neurosci.* 16: 673-782, 1985.

Degtyarenko, A. M., Simon, E. S., and Burke, R. E. Differential modulation of disynaptic cutaneous inhibition and excitation in ankle flexor motoneurons during fictive locomotion. *J. Neurophysiol.* 76: 2972-2985, 1996.

Delcomyn, F. Neural basis of rhythmic behavior in animals. *Science* 210: 492-498, 1980.

Dietz, V., Colombo, G., and Jensen, L. Locomotor activity in spinal man. *The lancet* 344: 1260-1263, 1994.

Dietz, V., Colombo, G., Jensen, L., and Baumgartner, L. Locomotor capacity of spinal cord in paraplegic patients. *Ann. Neurol.* 37: 574-582, 1995.

Douglas, J. R., Noga, B. R., Dai, X., and Jordan, L. M. The effects of intrathecal administration of excitatory amino acid agonists and antagonists on the initiation of locomotion in the adult cat. *J. Neurosci.* 13: 990-1000, 1993.

Drew, T. The role of the motor cortex in the control of gait modification in the cat. In: *Neurobiological basis of human locomotion*, edited by M. Shimamura, S. Grillner and V. R. Edgerton. Tokyo: Japan Scientific Societies Press, 1991a, p. 201-212.

Drew, T. Functional organization within the medullary reticular formation of the intact unanesthetized cat.III.Microstimulation during locomotion. *J. Neurophysiol.* 66: 919-938, 1991b.

Drew, T. Motor cortical activity during voluntary gait modifications in the cat.I. Cells related to the forelimbs. *J. Neurophysiol.* 70: 179-199, 1993.

Drew, T., Cabana, T., and Rossignol, S. Responses of medullary reticulospinal neurones to stimulation of cutaneous limb nerves during locomotion in intact cats. *Exp. Brain. Res.* 111: 153-168, 1996a.

Drew, T., Dubuc, R., and Rossignol, S. Discharge patterns of reticulospinal and other reticular neurons in chronic, unrestrained cats walking on a treadmill. *J. Neurophysiol.* 55: 375-401, 1986.

Drew, T., Jiang, W., Kably, B., and Lavoie, S. Role of the motor cortex in the control of visually triggered gait modifications. *Can. J. Physiol. Pharmacol.* 74: 426-442, 1996b.

Drew, T. and Rossignol, S. Phase-dependent responses evoked in limb muscles by stimulation of medullary reticular formation during locomotion in thalamic cats. *J. Neurophysiol.* 52: 653-675, 1984.

Dubuc, R., Cabelguen, J.-M., and Rossignol, S. Rhythmic antidromic discharges of single primary afferents recorded in cut dorsal roots filaments during locomotion in the cat. *Brain Res.* 359: 375-378, 1985.

Dubuc, R., Cabelguen, J.-M., and Rossignol, S. Rhythmic fluctuations of dorsal root potentials and antidromic discharges of single primary afferents during fictive locomotion in the cat. *J. Neurophysiol.* 60: 2014-2036, 1988.

Dubuc, R., Rossignol, S., and Lamarre, Y. The effects of 4-aminopyridine on the spinal cord: rhythmic discharges recorded from the peripheral nerves. *Brain Res.* 369: 243-259, 1986.

Durkovic, R. G. Classical conditioning of the flexion reflex in spinal cat: features of the reflex circuitry. *Neurosci. Lett.* 39: 155-160, 1983.

Duysens, J. and Pearson, K. G. Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. *Brain Res.* 187: 321-332, 1980.

Edgerton, V. R., de Guzman, C. P., Gregor, R. J., Roy, R. R., Hodgson, J. A., and Lovely, R. G. Trainability of the spinal cord to generate hindlimb stepping patterns in adult spinalized cats. In: *Neurobiological basis of human locomotion*, edited by M. Shimamura, S. Grillner and V. R. Edgerton. Tokyo: Japan scientific societies press, 1991, p. 411-423.

Edgerton, V. R., de Leon, R. D., Tillakaratne, N., Recktenwald, M. R., Hodgson, J. A., and Roy, R. R. Use-dependent plasticity in spinal stepping and standing. *Adv. Neurol.* 72: 233-247, 1997.

Edgley, S. A. and Jankowska, E. An interneuronal relay for group I and II muscle afferents in the midlumbar segments of the cat spinal cord. *J. Physiol.* 389: 647-674, 1987.

Eidelberg, E., Story, J. L., Meyer, B. L., and Nystel, J. Stepping by chronic spinal cats. *Exp. Brain. Res.* 40: 241-246, 1980.

Eken, T., Hultborn, H., and Kiehn, O. Possible functions of transmitter-controlled plateau potentials in alpha-motoneurones. *J. Physiol.* 80: 257-267, 1989.

Engberg, I. and Lundberg, A. An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. *Acta physiol. scand.* 75: 614-630, 1969.

Fenaux, F., Corio, M., Palisses, R., and Viala, D. Effects of an NMDA-receptor antagonist, MK-801,on central locomotor programming in the rabbit. *Exp.Brain Res.* 86: 393-401, 1991.

Forssberg, H. Stumbling corrective reaction: a phase-dependent compensatory reaction during locomotion. *J. Neurophysiol.* 42: 936-953, 1979.

Forssberg, H. and Grillner, S. The locomotion of the acute spinal cat injected with clonidine i.v. *Brain Res.* 50: 184-186, 1973.

Forssberg, H., Grillner, S., and Halbertsma, J. The locomotion of the low spinal cat. I. Coordination within a hindlimb. *Acta physiol. scand.* 108: 269-281, 1980a.

Forssberg, H., Grillner, S., Halbertsma, J., and Rossignol, S. The locomotion of the low spinal cat: II. Interlimb coordination. *Acta physiol. scand.* 108: 283-295, 1980b.

Forssberg, H., Grillner, S., and Rossignol, S. Phase dependent reflex reversal during walking in chronic spinal cats. *Brain Res.* 85: 103-107, 1975.

Forssberg, H., Grillner, S., and Sjostrom, A. Tactile placing reactions in chronic spinal kittens. *Acta physiol. scand.* 92: 114-120, 1974.

Forssberg, H. and Svartengren, G. Hardwired locomotor network in cat revealed by a retained motor pattern to gastrocnemius after muscle transposition. *Neurosci. Lett.* 41: 283-288, 1983.

Fung, J., Stewart, J. E., and Barbeau, H. The combined effects of clonidine and cyproheptadine with interactive training on the modulation of locomotion in spinal cord injured subjects. *J. Neurol. Sci.* 100: 85-93, 1990.

Giroux, N., Aloyz, R. S., Rossignol, S., and Reader, T. A. Serotonin 1a and $\alpha 1$ and $\alpha 2$-noradrenergic receptors in the spinal cord of spinalized cats. *Soc. Neurosci. Abstr.* 21: 926 no.369.31995.

Giroux, N., Rossignol, S., and Reader, T. An autoradiographic study of α_1-,α_2-noradrenergic and serotonin $_{1A}$ receptors in the spinal cord of normal and chronically transected cats. *J. Comp. Neurol.* submitted: 1997.

Goldberg, M. R. and Robertson, D. Yohimbine:A pharmacological probe for study of the α2-adrenoreceptor. *Pharmacol. Rev.* 35: 143-180, 1983.

Goldberger, M. E. Recovery of movement after CNS lesions in monkeys. In: *Recovery of function after neural lesion*, edited by D. Stein. New York: Academia, 1974, p. 265-337.

Goldberger, M. E. Autonomous spinal motor function and the infant lesion effect. In: *Development and plasticity of the mammalian spinal cord.Fidia Research Series.* edited by M. E. Goldberger, A. Gorio and M. Murray. Padova: Liviana Press, 1986, p. 363-380.

Goldberger, M. E. and Murray, M. Recovery of function and anatomical plasticity after damage to the adult and neonatal spinal cord. In: *Synaptic plasticity*, edited by C. Cotman. New York: Guilford Press, 1985, p. 77-110.

Gorska, T., Bem, T., and Majczynski, H. Locomotion in cats with ventral spinal lesions: support patterns and duration of support phases during unrestrained walking. *Acta Neurobiol. Exp.* 50: 191-200, 1990.

Gorska, T., Majczynski, H., Bem, T., and Zmyslowski, W. Hindlimb swing, stance and step relationships during unrestrained walking in cats with lateral funicular lesion. *Acta Neurobiol. Exp.* 53: 133-142, 1993.

Goslow, G. E., Reinking, R. M., and Stuart, D. G. The cat step cycle: hind limb joint angles and muscle lengths during unrestrained locomotion. *J. Morphol.* 141: 1-42, 1973.

Gossard, J.-P., Cabelguen, J.-M., and Rossignol, S. Phase-dependent modulation of primary afferent depolarization in single cutaneous primary afferents evoked by peripheral stimulation during fictive locomotion in the cat. *Brain Res.* 537: 14-23, 1990.

Gossard, J.-P. and Rossignol, S. Phase-dependent modulation of dorsal root potentials evoked by peripheral nerve stimulation during fictive locomotion in the cat. *Brain Res.* 537: 1-13, 1990.

Greve, J. M., Muszkat, R., Schmidt, B., Chiovatto, J., Barros Filho, T. E., and Batisttella, L. R. Functional electrical stimulation (FES): muscle histochemical analysis. *Paraplegia* 31: 764-770, 1993.

Grillner, S. Locomotion in the spinal cat. In: *Control of posture and locomotion.Adv.Behav.Biol.7:* edited by R. B. Stein, K. G. Pearson, R. S. Smith and J. B. Redford. New York: Plenum Press, 1973, p. 515-535.

Grillner, S. Some aspects of the descending control of the spinal circuits generating locomotor movements Neural control of locomotion Herman, R.;Grillner, S.;Stein, P.;Stuart, D. Plenum Press New York. *Adv. Behav. Biol.* 18: 351-375, 1976.

Grillner, S. Interaction between central and peripheral mechanisms in the control of locomotion. *Prog. Brain Res.* 50: 227-235, 1979.

Grillner, S. Control of locomotion in bipeds, tetrapods, and fish. In: *Handbook of physiology. The nervous system II.* edited by J. M. Brookhart, V. B. Mountcastle and V. B. Brooks. Bethesda: Amer. Physiol. Soc. 1981, p. 1179-1236.

Grillner, S. Neural control of vertebrate locomotion central mechanisms and reflex interaction with special reference to the cat. In: *Feedback and motor control in invertebrates and vertebrates*, edited by W. J. P. Barnes and M. H. Gladden. London: Croom Helm Ltd, 1985, p. 35-56.

Grillner, S., Hongo, T., and Lund, S. Descending pathways with monosynaptic action on motoneurones. *Acta physiol. scand.* 68: 60,1966.

Grillner, S., Hongo, T., and Lund, S. The vestibulospinal tract: effects on alpha-motoneurones in the lumbosacral spinal cord in the cat. *Exp. Brain. Res.* 10: 94-120, 1970.

Grillner, S. and Lund, S. The origin of a descending pathway with monosynaptic action on flexor motoneurones. *Acta physiol. scand.* 74: 274-284, 1968.

Grillner, S. and Matsushima, T. The neural network underlying locomotion in lamprey--synaptic and cellular mechanisms. *Neuron* 7: 1-15, 1991.

Grillner, S., McClellan, A., Sigvardt, K., Wallen, P., and Wilen, M. Activation of NMDA-receptors elicits "fictive locomotion" in lamprey spinal cord in vitro. *Acta physiol. scand.* 113: 549-551, 1981.

Grillner, S. and Rossignol, S. On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Res.* 146: 269-277, 1978.

Grillner, S. and Wallen, P. Does the central pattern generation for locomotion in lamprey depend on glycine inhibition. *Acta physiol. scand.* 110: 103-105, 1980.

Grillner, S. and Wallen, P. The ionic mechanisms underlying N-methyl-D-aspartate receptor-induced, tetrodotoxin-resistant membrane potential oscillations in lamprey neurons active during locomotion. *Neurosci. Lett.* 60: 289-294, 1985.

Grillner, S. and Zangger, P. On the central generation of locomotion in the low spinal cat. *Exp. Brain. Res.* 34: 241-261, 1979.

Grillner, S. and Zangger, P. The effect of dorsal root transection on the efferent motor pattern in the cat's hindlimb during locomotion. *Acta physiol. scand.* 120: 393-405, 1984.

Haggendal, J. and Dahlstrom, A. The time course of noradrenaline decrease in rat spinal cord following transection. *Neuropharmacology* 12: 349-354, 1973.

Halbertsma, J. M. The stride cycle of the cat: the modelling of locomotion by computerized analysis of automatic recordings. *Acta physiol. scand.* Suppl. 521: 1-75, 1983.

Harris-Warrick, R. M. and Cohen, A. H. Serotonin modulates the central pattern generator for locomotion in the isolated lamprey spinal cord. *J. Exp. Biol.* 116: 27-46, 1985.

Hiebert, G. W., Gorassini, M. A., Jiang, W., Prochazka, A., and Pearson, K. G. Corrective responses to loss of ground support during walking II. Comparison of intact and chronic spinal cats. *J. Neurophysiol.* 71: 611-622, 1994.

Hirayama, T., Ono, H., and Fukuda, H. Effects of adrenergic agents on ventral horn cells in rat spinal cord slices. *Biomed. Res.* 9: 343-351, 1988.

Hishinuma, M. and Yamaguchi, T. Cervical interneurons oligosynaptically excited from primary afferents and rhythmically active during forelimb fictive locomotion in the cat. *Neurosci. Lett.* 111: 287-291, 1990.

Hochman, S., Jordan, L. M., and Macdonald, J. F. N-Methyl-D-Aspartate receptor-mediated voltage oscillations in neurons surrounding the central canal in slices of rat spinal cord. *J. Neurophysiol.* 72: 565-577, 1994.

Hodgson, J. A., Roy, R. R., De Leon, R., Dobkin, B., and Edgerton, V. R. Can the mammalian lumbar spinal cord learn a motor task? *Med. Sci. Sports Exer.* 26: 1491-1497, 1994.

Hongo, T., Jankowska, E., and Lundberg, A. The rubrospinal tract. II. Facilitation of interneuronal transmission in reflex paths to motoneurons. *Exp. Brain. Res.* 7: 365-391, 1969.

Hounsgaard, J., Hultborn, H., Jespersen, J., and Kiehn, O. Bistability of alpha-motoneurones in the decerebrate cat and in the acute spinal cat after intravenous 5-hydroxytryptophan. *J. Physiol.* 405: 345-367, 1988.

Hultborn, H., Petersen, N., Brownstone, R., and Nielsen, J. Evidence of fictive spinal locomotion in the marmoset (Callithrix jacchus). *Soc. Neurosci. Abstr.* 19: 539 (no.225.1), 1993.

Hultborn, H. and Udo, M. Convergence in the reciprocal Ia inhibitory pathway of excitation from descending pathways and inhibition from motor axon collaterals. *Acta physiol. scand.* 84: 95-108, 1972.

Ishizuki, M. and Yanagisawa, M. Antinociceptive effects of tizanidine, diazepam and eperisone in isolated spinal cord-tail preparations of newborn rat. *Pain* 48: 101-106, 1992.

Jankowska, E., Jukes, M. G., Lund, S., and Lundberg, A. The effect of DOPA on the spinal cord. 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors. *Acta physiol. scand.* 70: 369-388, 1967.

Jankowska, E., Lund, S., and Lundberg, A. The effect of DOPA on the spinal cord. 4. Depolarization evoked in the central terminals of contralateral Ia afferent terminals by volleys in the flexor reflex afferents. *Acta physiol. scand.* 68: 337-341, 1966.

Jankowska, E., Riddell, J. S., Skoog, B., and Noga, B. R. Gating of transmission to motoneurones by stimuli applied in the locus coeruleus and raphe nuclei of the cat. *J. Physiol.* 461: 705-722, 1993.

Jiang, W. and Drew, T. Effects of bilateral lesions of the dorsal columns and dorsolateral funiculi at the level of the low thoracic spinal cord on the control of locomotion in the adult cat: I.Treadmill walking. *J.Neurophysiol.* 76(2):849-866, 1996.

Jordan, L. M. Brainstem and spinal cord mechanisms for the initiation of locomotion. In: *Neurobiological basis of human locomotion*, edited by M. Shimamura, S. Grillner and V. R. Edgerton. Tokyo: Japan scientific societies press, 1991, p. 3-20.

Jovanovic, K., Petrov, T., Greer, J. J., and Stein, R. B. Serotonergic modulation of the mudpuppy (Necturus maculatus) locomotor pattern in vitro. *Exp.Brain Res.* 111: 57-67, 1996.

Katakura, N. and Chandler, S. C. Iontophoretic analysis of the pharmacologic mechanisms responsible for initiation and modulation of trigemnical motoneuronal discharge evoked by intra-oral afferent stimulation. *Brain Res.* 549: 66-77, 1991.

Kehne, J. H., Gallager, D. W., and Davis, M. Spinalization unmasks clonidine's alpha1-adrenergic mediated excitation of the flexor reflex in rats. *J. Neurosci.* 5: 1583-1590, 1985.

Kemnitz, C. P. Dopaminergic modulation of spinal neurons and synaptic potentials in the lamprey spinal cord. *J. Neurophysiol.* 77: 289-298, 1997.

Kerkut, G. A. and Bagust, J. The isolated mammalian spinal cord. *Prog. Neurobiol.* 46: 1-48, 1995.

Kettler, J. and Jordan, L. M. Metabolic mapping of the brainstem during fictive locomotion. *Soc. Neurosci. Abstr.* 10: 633, 1984.

Kiehn, O. Plateau potentials and active integration in the 'final common pathway' for motor behaviour. *TINS* 14: 68-73, 1991.

Kiehn, O., Hounsgaard, J., and Sillar, K. Basic building blocks of vertebrate spinal central pattern generators. In: *Neurons, networks, and motor behavior*, edited by P. Stein, S. Grillner, A. Selverston and D. Stuart. Cambridge, Massachusetts: MIT Press, 1997, p. 47-59.

Kiehn, O., Hultborn, H., and Conway, B. A. Spinal locomotor activity in acutely spinalized cats induced by intrathecal application of noradrenaline. *Neurosci. Lett.* 143: 243-246, 1992.

Kiehn, O., Johnson, B. R., and Raastad, M. Plateau properties in mammalian spinal interneurons during transmitter-induced locomotor activity. *Neurosci.* 75: 263-273, 1996.

Kiehn, O. and Kjaerulff, O. Spatiotemporal characteristics of 5-HT and dopamin-induced rhythmic hindlimb activity in the in vitro neonatal rat. *J. Neurophysiol.* 75: 1472-1482, 1996.

Kremer, E. and Lev-Tov, A. Localization of the spinal network associated with generation of hindlimb locomotion in the neonatal rat and organization of its transverse coupling system. *J. Neurophysiol.* 77: 1155-1170, 1997.

Kudo, N. and Yamada, T. N-Methyl-D,L-aspartate-induced locomotor activity in a spinal cord -hindlimb muscles preparation of the newborn rat studied in vitro. *Neurosci. Lett.* 75: 43-48, 1987.

Kuhtz-Buschbeck, J. P., Boczek-Funcke, A., Illert, M., and Weinhardt, C. X-ray study of the cat hindlimb during treadmill locomotion. *Eur. J. Neurosc.* 6: 1187-1198, 1994.

Kuypers, H. G. J. M. Retrograde axonal transport of horseradish peroxydase from spinal cord to brain stem cell groups in the cat. *Neurosci. Lett.* 1: 9-14, 1975.

Kuypers, H. G. J. M. Anatomy of the descending pathways. In: *Handbook of physiology. The nervous system II.* edited by J. M. Brookhart, V. B. Mountcastle and

V. B. Brooks. Bethesda: American physiological society, 1981, p. 597-666.

Lovely, R. G., Gregor, R. J., Roy, R. R., and Edgerton, V. R. Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cat. *Brain Res.* 514: 206-218, 1990.

Lundberg, A. Reflex control of stepping. *The Nansen Memorial Lecture to the Norwegian Academy of Sciences and Letters, Universitets Forlaget Oslo,* 1969.

Lundberg, A. Half-centres revisited. In: *Regulatory functions of the CNS.Principles of motion and organization.Adv. Physiol. Sci. vol.1*, edited by J. Szentagothai, M. Palkovits and J. Hamori. Budapest: Pergamon Press, 1981, p. 155-167.

Marshall, K. C. Catecholamines and their actions in the spinal cord. In: *Handbook of the spinal cord: Pharmacology*, edited by R. A. Davidoff. New York: Marcel Dekker Inc. 1983, p. 275-328.

McClellan, A. and Farel, P. B. Pharmacological activation of locomotor patterns in larval and adult frog spinal cords. *Brain Res.* 332: 119-130, 1985.

Monroe, P. J., Smith, D. L., and Smith, D. J. Spinal imidazoline receptors do not mediate the antinociceptive action of intrathecal clondine in rat. *Ann. NY. Acad. Sci.* 763: 497-500, 1995.

Mori, S. Integration of posture and locomotion in acute decebrate cats and in awake, freely moving cats. *Prog. Neurobiol.* 28: 161-195, 1987.

Mori, S. Contribution of postural muscle tone to full expression of posture and locomotor movements:multi-faceted analyses of its setting brainstem-spinal cord

mechanisms in the cat. *Jpn. J. Physiol.* 39: 785-809, 1989.

Mori, S., Matsuyama, K., Kohyama, J., Kobayashi, Y., and Takakusaki, K. Neuronal constituents of postural and locomotor control systems and their interactions in cats. *Brain Dev.* 14: S109-S120, 1992.

Mori, S., Nishimura, N., Karakami, C., Yamamura, T., and Aoki, M. Controlled locomotion in the mesencephalic cat: distribution of facilitatory and inhibitory regions within pontine tegmentum. *J. Neurophysiol.* 41: 1580-1591, 1978.

Noga, B. R., Bras, H., and Jankowska, E. Transmission from group II muscle afferents is depressed by stimulation of locus coeruleus, kolliker-fuse and raphe nuclei in the cat. *Exp. Brain. Res.* 88: 502-516, 1992.

Noga, B. R., Fortier, P. A., Kriellaars, D. J., Dai, X., Detillieux, G. R., and Jordan, L. M. Field potential mapping of neurons in the lumbar spinal cord activated following stimulation of the mesencephalic locomotor region. *J. Neurosci.* 15: 2203-2217, 1995.

Norman, K. E. and Barbeau, H. Comparison of cyproheptadine,clonidine and baclofen on the modulation of gait pattern in subjects with spinal cord injury. In: *Spasticity:mechanisms and management*, edited by A. Thilmann, D. Burke and Z. Rymer. New York: Springer-Verlag, 1993, p. 410-425.

Nusbaum, M. P., El Manira, A., Gossard, J., and Rossignol, S. Presynaptic mechanism during rhythmic activity in vertebrates and invertebrates. In: *Neurons, networks, and motor behavior*, edited by P. Stein, S. Grillner, A. Selverston and D. G. Stuart. Cambridge, Massechusetts: MIT Press, 1997, p. 237-253.

O'Neill, T. P. and Haigler, H. J. Effects of clonidine on neuronal firing evoked by a noxious stimulus. *Brain Res.* 327: 97-103, 1985.

Ono, H. and Fukuda, H. Pharmacology of descending noradrenergic systems in relation to motor function. *Pharmac. Ther.* 68: 105-112, 1995.

Orlovsky, G. N. Activity of rubrospinal neurons during locomotion. *Brain Res.* 46: 99-112, 1972a.

Orlovsky, G. N. Activity of vestibulospinal neurons during locomotion. *Brain Rès.* 46: 85-98, 1972b.

Orlovsky, G. N. The effect of different descending systems on flexor and extensor activity during locomotion. *Brain Res.* 40: 359-371, 1972c.

Orlovsky, G. N. and Feldman, A. G. Role of afferent activity in the generation of stepping movements. *Neurophysiology* 4: 304-310, 1972.

Panchin, Y. V., Arshavsky, Y. I., Deliagina, T. G., Orlovsky, G. N., Popova, L. B., and Selverston, A. I. Control of locomotion in the marine mollusc Clione limacina. XI. Effects of serotonin. *Exp. Brain. Res.* 109: 361-365, 1996.

Panchin, Y. V., Perrins, R. J., and Roberts, A. The action of acetylcholine on the locomotor central pattern generator for swimming in *Xenopus* embryos. *J. Exp. Biol.* 161: 527-531, 1991.

Pascual, J., del Arco, C., Gonzalez, A. M., and Pazos, A. Quantative light microscopic autoradiographic localizatioin of α_2-adrenoceptors in the human brain. *Brain Res.* 585: 116-127, 1992.

Pearson, K. G. Common principles of motor control in vertebrates and invertebrates. *Annu. Rev. Neurosci.* 16: 265-297, 1993.

Pearson, K. G. and Rossignol, S. Fictive motor patterns in chronic spinal cats. *J. Neurophysiol.* 66: 1874-1887, 1991.

Perreault, M.-C., Drew, T., and Rossignol, S. Activity of medullary reticulospinal neurons during fictive locomotion. *J. Neurophysiol.* 69: 2232-2247, 1993.

Perreault, M.-C., Rossignol, S., and Drew, T. Microstimulation of the medullary reticular formation during fictive locomotion. *J. Neurophysiol.* 71: 229-245, 1994.

Perret, C. Centrally generated pattern of motoneuron activity during locomotion in the cat. In: *Neural origin of rhythmic movements.Soc.Exp.Biol.Symp.,37:* edited by A. Roberts and B. L. Roberts. Cambridge: Cambridge University Press, 1983, p. 405-422.

Perret, C. and Cabelguen, J.-M. Main characteristics of the hindlimb locomotor cycle in the decorticate cat with special reference to bifunctional muscles. *Brain Res.* 187: 333-352, 1980.

Philippson, M. L'autonomie et la centralisation dans le système nerveux des animaux. *Trav. Lab. Physiol. Inst. Solvay. (Bruxelles.)* 7: 1-208, 1905.

Poon, M. Induction of swimming in lamprey by L-DOPA and amino acids. *J. Comp. Physiol.* 136: 337-344, 1980.

Rasmussen, S., Chan, A. K., and Goslow, G. E. J. The cat step cycle: electromyographic patterns for hindlimb muscles during posture and unrestrained

locomotion. *J. Morphol.* 155: 253-270, 1978.

Rawlow, A. and Gorka, Z. Involvement of postsynaptic α-$_1$ and α-$_2$ adrenoceptors im the flexor reflex activity in the spinal rats. *J. Neurol. Transm.* 93-105, 1986.

Reddy, S. V. R., Maderdrut, J. L., and Yaksh, T. L. Spinal cord pharmacology of adrenergic agonist-mediated antinociception. *J. Pharmacol. Exper. Therap.* 213: 525-533, 1980.

Remy-Neris, O., Thiebaut, J. B., Boiteau, F., Bussel, B., and Barbeau, H. The effects of intrathecal clonidine on spinal reflexes and on locomotion in incomplete paraplegic subjects. *Abstracts from the 2nd European Meetings of Neuroscience* 1996.(Abstract)

Robinson, G. A. and Goldberger, M. E. The development and recovery of motor function in spinal cats. II. Pharmacological enhancement of recovery. *Exp. Brain. Res.* 62: 387-400, 1986.

Rossignol, S. Neural control of stereotypic limb movements. In: *Handbook of Physiology , section 12. Exercise: regulation and integration of multiple systems.* edited by L. B. Rowell and J. T. Sheperd. Bethesda: American Physiological Society, 1996, p. 173-216.

Rossignol, S., Barbeau, H., and Provencher, J. Locomotion in the adult chronic spinal cat. *Soc. Neurosci. Abstr.* 8: 163, no.47.1, 1982.

Rossignol, S., Chau, C., Brustein, E., Bélanger, M., Barbeau, H., and Drew, T. Locomotor capacity of cats after complete and partial lesions of the spinal cord. *Acta Neurobiol. Exp.* 56: 449-463, 1996. (See Appendix B)

Rossignol, S. and Dubuc, R. Spinal pattern generation. *Current Opinion in Neurobiology* 4: 894-902, 1994.

Rossignol, S., Lund, J. P., and Drew, T. The role of sensory inputs in regulating patterns of rhythmical movements in higher vertebrates. A comparison between locomotion, respiration and mastication. In: *Neural control of rhythmic movements in vertebrates*, edited by A. Cohen, S. Rossignol and S. Grillner. New York: Wiley and sons Co, 1988, p. 201-283.

Rossignol, S., Saltiel, P., Perreault, M.-C., Drew, T., Pearson, K., and Bélanger, M. Intralimb and interlimb coordination in the cat during real and fictive rhythmic motor programs. *Seminars in the neurosciences* 5: 67-75, 1993.

Roudet, C., Mouchet, P., Feuerstein, C., and Savasta, M. Normal distribution of alpha 2-adrenoreceptors in the rat spinal cord and its modification after noradrenergic demervation: a quantitative autoradiographic study. *J. Neurosci. Res.* 39: 319-329, 1994.

Roudet, C., Savasta, M., and Feuerstein, C. Normal distribution of alpha-1-adrenoceptors in the rat spinal cord and its modification after noradrenergic denervation: A quantitative autoradiographic study. *J. Neurosci. Res.* 34: 44-53, 1993.

Ruffolo, R.R.J. Interactions of agonists with peripheral α-adrenergic receptors. *Federation Proc.* 43:2910-2916, 1984.

Ruffolo, R. R. and Hieble, J. P. α-Adrenoreceptors. *Pharmac. Ther.* 61: 1-64, 1994.

Ruggiero, D. A., Regunathan, S., Wang, H., Milner, T., and Reis, D. Distribution of imidazoline receptor binding protein in the central nervous system. *Ann. New. York. Acad. Sci.* 763: 201-221, 1995.

Russo, R. E. and Hounsgaard, J. Plateau-generating neurones in the dorsal horn in an *in vitro* preparation of the turtle spinal cord. *J. Physiol. (Lond)* 493: 39-54, 1996a.

Russo, R. E. and Hounsgaard, J. Burst-generating neurons in the dorsal horn in an *in vitro* preparation of the turtle spinal cord. *J. Physiol. (Lond)* 55-66, 1996b.

Sakitama, K. Intrathecal noradrenaline facilitates and inhibits the flexor reflex mediated by group II afferents fibres via α1- and α2-receptors, respectively. *Japan. J. Pharmacol* 62: 131-136, 1993.

Saltiel, P. and Rossignol, S. Influence of proprioceptive inputs on fictive locomotion of the forelimb of the cat. *Soc. Neurosci. Abstr.* 14: 265 no.106.9, 1988.

Schmidt, B. J., Meyers, D. E. R., Tokuriki, M., and Burke, R. E. Modulation of short latency cutaneous excitation in flexor and extensor motoneurons during fictive locomotion in the cat. *Exp.Brain Res.* 77: 57-68, 1989.

Schomburg, E. D. and Steffens, H. The effect of DOPA and clonidine on reflex pathways from group II muscle afferents to alpha-motoneurones in the cat. *Exp.Brain Res.* 71: 442-446, 1988.

Schomburg, E. D. and Steffens, H. Bistable characteristics of motoneurone activity during DOPA induced fictive locomotion in spinal cats. *Neurosci. Res.* 26: 47-56, 1996.

Seki, K. and Yamaguchi, T. Cutaneous reflex activity of the cat forelimb during fictive locomotion. *Brain Res.* 753: 56-62, 1997.

Shefchyk, S., McCrea, D. A., Kriellaars, D., Fortier, P., and Jordan, L. Activity of interneurons within the L4 spinal segment of the cat during brainstem-evoked fictive locomotion. *Exp.Brain Res.* 80: 290-295, 1990.

Shefchyk, S. J. and Jordan, L. M. Spatial segregation of excitatory and inhibitory synaptic terminals producing locomotor drive potentials in alpha motoneurons. *Soc. Neurosci. Abstr.* 10: 633, 1984.

Shik, M. L. Action of the brainstem locomotor region on spinal stepping generators via propriospinal pathways. In: *Spinal cord reconstruction*, edited by C. C. Kao, R. P. Bunge and P. J. Reier. New York: Raven Press, 1983, p. 421-434.

Shik, M. L., Severin, F. V., and Orlovsky, G. N. Control of walking and running by means of electrical stimulation of the mid-brain. *Biophysics.* 11: 756-765, 1966.

Shik, M. L. and Yagodnitsyn, A. S. Neuron responses of the locomotor strip in the lower brain stem of the cat. *Neurophysiology* 10: 518, 1978.

Shurrager, P. S. Walking in spinal kittens and puppies. In: *Regeneration in the central nervous system*, edited by W. F. Windle. Springfield: C.C. Thomas, 1955, p. 208-218.

Shurrager, P. S. and Dykman, R. A. Walking spinal carnivores. *J. Comp. Physiol. Psychol.* 44: 252-262, 1951.

Skoog, B. and Noga, B. R. Dopaminergic control of transmission from group II

muscle afferents to spinal neurons in the cat and guinea-pig. *Exp.Brain Res.* 105: 39-47, 1995.

Smith, J. C. and Feldman, J. L. In vitro brainstem-spinal cord preparations for study of motor systems for mammalian respiration and locomotion. *J. Neurosci. Meth.* 21: 321-333, 1987.

Smith, J. L., Chung, S. H., and Zernicke, R. F. Gait-related motor pattern and hindlimb kinetics for the cat trot and gallop. *Exp.Brain Res.* 94: 308-322, 1993.

Smith, J. L., Smith, L. A., Zernicke, R. F., and Hoy, M. Locomotion in exercised and non-exercised cats cordotomized at two or twelve weeks of age. *Exp. Neurol.* 76: 393-413, 1982.

Smythe, J. W. and Pappas, B. A. Noradrenergic and serotonergic mediation of the locomotor and antinociceptive effects of clondine in infant and adult rats. *Pharmacol. Biochem. Behav.* 34: 413-418, 1989.

Sperry, R. W. The functional results of muscle transposition in the hindlimb of the rat. *J. Comp. Neurol.* 73: 379-404, 1940.

Sperry, R. W. The effects of crossing nerves to anatogonistic muscles in the hindlimb of the rat. *J. Comp. Neurol.* 75: 1-19, 1941.

Steeves, J. D. and Jordan, L. M. Autoradiographic demonstration of the projections from the mesencephalic locomotor region. *Brain Res.* 307: 263-276, 1984.

Steeves, J. D., Jordan, L. M., and Lake, N. The close proximity of catecholamine-containing cells to the "mesencephalic locomotor region" (MLR).

Brain Res. 100: 663-670, 1975.

Steeves, J. D., Schmidt, B. J., Skovgaard, B. J., and Jordan, L. M. Effect of noradrenaline and 5-hydroxytryptamine depletion on locomotion in the cat. *Brain Res.* 185: 349-362, 1980.

Stein, P. S. Central pattern generators in the spinal cord. In: *Handbook of the spinal cord*, edited by R. A. Davidoff. New York: Marcel Dekker Inc. 1984, p. 647-672.

Stein, R. B., Prochazka, A., Popovic, D., Edamura, M., LLewlundellyn, M. G. A., and Davis, L. A. Advances in external control of human extremities. In: *Technology transfer and development for walking using functional electrical stimulation*, edited by D. B. Popovic. Belgrade: Nauka, 1990, p. 161-175.

Stewart, J. E., Barbeau, H., and Gauthier, S. Modulation of locomotor patterns and spasticity with clonidine in spinal cord injured patients. *J. Can. Sci. Neurol.* 18: 321-332, 1991.

Timmermans, P.B.M.W.M. and Van Zwieten, P.A. Mini-Review: The postsynaptic a2-adrenoreceptor. *J.Auton.Pharmac.* 1:171-183, 1981.

Timmermans, P. B. M. W. M. and van Zwieten, P. A. $\alpha 2$ Adrenoceptors: Classification, Localization, Mechanisms, and Targets for Drugs. *J. Med. Chem* 25: 1389-1401, 1982.

Tremblay, L. E. and Bedard, P. J. Effects of clonidine on motoneuron excitability in spinalized rats. *Neuropharmacol.* 25: 41-46, 1986.

Udo, M., Matsukawa, K., Kamei, H., and Oda, Y. Cerebellar control of locomotion: effects of cooling cerebellar intermediate cortex in high decerebrate and awake walking cats. *J. Neurophysiol.* 44: 119-134, 1980.

Unnerstall, J. R., Fernandez, I., and Orensanz, L. M. The alpha-adrenergic receptor:radiohistochemical analysis of functional characteristic and biochemical differences. *Pharmacol. Biochem. Behav.* 22: 859-874, 1985.

Viala, D., Viala, G., and Fayein, N. Plasticity of locomotor organization in infant rabbits spinalized shortly after birth. In: *Development and plasticity of the mammalian spinal cord*, edited by M. Goldberger, A. Gorio and M. Murray. Padova: Liviana Press, 1986, p. 301-310.

Visintin, M. and Barbeau, H. The effects of body weight support on the locomotor pattern of spastic paretic patients. *Can. J. Neurol. Sci.* 16: 315-325, 1989.

Visintin, M. and Barbeau, H. The effects of parallel bars, body weight support and speed on the modulation of the locomotor pattern of spastic paretic gait. A preliminary communication. *Paraplegia* 32: 540-553, 1994.

Wallen, P., Buchanan, J. T., Grillner, S., Hill, R. H., Christenson, J., and Hokfelt, T. Effects of 5-hydroxytryptamine on the afterhyperpolarization, spike frequency regulation, and oscillatory membrane properties in lamprey spinal cord neurons. *J. Neurophysiol.* 61: 759-768, 1989.

Wallen, P. and Grillner, S. N-Methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. *J. Neurosci.* 7: 2745-2755, 1987.

Wernig, A. and Muller, S. Laufband locomotion with body weight support improved walking in persons with severe spinal cord injuries. *Paraplegia* 30: 229-238, 1992.

Wernig, A., Muller, S., Nanassy, A., and Cagol, E. Laufband therapy based on 'rules of spinal locomotion' is effective in spinal cord injured persons [published erratum appears in Eur J Neurosci 1995 Jun 1;7(6):1429]. *Eur. J. Neurosci.* 7: 823-829, 1995.

White, S. R., Fung, S. J., and Barnes, C. D. Norepinephrine effects on spinal motorneurons. *Prog. Brain Res.* 88: 343-350, 1991.

Wieler, M., Stein, R., and Dai, R. Multi-center clinical testing of functional electrical stimulation systems to assist walking. *Proceedings of the 12th International Congress of the World Confederation for Physical Therapy* 773, 1995.(Abstract)

Willard, A. L. Effects of serotonin on the genreation of the motor program for swimming by the medicinal leech. *J. Neurosci.* 1: 936-944, 1981.

Wisleder, D., Zernicke, R. F., and Smith, J. L. Speed-related changes in hindlimb intersegmental dynamics during the swing phase of cat locomotion. *Exp.Brain Res.* 79: 651-660, 1990.

Wolpaw, J. R. Adaptive plasticity in the primate spinal stretch reflex: reversal and re-development. *Brain Res.* 278: 299-304, 1983.

Wolpaw, J. R., Braitman, D. J., and Segal, R. F. Adaptive plasticity in primate spinal stretch reflex: initial development. *J. Neurophysiol.* 50: 1296-1311, 1983.

Wolpaw, J. R. and Carp, J. Adaptive plasticity in spinal cord. *Adv. Neurol.* 59: 163-174, 1993.

Wolpaw, J. R., Carp, J. S., and Lee, C. L. Memory traces in spinal cord produced by H-reflex conditioning: effects of post-tetanic potentiation. *Neurosci. Lett.* 103: 113-119, 1989.

Wolpaw, J. R. and Chong, L. L. Memory traces in primate spinal cord produced by operant conditioning of H-reflex. *J. Neurophysiol.* 61: 563-572, 1989.

Yumiya, H., Larsen, K. D., and Asanuma, H. Motor readjustment and input-output relationship of motor cortex follwoing cross-connection of forearm muscles in cats. *Brain Res.* 177: 566-570, 1979.

Zmyslowski, W., Gorska, T., Majczynski, H., and Bem, T. Hindlimb muscle activity during unrestrained walking in cats with lesions of the lateral funiculi. *Acta Neurobiol. Exp.* 53: 143-153, 1993.

Appendix A

Brain Research Bulletin, Vol. 30, pp. 387–393, 1993
Printed in the USA.

0361-9230/93 $6.00 + .00

Noradrenergic Agonists and Locomotor Training Affect Locomotor Recovery After Cord Transection in Adult Cats

H. BARBEAU,*† C. CHAU* AND S. ROSSIGNOL*[1]

*Centre de recherche en sciences neurologiques, Département de Physiologie, Université de Montréal H3C 3J7
†School of Physical and Occupational Therapy, McGill University, Montreal, Canada

BARBEAU, H., C. CHAU AND S. ROSSIGNOL. *Noradrenergic agonists and locomotor training affect locomotor recovery after cord transection in adult cats.* BRAIN RES BULL **30**(3/4) 387–393, 1993.—In one series of experiments, the effects of noradrenergic, serotonergic, and dopaminergic precursors and agonists on the initiation of locomotion were investigated within the first week after complete spinalization at +13 in five adult cats. In addition, the effects of clonidine and daily locomotor training were investigated during the first week after transection in another cat. The electromyographic (EMG) activity of vastus lateralis (VL) and semitendinosus (St) was recorded bilaterally through percutaneously implanted copper wires in all cats. The movement of the hindlimbs on the treadmill was also simultaneously videorecorded before and after the injection of drugs. Without drug injection, strong and sustained perineal or abdominal stimulation did not induce any prolonged episodes of coordinated stepping on the treadmill during the first week after spinalization. St often had sustained activity, in contrast to VL, in which minimal or no activity was present. Injection of apomorphine (0.3 to 0.5 mg/kg, $n = 3$), a dopaminergic agonist, or DL-5-HTP (50 mg/kg, $n = 2$), a serotonergic precursor, failed to induce locomotion at such an early stage after spinalization. In contrast, injection of either L-dopa (50–60 mg/kg, $n = 2$), a noradrenergic precursor, or clonidine (150 μg/kg, $n = 2$), a noradrenergic agonist, induced locomotion on the treadmill. The animal demonstrated bilateral foot placement on the soles and complete weight support of the hindquarters. The spinal cat could follow the treadmill speed up to 0.80 ms^{-1}. However, these effects disappeared when the NA drugs were tapered off. When the spinal cat was trained daily under the effect of clonidine, a stable locomotor pattern was established within 1 week post-transection, which was retained afterwards even without clonidine injection. This locomotor pattern is similar, in many aspects, to that observed in the chronic stage after an intensive program of locomotor training, 2 to 3 months posttransection, without clonidine injection (1). The present results confirm previous studies showing that locomotion cannot be elicited spontaneously in the first week after spinalization. We further demonstrate that L-dopa and clonidine, but not apomorphine or 5-HTP, are each capable of inducing locomotion in the first week after spinalization. These results also support that the combination of clonidine and locomotor training can accelerate the recovery of locomotion, since a well-developed locomotor pattern could be observed in as early as the first week post-transection.

Locomotion Spinalization Cat Norepinephrine Spinal cord Clonidine Apomorphine

RECENT studies (1,15) have documented in detail the recovery of locomotion after spinalization in adult cats from the first day post-spinalization up to the stage of full weight-bearing and plantar digitigrade walking. Between 3 weeks and 3 to 12 months, the spinal animals were capable of walking on the plantar surface of the feet and supporting the weight of the hindquarters, despite the loss of voluntary control and equilibrium. The range of angular motion and the EMG activity pattern of the hindlimb muscles were similar in many aspects to that observed in intact cats (6,8,10,13). Interactive locomotor training during the recovery process accelerated the recovery of locomotion with proper weight support and smooth locomotor movements (1). Lovely and collaborators (12) also found a marked improvement in the locomotor capability of the hindlimb with locomotor training. In contrast, in the first 2 weeks following the spinal cord transection only brief periods of coordinated air stepping and treadmill stepping were observed with strong perineal stimulation. Such reflexive stepping was not adequate for the spinal animal to walk bearing its full weight.

We concluded that the animal could never perform as well as in the chronic stage, except when clonidine, a noradrenergic agonist, was administered. The marked effect of clonidine (100 to 150 μg/kg) during the first days post-transection included a well-coordinated locomotor pattern, with full weight support of the hindquarters similar in many aspects to the locomotor pattern performed during the plateau or the chronic stage. When the effect of clonidine tapered off, the performance completely disappeared within a day and the locomotion regressed to that of the earlier stage.

The purpose of this report is twofold: firstly, to describe the effects of precursors and agonists of the noradrenergic, serotonergic, and dopaminergic systems on the initiation of locomotion

[1] To whom requests for reprints should be addressed.

within the first week after complete transection when spinal cats are not capable of spontaneous walking; and secondly, to present evidence that the dramatic effect observed with clonidine during the early period combined with locomotor training may accelerate the recovery of the locomotion. Preliminary results have been published (3,5).

METHOD

A complete spinal transection (T13) was performed on eight adult cats under pentobarbital (35 mg/kg) anesthesia. The complete spinalization procedure and animal care have been described previously (1,2,14). Briefly, a laminectomy was performed at T13, the dura was opened and part of a segment was removed so that the bottom of the spinal canal could clearly be visualized, and the space between the two ends of the spinal cord was filled with Surgicel. When the animals were sacrificed after the experiment, the spinal cord was removed for histological processing.

Not more than two spinal cats were kept simultaneously, and they were put in separate large cages with the bottom covered with foam mattresses and absorbent tissues. Their bladders were manually expressed daily.

Evaluation Procedures

The evaluation procedure, as well as electromyographic (EMG) recordings and analysis, movement recording and analysis, and drug injections were described extensively in previous studies (1,4,14). During the evaluation session, the spinal animals were positioned over the treadmill belt with the two forelimbs resting on a platform about 3 cm above the belt. The experimenter held the tail, particularly in the initial period when the animal was not capable of weight bearing or stepping, which showed a consequent drag of the hindfeet on the belt. Such a maneuver was not necessary after a well-coordinated locomotor pattern had been established between 1–3 months following spinalization.

Recording and Analyses

The EMGs were recorded, in each session, by percutaneous implantation (using 21-gauge needles) of pairs of enamel-insulated copper wires. The muscles recorded bilaterally were the semitendinosus, and sartorius, both knee flexors, and the vastus lateralis, a knee extensor. One other cat was reoperated under general anesthesia (sodium pentobarbital, 35 mg/kg) for chronic implantation of EMG electrodes. For this, multistranded stainless steel wires with Teflon insulation were sewn into the muscle bellies and the end of the wires soldered to a multipin connector cemented to the skull of the animal. The EMG signals were differentially amplified (Bandwidth of 300 Hz–10 kHz) and recorded on a 14-channel Honeywell FM tape recorder with a frequency response of 0–2500 Hz at the speed of recording.

The recorded data were played back on an electrostatic polygraph (Gould, Model ES 1000), and a representative record of the animal's performance at each speed, before and after the injection of a drug, was selected for further processing with a PDP-11/34 computer. The EMG signals were digitised at 1 kHz, and the onset and offset of the bursts were automatically detected. To evaluate changes in amplitude after the injection of a drug, the mean burst amplitude, defined as the integrated area under the rectified and filtered EMG bursts divided by the burst duration, was used.

Using ordinary white paint, light-reflecting spots were painted onto the ischiatic crest, the femoral head, the knee joint, the lateral malleolus, the tarso-metatarsal joint, and the tip of the toe. The movements of the animals were recorded using a shutter video camera (exposure time of 2 ms) which provided a sharp image for single field analysis (16.7 ms resolution). The synchronisation of the EMGs and the video images was achieved by recording a digital time code onto the FM and the video tapes simultaneously.

The X and Y coordinates of the light-reflecting markers were measured with a cursor directly from a video monitor at every field (1/60 s). It was then possible to reconstruct the display and typical sequences of movements in the form of stick diagrams or trajectories of marker points, and to illustrate the angular displacement at each joint. To display stick diagrams of a walking cat which is stationary relative to the camera, the distance travelled by the foot between each field was added to each marker point. In all displays of movements, a moving window average of five consecutive values was used to smooth the data.

Drug Injections and Locomotor Training

The effect of the serotonergic (5-HTP; 50 mg/kg), dopaminergic (dopa 60 mg/kg, apomorphine 0.5 mg/kg) and noradrenergic drugs (clonidine 100–150 μg/kg) on the walking pattern was evaluated during the first week following spinalization. Locomotor evaluation was performed during the maximal effect of the drugs injected intraperitoneally, from 30 to 60 min after the injection. The effects were assessed at the same speeds by comparing the locomotor pattern immediately preceding, and 30–60 min following, the injection of the drug using the same EMG electrodes, the same amplifier gains, and the same range of speeds. The effects of the agonist drugs generally lasted for at least 4–6 h.

The combined effects of daily injection of clonidine 100–150 μg/kg with locomotor training were assessed in one adult spinal cat from day 2 to day 9 post-transection. During the effect of clonidine, the animal was trained daily and walked sometimes for more than 1 h (four sessions of approximately 15 min) at different treadmill speeds. During each training session the weight support of the animal was provided by the experimenter who held the tail, and a separator was placed between the limbs to prevent the hindlimbs from impeding each other. The training consisted of a close interaction between the experimenter and the spinal cat such that the cat was allowed to walk with only the amount of weight that it could bear and to optimize the placement of the feet.

RESULTS

The Effect of Monoaminergic Drugs on the Initiation of Locomotion

During the first week after spinalization, even strong and continuous perineal or abdominal stimulation could not induce any significant or prolonged episodes of well-coordinated stepping on the treadmill without drug injections. As clearly illustrated in Fig. 1A–D, the knee flexor iSt was tonically active, whereas there was only minimal EMG activity in the knee extensor muscle VL during the control session.

As illustrated in Fig. 1A, injection of DL-5-HTP, 50 mg/kg, 2 days following spinalization, resulted in a marked tonic increase in both iSt and coSt amplitude when the treadmill belt started to move. Despite this increase in amplitude, no locomotor pattern was observed in this and in three other different experiments (not illustrated). Figure 1B shows that injection of apomorphine, at a dosage of 0.5 mg/kg, also failed to induce any episode of coordinated stepping at such an early period.

In contrast, injection of either the noradrenergic agonist clonidine (100 μg/kg) on day 6 post-spinalization, or the combination of nialamide (30 mg/kg) and L-dopa (60 mg/kg) on day 4 post-spinalization (Fig. 1C–D, respectively), the latter possibly acting as a noradrenergic precursor, could successfully induce

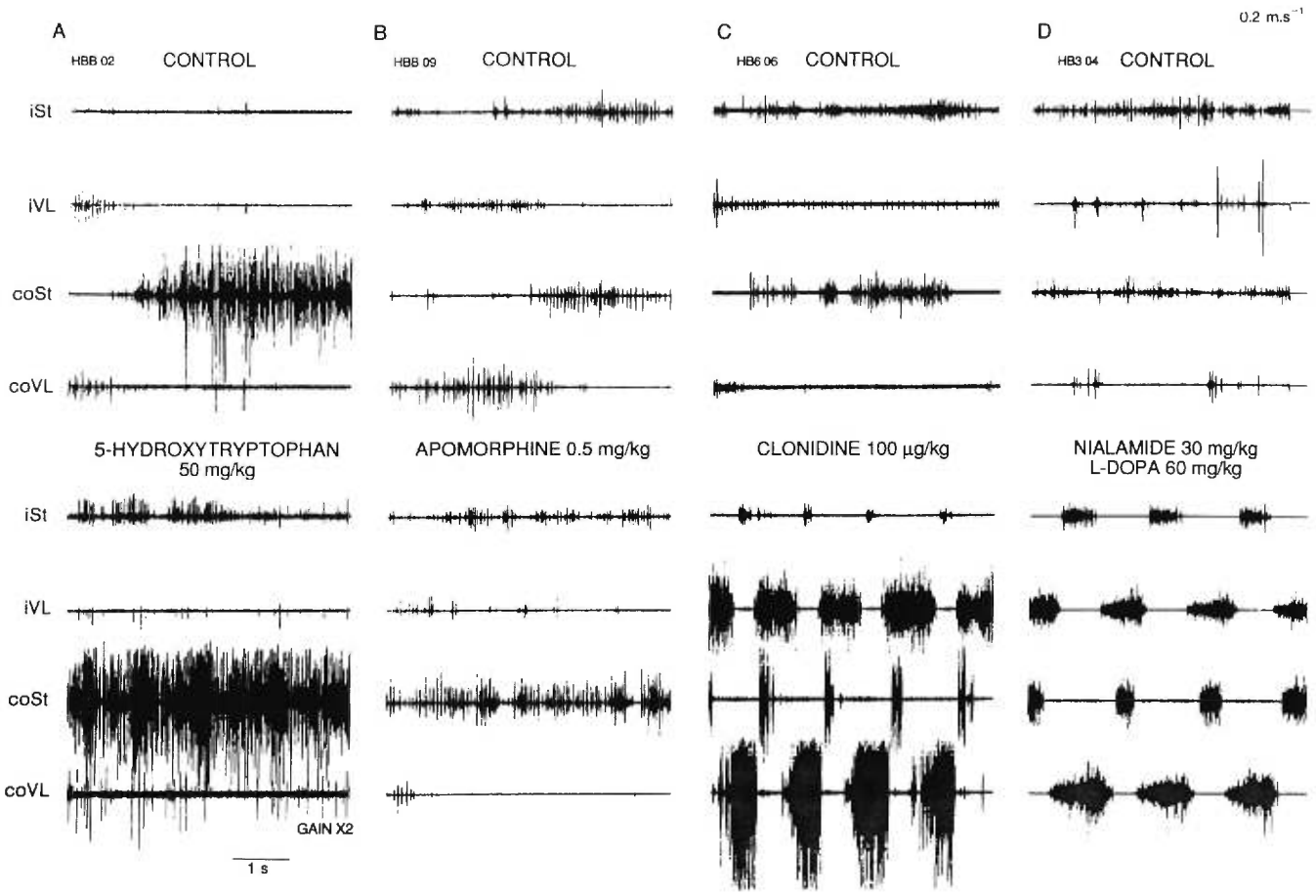


FIG. 1. Comparison of the effects of monoaminergic drugs on the initiation of the locomotor pattern in early spinal cats. (A) Changes induced by 5-HTP in cat HBB on day 2 post-spinalization. EMG activity of the hindlimb muscles (i and co St and VL) recorded at 0.20 $m \cdot s^{-1}$ during control and during 5-HTP. Amplifier gains are the same for all sets of records. (B) Changes induced by apomorphine in cat HBB on day 9 post-spinalization. (C) Changes induced by clonidine in cat HB6 on day 6 post-spinalization. (D) Changes induced by L-dopa in cat HB3 on day 4 post-spinalization. i: ipsilateral, co: contralateral.

locomotion in the spinal cat when the treadmill belt was moved (0.20 m · s). The spinal cat demonstrated a good bilateral foot placement with the sole of the foot and intermittent but complete weight support of the hindquarters for several minutes, requiring only light perineal stimulation. As shown in Fig. 1C, the EMG pattern is characterized by the alternation of long bursts of activity in the antagonist muscles St and VL.

Thus, clonidine, at an early stage after spinal transection, exerts a marked effect on the locomotor pattern such that the hindlimbs could walk in a well-coordinated manner with transient weight support. Irrespective of the amount of training, such behaviour is not seen at this stage without drug injection; it normally appears only at the end of the fourth week or later.

Figure 2 contrasts the kinematic pattern of three step cycles taken from the same adult spinal cat before and after the injection of clonidine 100–150 µg/kg on day 2 (Fig. 2A and D, respectively), day 7 (B and E, respectively) and day 9 (C and F, respectively). The stick diagram on the right of the figures illustrates a representative step cycle taken from the left. The angular plots (Fig. 2A) show that, at day 2, the total excursion at the hip, knee, and ankle was quite small. Stepping was possible only with very strong perineal stimulation, and the animal could not follow even the minimal treadmill speed. The hip remained mainly in extension with no weight support, and foot drag was observed during the step cycle with no lift during swing.

Following clonidine injection 150 µg/kg, there was a dramatic change in the kinematic pattern. There was a marked increase of the total excursion at the hip, knee, and ankle. The animal could walk with transient but complete weight support of the hindquarters. There was a foot drag during the early swing, but the foot could be lifted slightly over the treadmill belt during late swing.

On day 7, during the control period, there was a marked increase at the hip, knee, and ankle excursion (compare Fig. 2B with 2A). As illustrated in the stick diagram on the right, the foot drag during most of the swing. A small elevation of the foot from the surface of the belt could be observed only near the end of swing. The animal was still unable to bear weight and the experimenter had to support the animal by the tail.

Following injection of clonidine 150 µg/kg, there was a prolongation of the swing with a clear elevation of the foot from the surface of the belt during the second half of the swing. The animal could walk for a long period of time with weight support and foot placement on the treadmill at that treadmill speed.

During the control period of day 9, the angular excursion had become near normal except for the knee excursion which

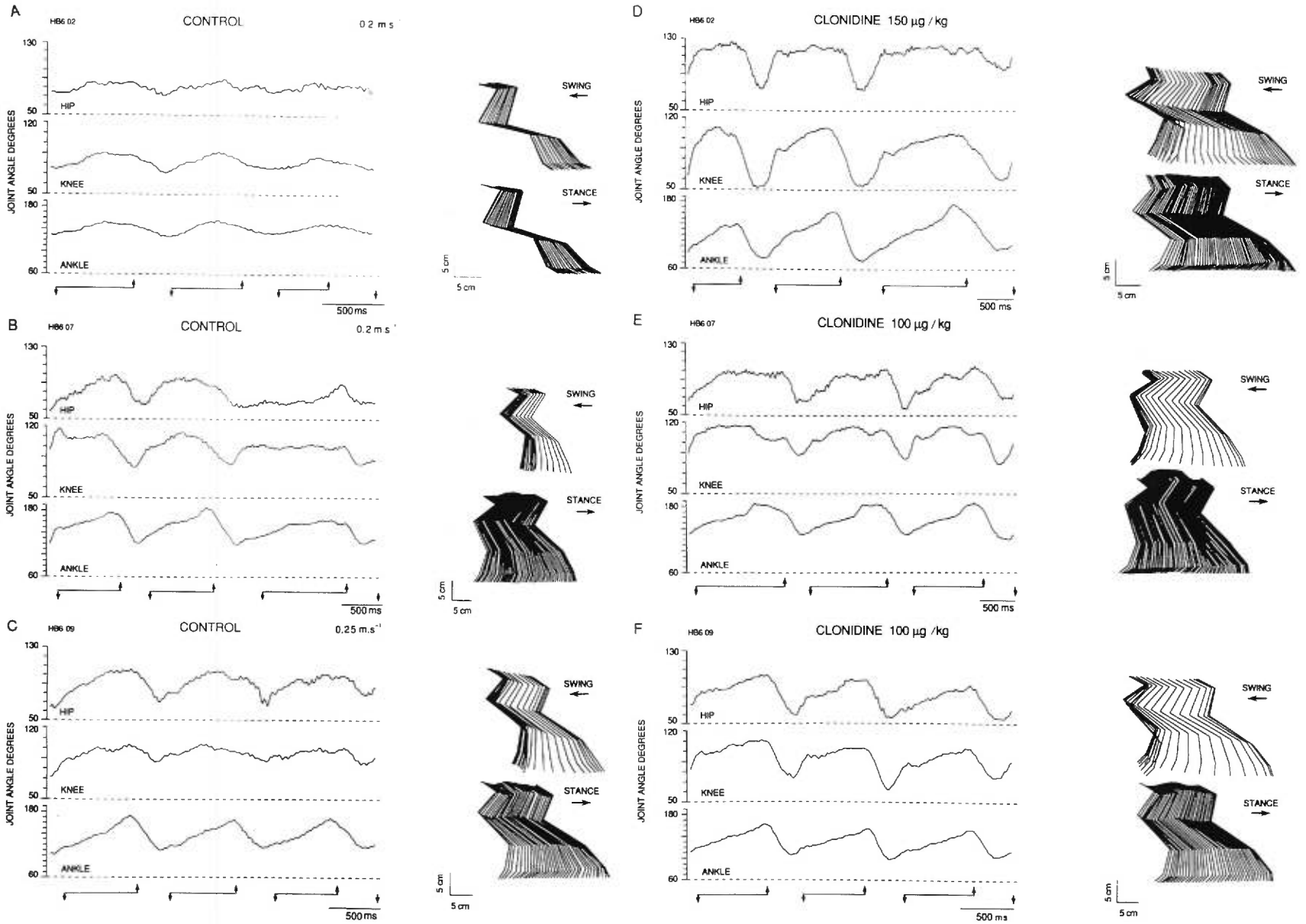


FIG. 2. Kinematic pattern of three successive step cycles at 0.2 m/s at 2, 7, and 9 days before (A, B, and C) and after clonidine injection (D, E, and F) in cat HB6. The plot of hip, knee, and ankle angles for three successive step cycles in the control period. Downgoing curves indicate flexion. The horizontal bars in the duty cycle indicate stance; the upgoing arrows, footlifts and downgoing arrows, foot contacts. The movement of one cycle in A to F has been reconstructed in a stick diagram form. Arrows indicate the duration of the limb movement in each component of the cycle. The horizontal scale is twice that of the vertical scale.

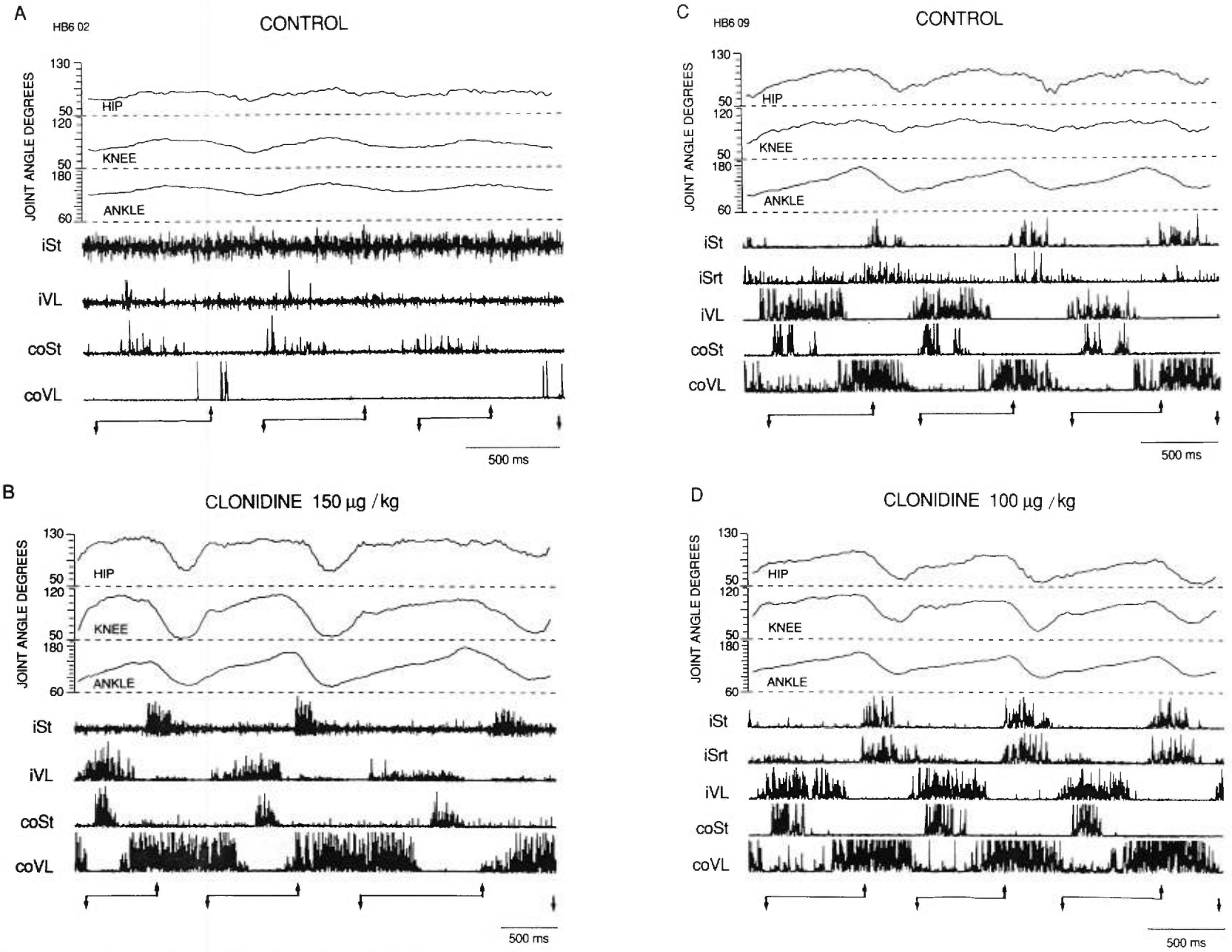


FIG. 3. Angular displacement of the hip, ankle, and knee joint in three successive step cycles at 0.2 m/s with the synchronized EMGs at 2 and 9 days postspinalisation before (A and C) and after clonidine injection (B and D) in cat HB6. The synchronized EMGs are full-wave rectified and filtered.

was quite small in amplitude (Fig. 2C). The animal still showed foot drag during early swing, but became capable of complete weight support of the hindquarters and foot placement on the plantar surface. Thus locomotor performance in such an early period (9 days postspinalization) could be observed for several minutes without clonidine injection.

Injection of clonidine (150 μg/kg) (Fig. 2F) produced an increase of the total excursion, mainly at the knee, with an increase in the swing duration. The animal could walk for a very long period of time with a stable and regular locomotor pattern as well as with foot placement on the plantar surface with full weight support of the hindquarters. However, some deficits such as foot drag during early swing were still present following clonidine injection.

Figure 3 contrasts the synchronized kinematic pattern and EMG activity of three step cycles from the same cat before and after clonidine injection on day 2 and day 9 following spinalization. As mentioned previously, there was very limited angular excursion at the hip, knee, and ankle joint on day 2. The St and VL bursts were tonically active throughout the swing and the stance phase, while the coSt showed some alternating activity.

In contrast, following clonidine injection, the hip, knee, and ankle joints started flexion slightly before liftoff followed by a progressive flexion pattern at the three joints during the first part of the swing. After foot contact, there is a progressive extension at the hip, knee, and ankle. During the first half of the stance, there is a small yield during the weight acceptance. The St burst was clearly phasic and occurred only in the silent periods of VL.

On day 9, the angular plots show that the total excursion at the hip, knee, and ankle markedly changes as compared to day 2. The EMG activity of lower limb muscle was also similar in many aspects to the normal pattern. Following clonidine injection (100 μg/kg), there was an increase of the total excursion at the hip, knee, and ankle, as well as a prolongation of the EMG burst activity. Interestingly, the cycle duration at that speed was very stable and the animal could maintain this performance for several minutes.

Thus clonidine, as early as the second day post-transection, exerts a dramatic effect on the locomotor pattern such that the hindlimbs could walk in a well-coordinated manner with transient but complete weight support. In one spinal cat which we trained under the influence of clonidine, we could accelerate the recovery of locomotion during the first 9 days post-spinalization. Such recovery of locomotion is not normally seen at this stage, but appears only by the third or the fourth week.

DISCUSSION

The present results confirm the importance of the noradrenergic system for the initiation of locomotion in the early stage following spinalization. Forssberg and Grillner (9) and Grillner and Zangger (11) demonstrated that both clonidine and L-dopa could trigger locomotion in adult acute spinal cats, purportedly through "a release of a spinal neuronal network generating locomotion." This effect is quite specific, as dopaminergic or serotonergic drugs failed to induce locomotion in such an early stage. Instead, serotonergic drugs have been found to increase the tonic activity in hindlimb muscles and dopaminergic drugs have been found to have minimal effect during this early period.

Under the influence of clonidine, the spinal cat demonstrated bilateral foot placement on the plantar surface of the foot and transient weight support of the hindquarters at a treadmill speed up to 0.80 $m \cdot s^{-1}$. The effect of clonidine lasted from 6 to 8 h, and these effects completely disappeared by the next day when the noradrenergic drugs had washed out and the locomotor performance regressed to that of the earlier stage. In another set of experiments, locomotor training had been found to accelerate the recovery of locomotor capacity of the hindlimbs in the adult spinal cat (1,14). An improvement in the locomotor capacity of the hindlimbs had also been reported by Lovely et al. (12) following locomotor training. After 3 weeks to 3 months, and up to 1 year depending on the animal, all were capable of walking on the plantar surface of the feet and supporting the weight of the hindquarters. Despite the obvious loss of voluntary control and equilibrium for which the experimenter partially compensated by maintaining the thorax and/or the tail, the cats could walk with a regular rhythm and a well-coordinated hindlimb alternation at speeds of 0.1–1.0 $m \cdot s^{-1}$. Cycle duration, as well as stance and swing duration, resembled those of normal cats at comparable speeds. The range of angular motion was also similar to that observed in intact cats, as was the coupling between different joints (6,10). The EMG activity of the hindlimb and lumbar axial muscles also retained the characteristics observed in the intact animal (6,7,8,13). Some deficits, such as a dragging of the foot in early swing and diminution of the angular excursion in the knee, were seen at later stages. Thus, it is suggested that proper interactive training greatly improves the recovery of locomotion and maintains smooth locomotor movements in adult spinal cats. This, together with the observation that clonidine can initiate locomotion as early as the second day post-spinalization, meant that it was of great interest to investigate if the combined regime of clonidine and locomotor training can accelerate the recovery of locomotor pattern during the early phase of post-spinalization.

In fact, one important new finding from this experiment was a progressive improvement in the locomotor pattern when the spinal cat was trained daily under the effect of clonidine. Within a week post-spinalization, with a regimen combining locomotor training and clonidine injection, a stable locomotor pattern similar in many aspects to that observed in the chronic stage was established, 2–3 months post-spinalization. This stable locomotor pattern was established within 9 days following spinalization, which was retained even without clonidine injection. Studies are in progress to differentiate the relative contribution of clonidine alone and clonidine plus locomotor training on the recovery of locomotor pattern.

In conclusion, clonidine permits the expression of a locomotor pattern in the early stage post-spinalization, the recovery of which could be accelerated with locomotor training.

ACKNOWLEDGEMENTS

This work was supported by a grant to a group in Neurological Sciences of the Medical Research Council and the Network of Centres of Excellence of Canada. H.B. is a scholar of the Fonds de la Recherche en Santé du Québec. We wish to thank J. Provencher for the care of the animals between experiments. We also wish to thank R. Bouchoux for technical expertise and J. Young for secretarial work.

REFERENCES

1. Barbeau, H.; Rossignol, S. Recovery of locomotion after chronic spinalization in the adult cat. Brain Res. 412:84–95; 1987.
2. Barbeau, H.; Rossignol, S. The effects of clonidine and yohimbine on locomotion and cutaneous reflexes in the adult chronic spinal cat. Brain Res. 437:83–96; 1987.
3. Barbeau, H.; Rossignol, S. Effects of noradrenergic, serotonergic and dopaminergic drugs on the initiation of locomotion in the adult spinal cat. Soc. Neurosci. Abstr. 15:393; 1989.
4. Barbeau, H.; Rossignol, S. The effects of serotonergic drugs on the locomotor pattern and cutaneous reflexes in the adult chronic spinal cat. Brain Res. 514:55–67; 1990.

5. Barbeau, H.; Rossignol, S. Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. Brain Res. 546:250–260; 1991.
6. Bélanger, M. A quantitative comparison of the locomotor patterns and the capacity for adaptation before and after spinalization of the cat. Ph.D. Thesis, 1990:198 p.
7. Carlson, H.; Halbertsma, J.; Zomlefer, M. Control of the trunk during walking in the cat. Acta Physiol. Scand. 105:251–253; 1979.
8. Engberg, I.; Lundberg, A. An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. Acta Physiol. Scand. 75:614–630; 1969.
9. Forssberg, H.; Grillner, S. The locomotion of the acute spinal cat injected with clonidine i.v. Brain Res. 50:184–186; 1973.
10. Goslow, G. E., Jr.; Reinking, R. M.; Stuart, D. G. The cat step cycle: Hindlimb joint angles and muscle lengths during unrestrained locomotion. J. Morphol. 141:1–42; 1973.
11. Grillner, S.; Zangger, P. On the central generation of locomotion in the low spinal cat. Exp. Brain Res. 34:241–261; 1979.
12. Lovely, R. G.; Gregor, R. J.; Roy, R. R.; Edgerton, V. R. Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. Exp. Neurol. 92:421–435; 1986.
13. Rasmussen, S.; Chan, A. K.; Goslow, G. E., Jr. The cat step cycle: Electromyographic patterns for hindlimb muscles during posture and unrestrained locomotion. J. Morphol. 155:253–270; 1978.
14. Rossignol, S.; Barbeau, H.; Julien, C. Locomotion of the adult chronic spinal cat and its modification by monoaminergic agonists and antagonists. In: Goldberger, M.; Gorio, A.; Murray, M., eds. Development and plasticity of the mammalian spinal cord. vol. 3. Padova, Italy: Liviana Press, Padova; 1986:323–346.
15. Rossignol, S.; Bélanger, M.; Barbeau, H.; Drew, T. Assessment of locomotor functions in the adult chronic spinal cat. In: Brown, M.; Goldberger, M. E., eds. Criteria for the assessment of recovery of function: Behavioral methods. American Paralysis Assn.; 1989: 62–65.

Appendix B

ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1996, 56: 449-463

Locomotor capacities after complete and partial lesions of the spinal cord

Serge Rossignol, Connie Chau, Edna Brustein, Marc Bélanger, Hughes Barbeau and **Trevor Drew**

Centre de recherche en sciences neurologiques, Université de Montréal, Québec, Canada H3C 3J7

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.

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

walk with their hindlimbs on a treadmill, much as the spinal kittens.

Along these lines of work, it was shown that the age of spinalisation and the amount of training on the treadmill (30 min 5 times a week) had important effects on the locomotor pattern (Smith et al. 1982, Bregman and Goldberger 1983, Robinson and Goldberger 1986b). Animals spinalised at 2 weeks of age had a much better locomotor performance than those spinalised at 12 weeks of age. Training in 12 week old cats had an observable effect on weight bearing during locomotion. The EMG pattern appeared normal in many respects, except that clonus was frequently observed. Even so, defects in the locomotor pattern such as uncoupling between the knee and ankle as well as an absence of yield in E2 phase of the step cycle were reported.

Work in adult chronic spinal cats (Rossignol et al. 1982, 1986, 1989a, Barbeau and Rossignol 1987, Belanger et al. 1996) has clearly established that the quality of locomotion is indeed improved by training and that the spinal locomotor pattern evolves with time. For instance, as time progresses, cats make larger steps at the same treadmill speed; this is achieved by a greater lengthening of the extensor burst relative to the flexor burst, a structure which resemble more the situation in the intact. In 3 cats it was found that cycle duration increased as a function of time but reached a plateau at around 3 months. All cats made plantigrade foot contact and could maintain the weight of the hindquarters by the third week. The important joint uncoupling described before and the absence of yield in E2 were not confirmed (see also: Lovely et al. 1990). However, foot drag was present in most cats during the first part of swing. This was interpreted as being due to a decrease in the delay between the onset of the knee flexor and the hip flexors so that both start more or less simultaneously in the spinal cat whereas normally the knee flexor first removes the foot from ground before it is brought forwards. It was also observed that Tibialis Anterior, an ankle flexor, also tended to be recruited earlier than in the intact which again could result in a foot drag if the foot is not already lifted from ground. In many other aspects 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-

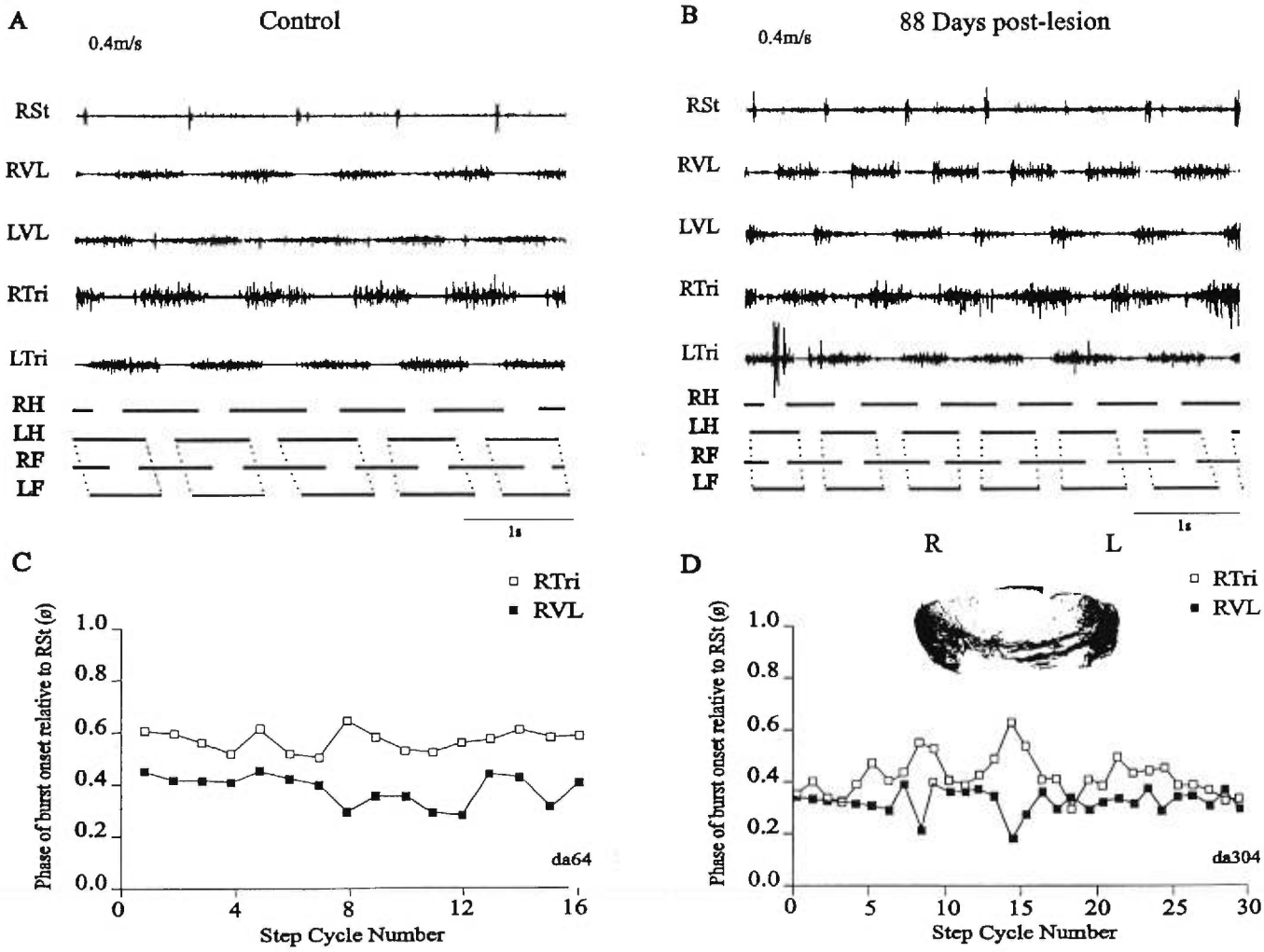


Fig. 1. EMG and foot fall pattern in a cat with a bilateral ventral and ventrolateral spinal cord lesion (the histological section is shown as an insert in D). A, control period. EMGs during walking at 0.4 m/s. St, Semitendinosus; VL, Vastus lateralis; Tri, Triceps brachii, lateral head. R, right; L, 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.

limb coordination. Cats tend to increase the control over the forelimbs and also tend to pace even several weeks after the lesion (see Fig.1). After such massive lesions of the ventral and ventrolateral pathways, the remaining dorsolateral pathways are thus capable of triggering voluntary hindlimb movements and in part coordinate all 4 limbs favouring the most stable locomotor pattern (increase in the number of feet on the ground at all times). We have not found any foot drag in these cats, although lesions of the dorsolateral funiculus (Jiang and Drew 1996) produce such foot drag much as in the complete spinal cat.

About a week after a spinal hemisection at the low thoracic level, cats can readily walk overground with both hindlimbs; after a second transection at the midthoracic level contralaterally to the first section, cats still can regain voluntary locomotor functions overground, although the fore- and hindlimbs coupling 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 incomplete means that the patients still have some sensory-motor functions whereas neurologically complete means that the patients have no sensory-motor functions although the spinal cord is not completely severed

Chronic partial spinal section in different species

Species	Type and level of lesions	References
MAN	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)
MAN	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)
MONKEYS	various quadrants	(Eidelberg et al. 1981b, Vilensky et al. 1992)
MONKEYS	hemisection	(Aoki et al. 1991)
CATS	various quadrants	(Eidelberg and Stein 1974, Eidelberg 1981, Eidelberg et al. 1981a, Eidelberg et al. 1985)
CATS	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)
CATS	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)
CATS	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),

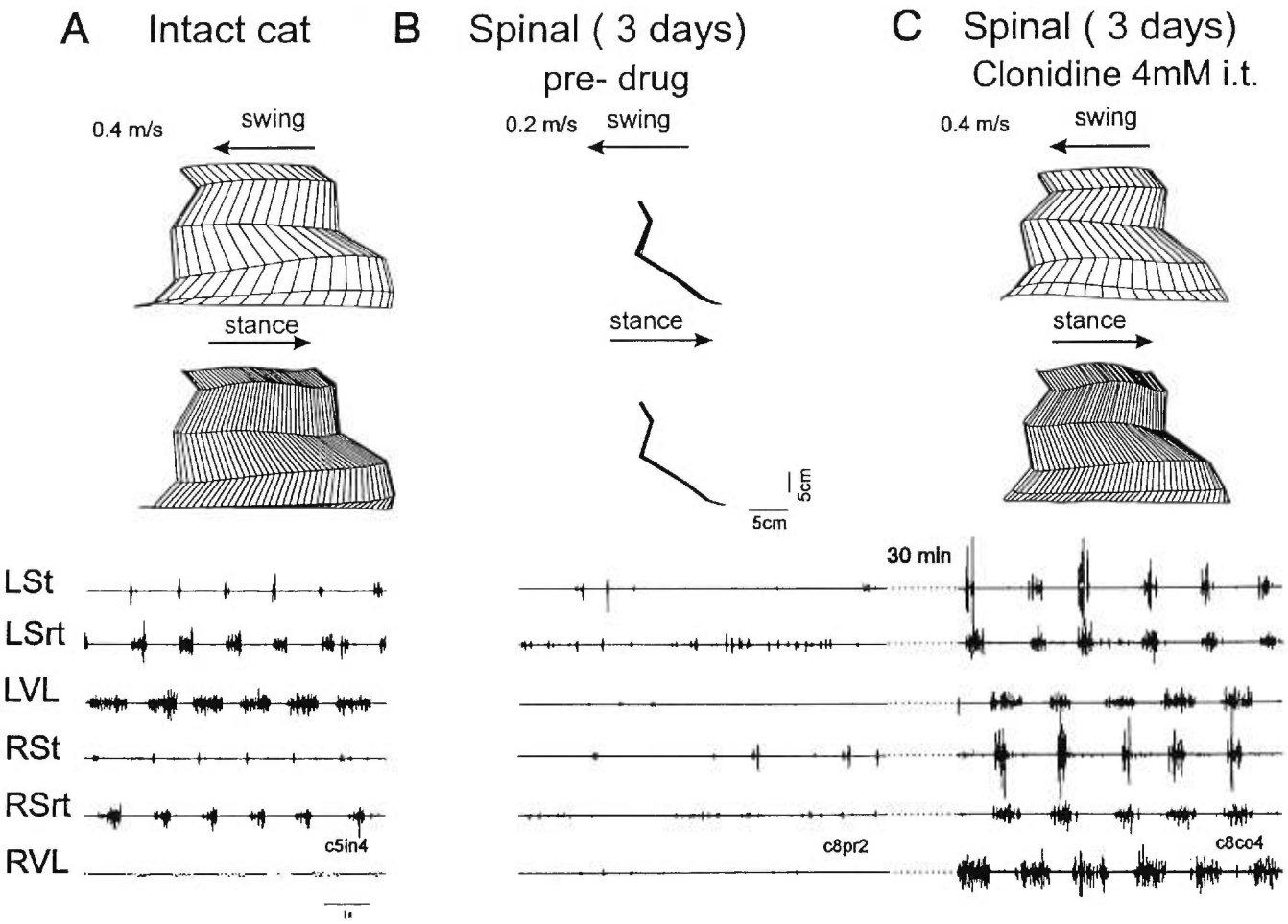


Fig. 2. Early-spinal (3 days) cat injected with intrathecal clonidine (4 mM). A, walking on a treadmill at 0.4 m/s during the control period before spinalisation. B, three days after spinalisation before injecting clonidine. C, thirty min. after i.t. clonidine. Muscles: Srt, Sartorius anterior; other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

NMDA in decerebrate cats fictive locomotion (Douglas et al. 1993), 5-HT and dopamine in rabbits (Viala and Buser 1969).

Figure 2 illustrates the effect of an intrathecal (i.t.) injection of clonidine in a complete spinal cat. The left panel illustrates the locomotor pattern of the cat during the control period before spinalisation. Three days after spinalisation the cat is virtually motionless on the treadmill, the limbs being passively dragged on the treadmill belt. A few minutes after the i.t. injection of clonidine, the cat can step with its hindlimbs on the treadmill in a well coordinated pattern 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).

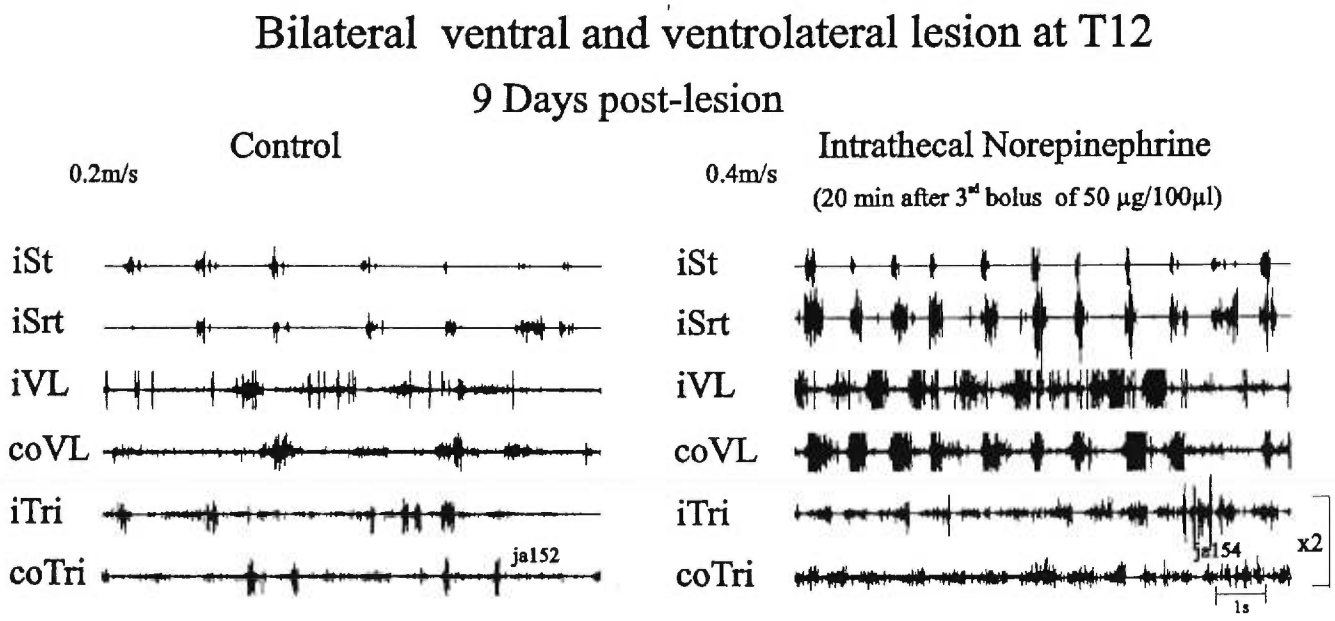


Fig. 3. Modulation of locomotion in a partially lesioned cat by i.t. administration of noradrenaline (NE) at 9 days post-lesion (large but yet undocumented spinal lesion because the cat is still alive). On the left is shown the EMG pattern of the cat walking at 0.2 m/s (its maximal speed performance) 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 most species, the locomotor program is innate and generated centrally in the spinal cord. After spinalisation, the locomotor capabilities change with time and training (plasticity) and locomotion can be adapted to speed and perturbations. Locomotion can be triggered by alpha-2 noradrenergic stimulation in early-spinal cats. In late-spinal cats, all neurotransmitter systems can on the other hand modulate the expression of the locomotor pattern. In normal conditions, it is possible that the locomotor program can be triggered through the action of several descending pathways. We have no indication that any particular descending pathway is unique or essential for triggering locomotion. Even cats with massive ventral and ventrolateral lesions can perform voluntary quadrupedal locomotion although there are major deficits in weight support and interlimb coordination. It is therefore possible to conclude that there is a spinal circuitry implicated in the generation of the locomotor rhythm, that this circuitry has some degree of plasticity which is essential for any potential benefit of locomotor training, that activity in this locomotor circuitry can be triggered or modulated by neurotransmitters as well as sensory inputs and finally that different descending pathways in the dorsolateral or the ventral-ventrolateral quadrants can trigger or modulate this circuitry. It is believed that these concepts are important and necessary for the design of locomotor rehabilitation strategies in patients with spinal cord injuries, especially when different approaches such as locomotor training, pharmacotherapy and functional electrical stimulation are combined (Barbeau and Rossignol 1994).

REFERENCES

Afelt Z. (1963) Variability of reflexes in chronic spinal frogs. In: Central and peripheral mechanisms of motor functions. House of the Czechoslovak Academy of Sciences, Prague, p. 37-41.

Afelt Z. (1970) Reflex activity in chronic spinal cats. Acta Neurobiol. Exp. 30: 129-144.

Afelt Z. (1974) Functional significance of ventral descending tracts of the spinal cord in the cat. Acta Neurobiol. Exp. 34: 393-407.

Aoki M., Fujito Y., Mizuguchi A., Satomi H. (1991) Recovery of hindlimb movement after spinal hemisection and collateral sprouting from corticospinal fibers in monkeys. In: Neurobiological basis of human locomotion (Eds. M. Shimamura, S. Grillner and V.R. Edgerton). Japan Scientific Societies Press, Tokyo, p. 401-405.

Armstrong D.M. (1986) Supraspinal contributions to the initiation and control of locomotion in the cat. Prog. Neurobiol. 26: 273-361.

Baker L. L., Chandler S. H., Goldberg L. J. (1984) L-Dopa induced locomotor-like activity in ankle flexor and extensor nerves of chronic and acute spinal cats. Exp. Neurol. 86: 515 -526.

Barbeau H., Chau C., Rossignol S. (1993) Noradrenergic agonists and locomotor training affect locomotor recovery after cord transection in adult cats. Brain Res. Bull. 30: 387-393.

Barbeau H., Fung J. (1994) Recovery of locomotion following spinal cord injury: new concepts and approaches in rehabilitation. In: Handbook of neurorehabilitation (Eds. D.C. Good and J.R. Couch). Marcel Dekker Inc., New York, p. 73-104.

Barbeau H., Julien C., Rossignol S. (1987) The effects of clonidine and yohimbine on locomotion and cutaneous reflexes in the adult chronic spinal cat. Brain Res. 437: 83-96.

Barbeau H., Rossignol S. (1987) Recovery of locomotion after chronic spinalization in the adult cat. Brain Res. 412: 84-95.

Barbeau H., Rossignol S. (1990) The effects of serotonergic drugs on the locomotor pattern and on cutaneous reflexes of the adult chronic spinal cat. Brain Res. 514: 55-67.

Barbeau H., Rossignol S. (1991) Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. Brain Res. 546: 250-260.

Barbeau H., Rossignol S. (1994) Enhancement of locomotor recovery following spinal cord injury. Curr. Opinion Neurol. 7: 517-524.

Belanger M., Drew T., Provencher J., Rossignol S. (1986) The study of locomotion and cutaneous reflexes in the same cat before and after spinalisation. Soc. Neurosci. Abstr. 12: no. 241.13,880.

Belanger M., Drew T., Provencher J., Rossignol S. (1987) Cutaneous reflexes evoked by mechanical stimulation during locomotion in the same chronically implanted cats before and after spinalisation. Soc. Neurosci. Abstr. 13: 1176 no. 321.11.

Belanger M., Drew T., Provencher J., Rossignol S. (1988a) Locomotion on an inclined plane before and after spinalisation in the same cat. Soc. Neurosci. Abstr. 14: 265 no. 106.7.

Belanger M., Drew T., Rossignol S. (1988b) Spinal locomotion: a comparison of the kinematics and the electromyographic activity in the same animal before and after spinalization. Acta Biol. Hungarica 39: 151-154.

Belanger M., Drew T., Rossignol S. (1988c) A comparative study of the response to mechanical perturbations during locomotion in the same chronically implanted cats before and after spinalisation. Can. J. Physiol. Pharmacol. 66: AV.

Belanger M., Drew T., Rossignol S. (1989) Adaptation to treadmill speed of chronically implanted cats before and after spinalisation. Soc. Neurosci. Abstr. 15: 393-no. 160.8.

Belanger M., Drew T., Rossignol S. (1996) A comparison of treadmill locomotion in adult cats before and after spinalization. J. Neurophysiol. (in press)

Bem T., Górska T., Majczyński H., Zmysłowski W. (1995) Different patterns of fore-hindlimb coordination during overground locomotion in cats with ventral and lateral spinal lesions. Exp.Brain Res. 104: 70-80.

Bregman B.S., Goldberger M.E. (1983) Infant lesion effect: I. Development of motor behavior following neonatal spinal cord damage in cats. Developmental Brain Res. 9: 103-117.

Bregman B.S., Kunkel-Bagden E., Reier P.J., Ning Dai H., McAtee M., Gao D. (1993) Recovery of function after spinal cord injury: mechanisms underlying transplant-mediated recovery of function differ after spinal cord injury in newborn and adult rats. Exp. Neurol. 123: 3-16.

Brown T.G. (1911) The intrinsic factors in the act of progression in the mammal. Proc. Roy. Soc. London B. 84: 308-319.

Brustein E., Lavoie S., Lebel F., Provencher J., McFadyen B., Rossignol S. (1995) The recovery of locomotion in adult cats subjected to bilateral lesions of the ventral and ventrolateral spinal quadrants. Soc. Neurosci. Abstr. 25.

Brustein E., Provencher J., Lebel F., Rossignol S. (1993) Recovery of locomotion in cats after lesions of the ventral spinal cord. Soc. Neurosci. Abstr. 19: 147 no. 63.15.

Brustein E., Provencher J., Lebel F., Rossignol S. (1994) Adjustments of the locomotor pattern in cats with a chronic lesion of the ventral spinal cord. Soc. Neurosci. Abstr. 20: 573 no. 241.13.

Bussel B.C., Roby-Brami A., Yakovleff A., Bennis N. (1988) Evidences for the presence of a spinal stepping generator in patients with a spinal cord section. In: Posture and gait: development, adaptation and modulation (Eds. B. Amblard, A. Berthoz and F. Clarac). Elsevier, North Holland, p. 273-278.

Calancie B., Needham-Shropshire B., Jacobs P., Willer K., Zych G., Green B.A. (1994) Involuntary stepping after chronic spinal cord injury. Evidence for a central rhythm generator for locomotion in man. Brain 117: 1143-1159.

Cazalets J.R., Sqalli-Houssaini Y., Clarac F. (1992) Activation of the central pattern generators for locomotion by serotonin and excitatory amino acids in neonatal rat. J. Physiol. 455: 187-204.

Chau C., Provencher J., Lebel F., Jordan L., Barbeau H., Rossignol S. (1994) Effects of intrathecal injection of NMDA receptor agonist and antagonist on locomotion of adult chronic spinal cats. Soc. Neurosci. Abstr. 20 no. 241.14: 573.

Contamin F. (1983) Sections médullaires incompletes et locomotion chez le chat. Bull. Acad. Natl. Med. 167: 727-730.

Dietz V., Colombo G., Jensen L. (1994) Locomotor activity in spinal man. Lancet 344: 1260-1263.

Dietz V., Colombo G., Jensen L., Baumgartner L. (1995) Locomotor capacity of spinal cord in paraplegic patients. Ann. Neurol. 37: 574-582.

Dobkin B.H., Edgerton V.R., Fowler E. (1992) Sensory input during treadmill training alters rhythmic locomotor EMG output in subjects with complete spinal cord injury. Soc. Neurosci. Abst. 18: 1403.

Douglas J.R., Noga B.R., Dai X., Jordan L.M. (1993) The effects of intrathecal administration of excitatory amino acid agonists and antagonists on the initiation of locomotion in the adult cat. J. Neurosci. 13: 990-1000.

Duysens J., Pearson K.G. (1980) Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. Brain Res. 187: 321-332.

Edgerton V.R., de Guzman C.P., Gregor R.J., Roy R.R., Hodgson J.A., Lovely R.G. (1991) Trainability of the spinal cord to generate hindlimb stepping patterns in adult spinalized cats. In: Neurobiological basis of human locomotion (Eds. M. Shimamura, S. Grillner and V.R. Edgerton). Japan Scientific Societies Press, Tokyo, p. 411-423.

Eidelberg E. (1981) Consequences of spinal cord lesions upon motor function, with special reference to locomotor activity. Prog. Neurobiol. 17: 185-202.

Eidelberg E. (1983) Loss and recovery of locomotor function after spinal cord lesions in cats and monkeys. In: Nerve organ and tissue regeneration: research perspectives. (Ed. F.J. Seil). Academic Press, New York, p. 231-242.

Eidelberg E., Jones D.J., Keenan R.W., Schwartzman R.J. (1985) Report from the spinal cord injury research program. Eur. J. Neurosci. 225-234.

Eidelberg E., Stein D.G. (1974) Functional recovery after lesions of the nervous system. Neurosci. Res. Prog. Bull. 12: 191-303.

Eidelberg E., Story J.L., Meyer B.L., Nystel J. (1980) Stepping by chronic spinal cats. Exp. Brain. Res. 40: 241-246.

Eidelberg E., Story J.L., Walden J.G., Meyer B.L. (1981a) Anatomical correlates of return of locomotor function after partial spinal cord lesions in cats. Exp. Brain. Res. 42: 81-88.

Eidelberg E., Walden J.G., Nguyen L.H. (1981b) Locomotor control in macaque monkeys. Brain 104: 647-663.

English A.W. (1980) Interlimb coordination during stepping in the cat: effects of dorsal column section. J. Neurophysiol. 44: 270-279.

English A.W. (1985) Interlimb coordination during stepping in the cat. The role of the dorsal spinocerebellar tract. Exp. Neurol. 87: 96-108.

Fayein G.A., Viala D. (1976) Development of locomotor activities in young chronic spinal rabbits. Neurosci. Lett. 3: 329-333.

Forssberg H., Grillner S. (1973) The locomotion of the acute spinal cat injected with clonidine i.v. Brain Res. 50: 184-186.

Forssberg H., Grillner S., Halbertsma J. (1980a) The locomotion of the low spinal cat. I. Coordination within a hindlimb. Acta Physiol. Scand. 108: 269-281.

Forssberg H., Grillner S., Halbertsma J., Rossignol S. (1980b) The locomotion of the low spinal cat: II. Interlimb coordination. Acta Physiol. Scand. 108: 283-295.

Forssberg H., Grillner S., Sjostrom A. (1974) Tactile placing reactions in chronic spinal kittens. Acta Physiol. Scand. 92: 114-120.

Freeman L.W. (1952) Return of function after complete transection of the spinal cord of the rat, cat and dog. Ann. Surg. 136: 193-205.

Freeman L.W. (1954) Functional recovery in spinal rats. In: Regeneration in the central nervous system (Eds. W.F. Windle and C.C. Thomas). Springfield, Illinois, p. 195-207.

Fung J., Stewart J.E., Barbeau H. (1990) The combined effects of clonidine and cyproheptadine with interactive training on the modulation of locomotion in spinal cord injured subjects. J. Neurol. Sci. 100: 85-93.

Giuliani C.A., Smith J.L. (1987) Stepping behaviors in chronic spinal cats with one hindlimb deafferented. J. Neurosci. 7: 2537-2546.

Goldberger M.E. (1986) Autonomous spinal motor function and the infant lesion effect. In: Development and plasticity of the mammalian spinal cord. Fidia Research Series. (Eds. M.E. Goldberger, A. Gorio and M. Murray). Liviana Press, Padova, p. 363-380.

Górska T., Bem T., Majczyński H. (1990) Locomotion in cats with ventral spinal lesions: support patterns and duration of support phases during unrestrained walking. Acta Neurobiol. Exp. 50: 191-200.

Górska T., Bem T., Majczyński H., Zmysłowski W. (1993a) Unrestrained walking in cats with partial spinal lesions. Brain Res. Bull. 32: 241-249.

Górska T., Majczyński H., Bem T., Zmysłowski W. (1993b) Hindlimb swing, stance and step relationships during unrestrained walking in cats with lateral funicular lesion. Acta Neurobiol. Exp. 53: 133-142.

Grillner S. (1973) Locomotion in the spinal cat. In: Control of posture and locomotion. Adv. Behav. Biol. 7: (Eds. R.B. Stein, K.G. Pearson, R.S. Smith and J.B. Redford). Plenum Press, New York, p. 515-535.

Grillner S. (1981) Control of locomotion in bipeds, tetrapods, and fish. In: Handbook of physiology. The nervous system II. (Eds. J.M. Brookhart and V.B. Mountcastle). Am. Physiol. Soc., Bethesda, p. 1179-1236.

Grillner S., Dubuc R. (1988) Control of locomotion in vertebrates: spinal and supraspinal mechanisms. In: Functional recovery in neurological disease (Ed. S.G. Waxman). Raven Press, New York, p. 425-453.

Grillner S., Zangger P. (1979) On the central generation of locomotion in the low spinal cat. Exp. Brain. Res. 34: 241-261.

Hanna J.P., Frank J.I. (1995) Automatic stepping in the pontomedullary stage of central herniation. Neurology 45: 985-986.

Helgren M.E., Goldberger M.E. (1993) The recovery of postural reflexes and locomotion following low thoracic hemisection in adult cats involves compensation by undamaged primary afferent pathways. Exp. Neurol. 123: 17-34.

Hinsey J.C., Cutting C.C. (1932) The spinal rabbit and its reflexes. Proc. Soc. Exp. Biol. Med. 30: 134-135.

Hinsey J.C., Cutting C.C. (1936) Reflexes in the spinal opossum. J. Comp. Neurol. 64: 375-387.

Holmes G. (1915) Spinal injuries of welfare. Br. Med. J. 2: 815-821.

Hultborn H., Petersen N., Brownstone R., Nielsen J. (1993) Evidence of fictive spinal locomotion in the marmoset (Callithrix jacchus). Soc. Neurosci. Abstr. 19: 539 no. 225.1.

Jiang W., Drew T. (1996) Effects of bilateral lesions of the dorsal columns and dorsolateral funiculi at the level of the low thoracic spinal cord on the control of locomotion in the adult cat: I. Treadmill walking. J. Neurophysiol. (in press)

Kato M. (1988) Longitudinal myelotomy of lumbar spinal cord has little effect on coordinated locomotor activities of bilateral hindlimbs of the chronic cats. Neurosci. Lett. 93: 259-263.

Kato M. (1989) Chronically isolated lumbar half spinal cord produced by hemisection and longitudinal myelotomy generates locomotor activities of the ipsilateral hindlimb of the cat. Neurosci. Lett. 98: 149-153.

Kato M. (1991) Chronically isolated lumbar half spinal cord and locomotor activities of the hindlimb. In: Neurobiological basis of human locomotion (Eds. M. Shimamura, S. Grillner and V.R. Edgerton). Japan Scientific Societies Press, Tokyo, p. 407-410.

Kato M., Murakami S., Hirayama H., Hikino K. (1985) Recovery of postural control following chronic bilateral hemisections at different spinal cord levels in adult cats. Exp. Neurol. 90: 350-364.

Kato M., Murakami S., Yasuda K., Hirayama H. (1984) Disruption of fore-and hindlimb coordination during overground locomotion in cats with bilateral serial hemisection of the spinal cord. Neurosci. Res. 2: 27-47.

Kehne J.H., Gallager D.W., Davis M. (1985) Spinalization unmasks clonidine's alpha1-adrenergic mediated excitation of the flexor reflex in rats. J. Neurosci. 5: 1583-1590.

Kellogg W.N., Deese J., Pronko N.H. (1946) On the behavior of the lumbo-spinal dog. J. Exp. Psychol. 36: 503-511.

Kozak W., Westerman R. (1966) Basic patterns of plastic change in the mammalian nervous system. Symposia Soc. Exp. Biol. 20: 509-544.

Kuhn R.A. (1950) Functional capacity of the isolated human spinal cord. Brain 73: 1-51.

Kunkel-Bagden E., Dai H.-N., Bregman B.S. (1993) Methods to assess the development and recovery of locomotor function after spinal cord injury in rats. Exp. Neurol. 119: 153-164.

Laughton N.B. (1924) Studies on the nervous regulation of progression in mammals. Am. J. Physiol. 70: 358-384.

Lovely R.G., Gregor R.J., Roy R.R., Edgerton V.R. (1986) Effects of training on the recovery of full-weight-bearing stepping in the adult spinal cat. Exp. Neurol. 92: 421-435.

Lovely R.G., Gregor R.J., Roy R.R., Edgerton V.R. (1990) Weight-bearing hindlimb stepping in treadmill-exercised adult spinal cat. Brain Res. 514: 206-218.

Mandel S., Arenas A., Scasta D. (1982) Spinal automatism in cerebral death. New Engl. J. Med. 307: 501.

Masamichi K., Murakami S., Yasuda K., Hirayama H. (1984) Disrupting of fore- and hindlimb coordination during overground locomotion in cats with bilateral serial hemisection of the spinal cord. Neurosci. Res. 2: 27-47.

McCouch G.P. (1947) Reflex development in the chronically spinal cat and dog. J. Neurophysiol. 10: 425-428.

Meisel R.L., Rakerd B. (1982) Induction of hindlimb stepping movements in rats spinally transected as adults or as neonates. Brain Res. 240: 353-356.

Miller S., Van der Meche F.G.A. (1976) Coordinated stepping of all four limbs in the high spinal cat. Brain Res. 109: 395-398.

Naito A., Shimizu Y. (1991) Analyses of the stepping movements in adult spinal dogs. In: Neurobiological basis of human locomotion (Eds. M. Shimamura, S. Grillner and V.R. Edgerton). Japan Scientific Societies Press, Tokyo, p. 395-399.

Naito A., Shimizu Y., Handa Y. (1990) Analyses of treadmill locomotion in adult spinal dogs. Neurosci. Res. 8: 281-290.

Nathan P.W. (1994) Effects on movement of surgical incisions into the human spinal cord. Brain 117: 337-346.

Norman K.E., Barbeau H. (1992) Comparison of cyproheptadine, clonidine and baclofen on the modulation of gait pattern in subjects with spinal cord injury. In: Spasticity (Eds. A. Thilmann, D. Burke and Z. Rymer). Springer-Verlag, New York, p. 410-425.

Pearson K.G. (1993) Common principles of motor control in vertebrates and invertebrates. Ann. Rev. Neurosci. 16: 265-297.

Philippson M. (1905) L'autonomie et la centralisation dans le system nerveux des animaux. Trav. Lab. Physiol. Inst. Solvay. (Bruxelles) 7: 1-208.

Ranson S.W., Hinsey J.C. (1930) Reflexes in the hind limbs of cats after transection of the spinal cord at various levels. Am. J. Physiol. 94: 471-495.

Robinson G.A., Goldberger M.E. (1986a) The development and recovery of motor function in spinal cats. II. Pharmacological enhancement of recovery. Exp. Brain. Res. 62: 387-400.

Robinson G.A., Goldberger M.E. (1986b) The development and recovery of motor function in spinal cats. I. The infant lesion effect. Exp. Brain. Res. 62: 373-386.

Roby-Brami A., Bussel B. (1987) Long-latency spinal reflex in man after flexor reflex afferent stimulation. Brain 110: 707-725.

Rosenfeld J.E., Sherwood A.M., Halter J.A., Dimitrijevic M.R. (1995) Evidence of a pattern generator in paralyzed subjects with spinal cord injury during spinal cord stimulation. Soc. Neurosci. Abstr. 21: 688.

Rossignol S. (1996) Neural control of stereotypic limb movements. In: Handbook of physiology. Section 12. Exercise: Regulation and integration of multiple systems (Eds. L.B. Rowell and J.T. Sheperd). Am. Physiol. Soc. (in press)

Rossignol S., Barbeau H. (1993) Pharmacology of locomotion: an account of studies in spinal cats and spinal cord injured subjects. The journal of the american paraplegia society. 16: 190-196.

Rossignol S., Barbeau H. (1995) New approaches to locomotor rehabilitation in spinal cord injury. Ann. Neurol. 37: 555-556.

Rossignol S., Barbeau H., Chau C. (1995) Pharmacology of locomotion in chronic spinal cat. In: Alpha and gamma motor systems (Eds. A. Taylor, M.H. Gladden and R. Durbaba). Plenum Press, New York, p. 449-455.

Rossignol S., Barbeau H., Julien C. (1986) Locomotion of the adult chronic spinal cat and its modification by monoaminergic agonists and antagonists. In: Development and plasticity of the mammalian spinal cord (Eds. M. Goldberger, A. Gorio and M. Murray). Fidia Research Series III. Liviana Press, Padova, p. 323-345.

Rossignol S., Barbeau H., Provencher J. (1982) Locomotion in the adult chronic spinal cat. Soc. Neurosci. Abstr. 8: no. 47.1,163.

Rossignol S., Belanger M., Barbeau H., Drew T. (1989a) Assessment of locomotor functions in the adult chronic spinal cat. In: Conference proceedings: criteria for assessing recovery of function: behavioral methods (Eds. M. Brown and M.E. Goldberger). A.P.A., Springfield, p. 10-11.

Rossignol S., Belanger M., Barbeau H., Drew T. (1989b) Assessment of locomotor functions in the adult chronic spinal cat. In: Conference proceedings: criteria for assessing recovery of function: behavioral methods (Eds. M. Brown and M.E. Goldberger). A.P.A, Springfield, p. 62-65.

Rossignol S., Dubuc R. (1994) Spinal pattern generation. Curr. Opinion Neurobiol. 4: 894-902.

Rossignol S., Lund J.P., Drew T. (1988) The role of sensory inputs in regulating patterns of rhythmical movements in higher vertebrates. A comparison between locomotion, respiration and mastication. In: Neural control of rhythmic

movements in vertebrates (Eds. A. Cohen, S. Rossignol and S. Grillner). Wiley and Sons Co., New York, p. 201-283.

Roy R.R., Hodgson J.A., Lauretz S.D., Pierotti D.J., Gayek R.J., Edgerton V.R. (1992) Chronic spinal cord-injured cats: surgical procedures and management. Lab. Anim. Sci. 42: 335-343.

Sherrington C.S. (1899) On the spinal animal. Medico-Chirugical. Transactions 82: 449-486.

Sherrington C.S. (1910a) Remarks on the reflex mechanism of the step. Brain 33: 1-25.

Sherrington C.S. (1910b) Flexion-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. J. Physiol. 40: 28-121.

Shimizu Y. (1991) Locomotive movements of the hindlimbs after complete transection of the spinal cord in adult dogs. In: Neurobiological basis of human locomotion (Eds. M. Shimamura, S. Grillner and V.R. Edgerton). Japan Scientific Societies Press, Tokyo, p. 387-394.

Shurrager P.S., Dykman R.A. (1951) Walking spinal carnivore. J. Comp. Physiol. Psychol. 44: 252-262.

Smith J.L., Smith L.A., Zernicke R.F., Hoy, M. (1982) Locomotion in exercised and non-exercised cats cordotomized at two or twelve weeks of age. Exp. Neurol. 76: 393-413.

Stelzner D.J., Ershler W.B., Weber E.D. (1975) Effects of spinal transection in neonatal and weanling rats: survival of functionn, Exp. Neurol. 46: 156-177.

Stewart J.E., Barbeau H., Gauthier S. (1991) Modulation of locomotor patterns and spasticity with clonidine in spinal cord injured patients. Can. J. Neurol. Sci. 18: 321-332.

Ten Cate J. (1939) Quelques observations sur la locomotion des chiens dont la moelle épinière est sectionnée transversalement. Arch. Neerl. Physiol. 24: 476-485.

Ten Cate J. (1960) Locomotor movements in the spinal pigeon. J. Exp. Biol. 37: 609-613.

Ten Cate J. (1962) Innervation of locomotor movements by the lumbosacral cord in birds and mammals. J. Exp. Biol. 39: 239-242.

Ten Cate J. (1964) Locomotory movements of the hindlimbs in rabbits after isolation of the lumbosacral cord. J. Exp. Biol. 41: 359-362.

Viala D., Buser P. (1969) The effects of DOPA and 5-HTP on rhythmic efferent discharges in hindlimb nerves in the rabbit. Brain Res. 12: 437-443.

Viala D., Viala G., Fayein N. (1986) Plasticity of locomotor organization in infant rabbits spinalized shortly after birth. In: Development and plasticity of the mammalian spinal cord (Eds. M. Goldberger, A. Gorio and M. Murray). Liviana Press, Padova, p. 301-310.

Vilensky J.A., Moore A.M., Eidelberg E., Walden J.G. (1992) Recovery of locomotion in monkeys with spinal cord lesions. J. Motor Behav. 24: 288-296.

Wainberg M., Barbeau H., Gauthier S. (1990) The effects of cyproheptadine on locomotion and on spasticity in patients with spinal cord injuries. J. Neurol. Neurosurg. Psychiat. 53: 754-763.

Weber E.D., Stelzner D.J. (1977) Behavioral effects of spinal cord transection in the developing rat. Brain Res. 125: 241-255.

Wernig A., Muller S. (1992) Laufband locomotion with body weight support improved walking in persons with severe spinal cord injuries. Paraplegia 30: 229-238.

Wernig A., Muller S., Nanassy A., Cagol E. (1995) Laufband therapy based on 'rules of spinal locomotion' is effective in spinal cord injured persons. Eur. J. Neurosci. 7: 823-829.

Windle W.F., Smart J.O., Beers J.J. (1958) Residual function after subtotal spinal cord transection in adult cats. Neurology 8: 518-521.

Wolpaw J.R., Lee C.L. (1989) Memory traces in primate spinal cord produced by operant conditioning of H-reflex. J. Neurophysiol. 61: 563-572.

Zangger P. (1981) The effect of 4-aminopyridine on the spinal locomotor rhythm induced by L-Dopa. Brain Res. 215: 211-223.

Zhang A.A., Kirkpatrick G., Zhong V.H., Nguyen V.T., Dobkin B.H., Edgerton V.R. (1994) Cinematographic analysis of hindlimb stepping in spinal (7 days post-natal) rats. Soc. Neurosci. Abstr. 20 No. 241.1: 571.

Zmysłowski W., Górska T., Majczyński H., Bem T. (1993) Hindlimb muscle activity during unrestrained walking in cats with lesions of the lateral funiculi. Acta Neurobiol. Exp. 53: 143-153.

Paper presented at the 2nd International Congress of the Polish Neuroscience Society; Session: Plascity of the spinal cord

Rossignol, S., Chau, C., and Barbeau, H. Pharmacology of locomotion in chronic spinal cats. In: *Alpha and gamma motor systems*, edited by Taylor, A., Gladden, M.H., and Durbaba, R.New York, London:Plenum Press, 1995,p. 449-455.

Appendix C

S. Rossignol[1], C. Chau[1] and H. Barbeau[2]

[1]Department of Physiology, CNRS
Faculty of Medicine, Université de Montréal
[2]School of Occupational Therapy, McGill University
Montréal, Canada

This short review will summarize some of the work we and others have performed in the field of pharmacology of locomotion in cats and indicate the potential benefits of such an approach in clinical situations where we believe that a rational locomotor pharmacotherapy can be developed.

GENERATION AND MODULATION OF LOCOMOTION BY NORADRENERGIC DRUGS

In 1931, Hinsey, Ranson & Zeiss (1931) showed that the i.v. injection of ephedrine increased the ability of acute spinal cats (C1) to stand and even to display rhythmic movements of the forelimbs and hindlimbs. Although the possibility exists that the effects of ephedrine could be explained by an increase in blood pressure, the authors suggested an independent central action unrelated to cardiovascular effects. Comparing the behaviour of high spinal cats with and without ephedrine, they concluded that "the reflex pathways necessary for these responses are already present and ephedrine, whatever its action may be, is instrumental only in making possible their demonstration". This appears to be one of the earliest studies suggesting an important role of catecholamines in motor control and their potential role in facilitating spinal circuits important for stepping. In 1967, classical papers on the effects of l-DOPA on the spinal cord suggested that, through the release of noradrenaline, DOPA could activate spinal pathways capable of generating alternating bursts in flexor and extensor motor nerves as during locomotion (Jankowska, Jukes, Lund & Lundberg, 1967a,b). After potentiating DOPA with a monoamine oxidase inhibitor, nialamide, Grillner and Zangger (1975;1979) established that the spinal cord was capable of generating, in the absence of phasic afferent feedback, a detailed locomotor pattern with a characteristic ratio of flexor/extensor burst durations and a bilaterally organised alternating activity with muscles being activated at their respective characteristic times within the cycle.

This was confirmed in different preparations by several authors (Baev, 1977, Fleshman, Lev-Tov & Burke, 1984, Pearson & Rossignol, 1991).

The α_2 noradrenergic agonist clonidine was then used by Forssberg and Grillner (1973) to induce locomotion successfully in acute spinal cats. We have also used intraperitoneal clonidine to induce treadmill locomotion of the hindlimbs within the first week after spinalisation at the last thoracic segment (T13) of adult cats chronically implanted with EMG electrodes (Barbeau & Rossignol, 1991). We found that within that week the locomotor pattern evolved rather rapidly. When given during the first few days after spinalisation, the locomotor pattern is often not as well developed as later on. A stronger perineal stimulation is often needed and the rhythmic movements of the hindlimbs are often performed with the hips more extended than in the normal. This progresses rapidly so that after the first week, the locomotor movements have a greater amplitude and the hip excursion is more in the normal locomotor range. Similarly, the fictive locomotor pattern induced by DOPA or clonidine in paralysed cats (Pearson & Rossignol, 1991) changes with time after spinalisation. Indeed, in early-spinal cats, the rhythmic pattern (recorded in peripheral nerves with nerve cuffs) can be more rudimentary with flexor and extensor bursts often of similar duration. In late-spinal cats, the flexor bursts are typically much shorter than the extensor bursts, as in the normal walking cat.

The above results suggest that there is some degree of plasticity in the locomotor circuitry which evolves as a function of time after spinalisation. We have taken advantage of

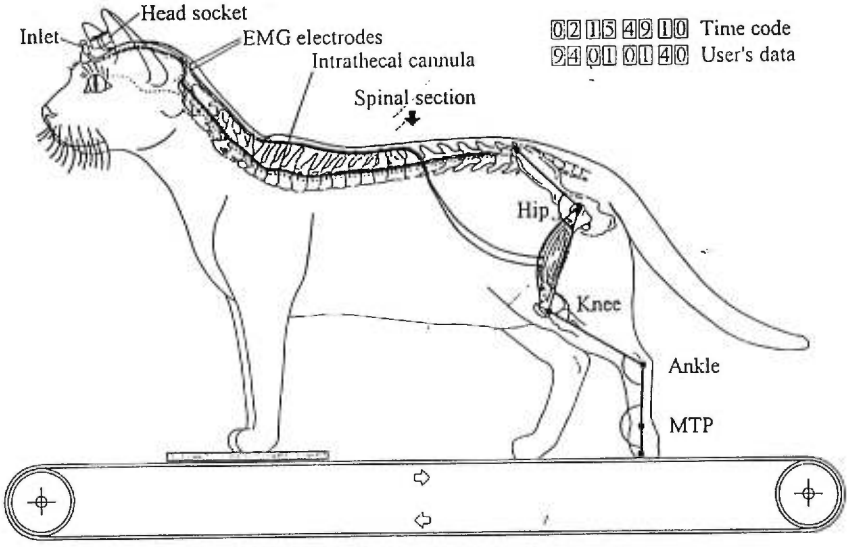


Figure 1. Methodology for EMG and video recordings in chronic spinal cats implanted with an intrathecal cannula. The cats are chronically implanted with bipolar EMG electrodes in selected hindlimb muscles and the leads are connected to a multipin head socket cemented on the skull. The intrathecal cannula consists of a Teflon tubing (24 LW) inserted through the atlanto-occipital ligament with its port of entry cemented on the head next to the EMG socket. The tip of the cannula is inserted in the intrathecal space down to approximately L4. The locomotor movements on the treadmill are recorded on videotape with a synchronised SMPTE time code and other user's data such as dates and the speed of the treadmill. Digitisation with a Peak Performance system of the light-reflecting spots glued to the various joint axes permit the reconstruction of the kinematics and the synchronisation of these mechanical events to the EMG activity with a resolution of one video field (16.7 ms). Usually a single bolus of 100 µl of the drug solution is injected through the inlet and is pushed out of the cannula with another bolus of 100 µl of sterile saline to fill the dead space of the cannula. Note that only the hindlimbs are on the treadmill, the forelimbs remain on a fixed platform above the belt.

this situation to train cats to walk daily on a treadmill (75-110 mins/day) with a daily i.p. injection of clonidine (Barbeau, Chau & Rossignol, 1993). Following such intense training, some cats could walk, with their hindlimbs on the treadmill, without drugs after 7-11 days and maintain this performance for several weeks.

The need to inject drugs daily and the variable importance of side effects of clonidine i.p. in cats (sleepiness and nausea) led us to use a chronic intrathecal (i.t.) cannula which is illustrated in Figure 1 with other essential points of methodology. The following examples illustrate three distinct situations where different aspects of these drugs can be considered: initiation of locomotion, modulation of an existing locomotor pattern and improvement of a defective locomotor pattern.

Figure 2 illustrates the initiation of locomotion after an i.t. injection of clonidine (100 µg/100µl ≈ 4mM) in a cat spinalised 3 days prior to the experiment. The stick figures and EMG records of Figure 2A show that, prior to the injection, the cat is incapable of any significant rhythmic movement on the treadmill. Thirty minutes after the injection, the cat can walk vigorously when the treadmill belt is moved. The EMGs are well organised bilaterally and the stick diagrams of the swing and stance of one step illustrate the amplitude of the overall limb movement. In some cases we could demonstrate that within only 3-5 minutes of the i.t. injection of clonidine, the cats' hindlimbs which had been completely paralysed were walking on the treadmill. Since the intrathecal route opens up the possibility of using chemicals that do not cross the blood brain barrier, we have started to evaluate in more detail the effects of various other α_2 noradrenergic agonists such as tizanidine and oxymetazoline. Whereas tizanidine has about the same time course as clonidine (about 6

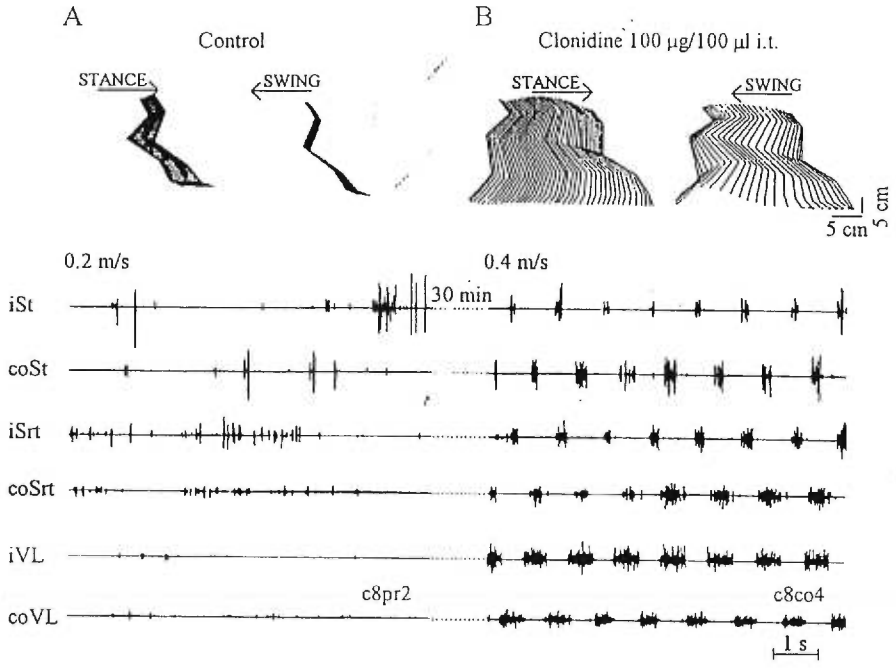


Figure 2. Initiation of locomotion in a 3-day post-spinal cat with an intrathecal injection of clonidine. A. Stick figures of the hindlimb representing the swing and stance phase of 1 step cycle and the raw electromyogram (EMG) of hindlimb muscles of a spinal cat during treadmill locomotion before clonidine injection. B. At 30 minutes after 100µg of clonidine (i.t. 100µg/100 µl, ≈ 4mM), with perineal stimulation, a good locomotor pattern is triggered. Abbreviations of the muscles are St: Semitendinosus; Srt: Sartorious; VL: Vastus Lateralis.i: ipsilateral; co: contralateral.

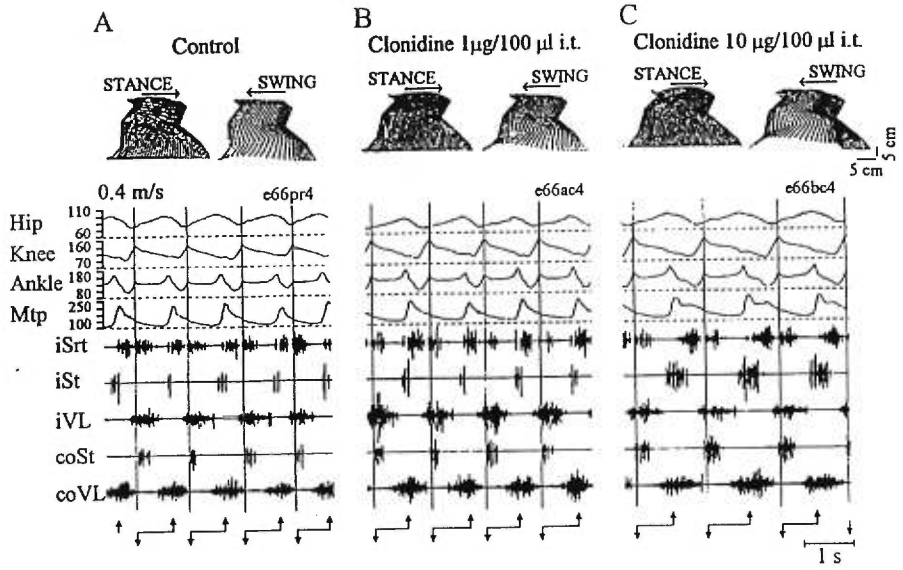


Figure 3. Effects of increasing doses of intrathecal clonidine on the locomotor pattern in a cat 275 days post-spinalisation. A. Stick figures showing joint angle displacement synchronised with the raw EMG of various hindlimb muscles, before clonidine injection (mean duration of 8 cycles = 1.07s ± S.D. 0.030s). B. At 22 minutes after 1 µg clonidine (i.t. 1µg/100µl), a slight increase in cycle duration is seen (mean duration of 7 cycles = 1.17s ±S.D. 0.026s). C. At 23 minutes after 10 µg clonidine (i.t 10µg/100µl, injected at 31 minutes following the previous 1 µg clonidine injection), the cycle duration, particularly the swing phase, was prolonged (mean cycle duration (n=8) increased to 1.462 s (± S.D. 0.035s). A marked increase in toe drag during the initial swing phase was also observed. The vertical lines delineate each step cycle, horizontal bars indicate stance; upward arrows indicate foot lift; downward arrows indicate foot contact.

hours), oxymetazoline has a slower onset of action but can last for more than 48 hours. This means that during that effective period, the cat can readily walk when its hindlimbs are placed over the moving treadmill belt. It should be emphasised that although rhythmic movements are easily elicited during that period by various stimuli when the cat is lying down in its cage or on a treadmill, spontaneous rhythmic movements are rare. Therefore, it should not be imagined that such drugs elicit a perpetual stepping pattern; the cats indeed need the peripheral afferent inputs normally provided by the treadmill or other stimuli such as perineal stimulation to express the locomotor pattern. The activation of the α_2 noradrenergic receptors prime the relevant locomotor circuits which are then activated when appropriate afferent signals are given.

We have shown before (Barbeau, Julien & Rossignol, 1987, Rossignol, Barbeau & Julien, 1986) that when clonidine is given i.p. to a late-spinal cat which has already established a stable locomotor pattern (Barbeau & Rossignol, 1987), there is a marked increase in the duration of muscle discharges, especially of flexor muscles. Therefore, the whole step cycle duration is increased although the amplitude of the EMGs is almost unchanged. Figure 3 shows how clonidine can modulate the expression of the locomotor pattern in a cat which has reached a good stable locomotor performance (Figure 3A) after being trained on a treadmill for several weeks. A very small dose of clonidine (1 µg/100µl i.t.) is sufficient to induce some changes in the step cycle duration. Note how the foot is placed at a longer distance in front of the hip joint and also how the ankle is slightly more extended. With higher doses, a deterioration of the walking pattern can actually be

observed. Indeed, even before any drug, the cat can typically have a short period of foot drag. After clonidine this foot drag can be exaggerated (see in particular the metatarso-phalangeal (Mtp) joint in Figure 3C). Cycle duration is much increased due principally to a prolongation of the flexor bursts, especially semitendinosus muscles on both sides.

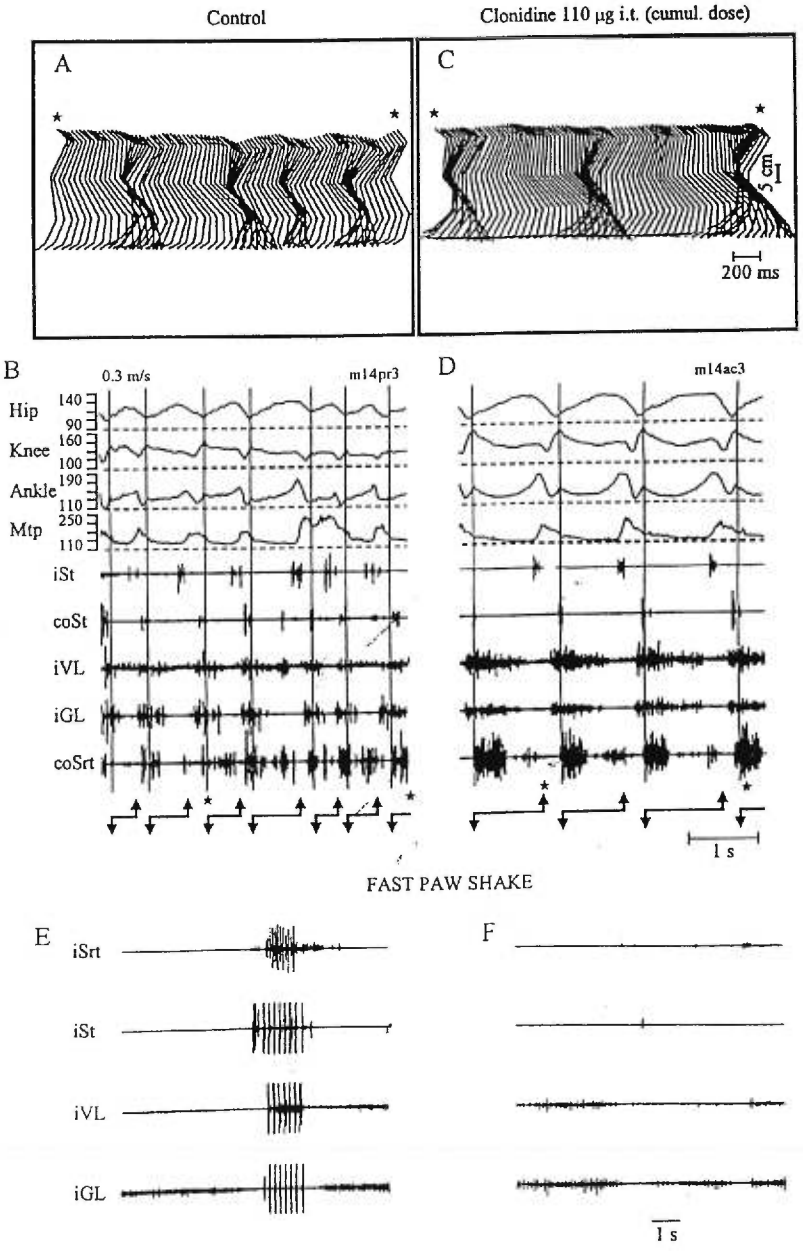


Figure 4. Improvement of the locomotor pattern in a spinal cat. A. Stick figures representing a continuous sequence (about 3.3s) of irregular stepping of the hindlimb on the treadmill. B. The corresponding sequence after clonidine. Stars indicate the starting and ending point of the kinematic analysis in A. C. Kinematics following three successive doses of 10µg, 25µg, and 75µg of clonidine injected within an hour. D. Corresponding angular and EMG data. E. Fast paw shake response which was abolished after clonidine (F).

TOWARDS A LOCOMOTOR PHARMACOTHERAPY?

Figure 4 illustrates an example where clonidine improved the characteristics of a walking pattern which had deteriorated and had become somewhat irregular due to a lack of training. In the control situation (Figure 4A & B), the cat had irregular steps with inconsistent foot placement, little weight support and a generally disorganised EMG pattern. This cat also had very brisk extensor reflexes as can be seen from the violent paw shake (Figure 4E) induced by dipping the foot in water. After clonidine, there was a general improvement of the walking pattern: it was more regular, the steps were larger and the weight support was increased as can be seen from the larger joint excursions and the "longer" legs in Figure 4C. These changes are, of course, reflected by a much better organised EMG pattern. Consistent with our previous findings using i.p. clonidine injection (Barbeau et al., 1987), there was a decrease in cutaneous reflex excitability, which is best exemplified by the complete abolition of the fast paw shake (Figure 4F).

Thus, the present results clearly indicate that the activation of noradrenergic α_2 receptors is one of the most potent means of inducing locomotion in spinal cats, that it can modulate the duration of an existing locomotor pattern and improve the rhythmicity of poor locomotor patterns. It is believed that the effects of α_2 noradrenergic stimulation are the manifestations of changes occurring in reflex pathways and rhythm generation circuits.

What about other neurotransmitter systems? In early-spinal cats, we have been unable to initiate locomotion with serotonergic precursor 5-HTP or agonists such as quipazine or 5-MeO-DMT (Barbeau & Rossignol, 1990). However, in late-spinal cats, serotonergic agonists markedly increase the output amplitude of muscles and, consequently, the cycle duration. We have shown that the combination of noradrenergic and serotonergic agonists can increase simultaneously the duration of the cycle and the amplitude of the EMGs (Barbeau & Rossignol, 1991). The dopaminergic agonist apomorphine did not induce locomotion either, although a marked hyperflexion developed (Rossignol et al., 1986, Barbeau & Rossignol, 1991).

In recent experiments (Chau, Provencher, Lebel, Jordan, Barbeau & Rossignol, 1994), the i.t. administration of excitatory amino acids (EAA) did not induce locomotion in early-spinal cats although NMDA had been shown before to initiate locomotion in decerebrate paralysed cats (Douglas, Nogas, Dai & Jordan, 1994). It is possible that activation of NMDA receptors in this situation leads to a widespread excitation of many conflicting pathways and that disorganised movements generated by NMDA such as paw-shake might have prevented the expression of the locomotor pattern. However, in walking late-spinal cats AP5 (15-20 mM), an NMDA receptor blocker, could stop locomotion which could, however, be partially reinstated with a further i.t. injection of NMDA (10-15 mM).

Recent clinical work in spinal cord injured patients also suggests that the combination of a noradrenergic agonist and a serotonergic antagonist such as cyproheptadine, together with locomotor training on treadmill, might constitute a valid approach to locomotor pharmacotherapy (see Rossignol & Barbeau, 1993 and Barbeau & Rossignol, 1994 for brief reviews), and even allow some patients to benefit from other rehabilitation procedures such as functional electrical stimulation from which they could not otherwise benefit. It is possible that such effects on locomotion may be related to the decrease in reflex excitability (spasticity) that would otherwise prevent the smooth expression of locomotion, the priming of relevant spinal circuits that could be entrained by the proper afferent feedback and the ensuing beneficial effect of locomotor training. More research is needed to understand the relative importance of all these interlinked aspects.

ACKNOWLEDGEMENTS

This work has been supported by The Network of Centres of Excellence (NCE) in Neural Regeneration and Functional recovery and a Group Grant from the Medical Research Council of Canada. C.C. is an NCE trainee partly funded by the FCAR Group; H.B. is an FRSQ chercheur-boursier. All our thanks to Janyne Provencher and France Lebel for their active participation in all experiments and G. Filosi for his graphics skills.

REFERENCES

BAEV, K. V. (1977). Rhythmic discharges in hindlimb motor nerves of the decerebrate, immobolized cat induced by intravenous injection of DOPA. *Neurophysiol.* **9**, 165-167.

BARBEAU, H., JULIEN, C. & ROSSIGNOL, S. (1987). The effects of clonidine and yohimbine on locomotion and cutaneous reflexes in the adult chronic spinal cat. *Brain Res.* **437**, 83-96.

BARBEAU, H., CHAU, C. & ROSSIGNOL, S. (1993). Noradrenergic agonists and locomotor training affect locomotor recovery after cord transection in adult cats.. *Brain Res. Bull.* **30**, 387-393.

BARBEAU, H. & ROSSIGNOL, S. (1987). Recovery of locomotion after chronic spinalization in the adult cat. *Brain Res.* **412**, 84-95.

BARBEAU, H. & ROSSIGNOL, S. (1990). The effects of serotonergic drugs on the locomotor pattern and on cutaneous reflexes of the adult chronic spinal cat. *Brain Res.* **514**, 55-67.

BARBEAU, H. & ROSSIGNOL, S. (1991). Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. *Brain Res.* **546**, 250-260.

BARBEAU, H. & ROSSIGNOL, S. (1994) Spinal cord injury: enhancement of locomotor recovery. *Current Opinion in Neurology.* **7**, 517-524.

CHAU, C., PROVENCHER, J., LEBEL, F., JORDAN, L., BARBEAU, H. & ROSSIGNOL, S. (1994). Effects of intrathecal injection of NMDA receptor agonist and antagonist on locomotion of adult chronic spinal cats. *Soc. Neurosci. Abstr.* **20**, 573.

DOUGLAS, J. R., NOGAS, B. R., DAI, X. & JORDAN, L. M. (1993). The effects of intrathecal administration of excitatory amino acid agonists and antagonists on the initiation of locomotion in the adult cat. *J. Neurosci.* **13**, 990-1000.

FLESHMAN, J. W., LEV-TOV, A. & BURKE, R. E. (1984). Peripheral and central control of flexor digitorium longus and flexor hallucis longus motoneurons: the synaptic basis of functional diversity. *Exp. Brain Res.* **54**, 133-149.

FORSSBERG, H. & GRILLNER, S. (1973). The locomotion of the acute spinal cat injected with clonidine i.v.. *Brain Res.* **50**, 184-186.

GRILLNER, S. & ZANGGER, P. (1975). How detailed is the central pattern generation for locomotion?. *Brain Res.* **88**, 367-371.

GRILLNER, S. & ZANGGER, P. (1979). On the central generation of locomotion in the low spinal cat. *Exp. Brain Res.* **34**, 241-261.

HINSEY, J. C., RANSON, S. W. & ZEISS, F. R. (1931). Observations on reflex activity and tonicity in acute decapitate preparations with and without ephedrine. *J. Comp. Neurol.* **53**, 401-407.

JANKOWSKA, E., JUKES, M. G., LUND, S. & LUNDBERG, A. (1967a). The effect of DOPA on the spinal cord. 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors. *Acta physiol. scand.* **70**, 369-388.

JANKOWSKA, E., JUKES, M. G., LUND, S. & LUNDBERG, A. (1967b). The effects of DOPA on the spinal cord. 6. Half centre organization of interneurones transmitting effects from the flexor reflex afferents. *Acta physiol. scand.* **70**, 389-402.

PEARSON, K. G. & ROSSIGNOL, S. (1991). Fictive motor patterns in chronic spinal cats. *J. Neurophysiol.* **66**, 1874-1887.

ROSSIGNOL, S., BARBEAU, H. & JULIEN, C. (1986). Locomotion of the adult chronic spinal cat and its modification by monoaminergic agonists and antagonists. In *Development and plasticity of the mammalian spinal cord*, eds. GOLDBERGER, M., GORIO, A. & MURRAY, M., pp. 323-345. Liviana Press, Padova.

ROSSIGNOL, S. & BARBEAU, H. (1993). Pharmacology of locomotion: an account of studies in spinal cats and spinal cord injured subjects. *J. Am. Paraplegia Soc.* **16**, 190-196.