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Contribution du cortex moteur et des afférences cutanées dans le contrôle et la plasticité de la locomotion chez le chat

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Sommaire

La locomotion est générée par un réseau dans la moelle épinière sous l'influence des informations sensorielles et sous le contrôle descendant de plusieurs structures supraspinales. Dans cette thèse nous nous sommes plus particulièrement intéressés à la contribution du cortex moteur dans le contrôle de la patte postérieure lors de la locomotion et à la modulation corticale des informations cutanées chez le chat intact. Enfin, nous avons également abordé le rôle du cortex moteur dans la récupération fonctionnelle de la marche à la suite d'une dénervation cutanée de l'extrémité de la patte.

Pour déterminer la contribution du cortex moteur dans le contrôle de la locomotion, nous avons enregistré les réponses musculaires évoquées par une microstimulation intracorticale appliquée dans la représentation de la patte postérieure. Cette stimulation modifie l'amplitude et la durée des activités électromyographiques en fonction de la phase du cycle locomoteur, suggérant que le cortex influence la structure du patron de marche. Elle est également capable de raccourcir la durée du cycle de marche, suggérant un contrôle cortical sur le rythme locomoteur. Ceci suggère un contrôle cortical sur la locomotion du membre postérieur semblable à celui du membre antérieur.

Afin d'étudier les interactions entre les inputs corticaux et cutanés lors de la marche, nous avons conditionné les stimulations de différents nerfs cutanés à différents délais (0-50 ms) par rapport à celle du cortex moteur et de la pyramide. La stimulation du cortex moteur et de la pyramide modulait en facilitant ou déprimant l'amplitude des réflexes évoqués par les nerfs cutanés. La nature de cette modulation apparaissait de

plus être différente selon le site cortical, le nerf cutané, ainsi que le muscle enregistré, suggérant une convergence de ces inputs dans des sous-populations interneuronales spinales.

Troisièmement, nous avons investigué la contribution du cortex moteur dans la récupération de la marche après une dénervation cutanée unilatérale. Les réponses évoquées par une stimulation corticale, via des microfils implantés chroniquement, étaient enregistrées durant une période contrôle de plusieurs mois, afin de s'assurer de leur stabilité avant dénervation. Après dénervation, nous avons observé une augmentation dans l'amplitude des réponses évoquées par la stimulation du cortex moteur, ce qui suggère une augmentation d'efficacité corticospinale. Nous suggérons que cette augmentation contribue à la récupération locomotrice, car elle semble parallèle aux changements survenant dans l'activité musculaire de base et que la récupération est abolie après une pyramidotomie. De plus, l'absence ou les faibles changements dans les réponses évoquées par les réflexes cutanés croisés (de la patte non dénervée) ou la stimulation de la pyramide suggère que les changements corticospinaux résultent en grande partie d'une augmentation d'excitabilité corticale, mais qu'il pourrait aussi résulter de changements d'excitabilité dans certaines sous-populations d'interneurones spinaux.

Le cortex moteur semble donc contribuer de manière importante au contrôle locomoteur en modifiant la structure et le rythme du cycle de marche, en étant capable de moduler les réflexes cutanés et de compenser les déficits d'une dénervation cutanée.

Mots clés: cortex moteur, moelle épinière, dénervation, récupération fonctionnelle, locomotion, réflexes cutanés, électrophysiologie, chat.

Abstract

Locomotion is generated by a spinal interneuronal network that is influenced both by peripheral afferent feed-back and supraspinal descending pathways. In this thesis, we investigated the contribution of the motor cortex to the control of the hindlimb during locomotion and to the cortical modulation of hindlimb cutaneous reflexes in the intact cat. We also investigated the role of the motor cortex in the functional recovery of locomotion following a cutaneous denervation of the hindpaw.

To determine the contribution of the motor cortex to locomotor control, we recorded the responses evoked in hindlimb muscles by intracortical microstimulation applied throughout the hindlimb representation of the motor cortex. This stimulation modified the amplitude and duration of electromyographic activity as a function of the phase of the step cycle, suggesting that the cortex influences the structure of the locomotor pattern. The stimulation was also able to shorten the step cycle duration, suggesting that the motor cortex also influences the locomotor rhythm. These results suggest a cortical control over hindlimb locomotion similar to that of the forelimb.

To study the interactions between cortical and cutaneous inputs during locomotion, cutaneous stimulation was delayed (0-50 ms) with respect to that of the motor cortex or the pyramid. Stimulation of the motor cortex, as well as of the pyramid, produced a mixture of facilitation and depression of the amplitude of the reflexes evoked by cutaneous stimulation. The nature of this modulation was different according to the cortical site, the cutaneous nerve and to the recorded muscle, suggesting a convergence of inputs onto subpopulations of spinal interneuronal networks.

Finally, we investigated the contribution of the motor cortex to the recovery of locomotion following a unilateral cutaneous denervation. Responses evoked in hindlimb muscles by stimulation of the motor cortex, via chronically implanted microwire electrodes, were recorded over a period of several months to ensure stability in the responses before denervation. After denervation, the amplitude of the cortically evoked responses increased, suggesting an increased corticospinal efficacy. This increase is suggested to contribute to the functional recovery, because it had similar time course to the changes observed in the background locomotor activity and the recovery was abolished after a pyramidotomy. Changes in the magnitude of the crossed cutaneous reflexes (from the non-denevated hindlimb) or the responses evoked by the stimulation of the pyramid were weak or absent. This suggests that the increased corticospinal efficacy is due, in part, to an increased cortical excitability, although changes in the excitability of specific spinal interneuronal networks probably also contribute.

In summary, the motor cortex makes an important contribution to the locomotor control of the hindlimb by influencing the structure and the rhythm of the locomotion and in modulating cutaneous reflexes. It also has the capacity to contribute to the compensation of the deficits produced by a cutaneous denervation of the hindpaw.

Keyboards: motor cortex, spinal cord, cutaneous reflexes, denerveration, functional recovery, locomotion, electrophysiology, cat

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Listes des abréviations

EMG : Électromyogramme

CPG : Central Pattern Generator

TES : Stimulation Transcorticale Électrique

TMS : Stimulation Transcorticale Magnétique

GABA : Acide Gamma Amino Butyrique

Saph : Saphenus

SP : Superficial Peroneus

TP : Tibial Posterior

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À Isabelle

Introduction

La locomotion est un mouvement complexe qui requiert la coordination de différents muscles agissant sur différentes articulations chez l'homme comme chez l'animal. Cette activité musculaire est modulée spécifiquement en relation avec chacun des autres muscles afin d'ajuster la flexibilité et l'adaptabilité du système au contrôle de la marche. Cette flexibilité dans le contrôle de la marche résulte de l'intégration des activités des circuits interneuronaux spinaux, des informations sensorielles telles que les informations cutanées, ainsi que du contrôle descendant supraspinal tel que le cortex moteur.

Cette intégration offre donc une grande flexibilité dans le système lui permettant de s'adapter aux contraintes rencontrées dans l'environnement ainsi qu'aux altérations ou blessures du système nerveux périphérique ou central. Dans cette thèse, nous aborderons plusieurs aspects importants de cette capacité du système à s'adapter à différentes circonstances.

Dans un premier chapitre, nous aborderons la contribution du cortex moteur dans le contrôle de la marche chez le chat intact. À savoir s'il est capable de modifier l'amplitude et la durée des activités musculaires lors de la marche, ainsi que sa capacité à agir sur le rythme locomoteur.

Dans un second chapitre, nous aborderons la capacité du cortex moteur à modifier les effets des réflexes cutanés lors de la marche chez le chat intact. Nous discuterons des évidences indirectes suggérant que le contrôle descendant cortical et les informations

cutanées pourraient converger sur des réseaux interneuronaux spinaux spécifiques lors de la marche.

Dans un troisième chapitre, nous aborderons les mécanismes de plasticité du cortex moteur et des réseaux interneuronaux spinaux contribuant à la récupération fonctionnelle de la marche à la suite d'une dénervation cutanée de la patte postérieure chez le chat.

En guise d'introduction générale, nous décrirons les concepts et les évidences suggérant l'existence de réseaux interneuronaux spinaux à l'origine de la genèse de la locomotion. Nous décrirons ensuite les signaux périphériques et les structures supraspinales contribuant à son contrôle. Nous nous attarderons plus particulièrement sur le rôle du cortex moteur, ainsi que de ses interactions avec les afférences cutanées lors de la marche. Nous traiterons par la suite des évidences d'une plasticité supraspinale et spinale dans le système locomoteur. Nous aborderons aussi les études de plasticité du cortex moteur survenant à la suite d'une altération du système nerveux périphérique. Dans la dernière partie de cette introduction, nous exposerons enfin nos hypothèses de recherches concernant la contribution et la plasticité du cortex moteur et des afférences cutanées lors de la marche du membre postérieur chez le chat.

La locomotion

La locomotion semble résulter de l'intégration des activités des circuits interneuronaux spinaux, des informations sensorielles, ainsi que du contrôle descendant supraspinal.

Circuits interneuronaux spinaux ou ``Générateur Central de Patron`` (CPG)

Les premiers travaux de Sherrington au début du 20^{e} siècle ont montré que la moelle épinière est capable d'évoquer un patron alternatif de flexion et d'extension en absence d'information provenant du cerveau (Sherrington 1910). Cette alternance de flexion et d'extension a été également observé chez le chat spinal et déafférenté (Brown 1911), suggérant que la moelle épinière serait capable en absence d'inputs des centres supérieurs et de la périphérie de générer un patron locomoteur. Ces observations ont conduit Graham Brown (1911) à suggérer que la marche serait générée par des demi-centres fléchisseurs et extenseurs qui s'inhiberaient mutuellement pour permettre l'activation alternée de la flexion et de l'extension lors de la marche. Les travaux du groupe de Lundberg dans les années 1960 ont renouvelé la théorie des demi-centres, en montrant d'une part qu'une stimulation électrique des afférences du réflexe de flexion chez le chat spinal engendrait une courte séquence de rythmicité montrant une alternance de flexion et d'extension (Eccles et Lundberg 1959; voir Hultborn et al. 1998) et d'autre part en identifiant une population d'interneurones inhibiteurs spinaux

qui pourrait sculpter l'activité musculaire (Anden et al. 1966a,b ; Jankowska et al. 1967a,b). Sur la base de ces observations, Lundberg (1969) a proposé que les demi-centres fléchisseurs et extenseurs de la moelle épinière génèreraient un simple patron de flexion-extension et que l'activité générée serait sculptée par le feed-back sensoriel pour produire le patron d'activité observé chez le chat intact.

Cependant, dans une série d'expériences importantes, le groupe de Grillner a montré que les patrons locomoteurs chez le chat paralysé ainsi que chez le chat spinal déafférenté (Grillner et Zangger 1975, 1979 ; voir aussi Pearson et Rossignol 1991) sont semblables à celui du chat intact marchant librement sur tapis roulant. La moelle épinière isolée semble donc être capable de générer un patron locomoteur complexe sans aucun feed-back sensoriel. Ces observations ont conduit Grillner (1981) à proposer le concept de CPG ou générateur central de patron, qui serait constitué de réseaux de neurones impliqués dans la genèse intrinsèque d'un rythme locomoteur en tant qu'oscillateur, ainsi que dans la génération d'un patron d'activité musculaire, c'est à dire une séquence spécifique d'activité musculaire. La théorie de Grillner a constitué le cadre conceptuel d'une intense recherche depuis 25 ans visant à supporter, clarifier ou déterminer la nature du CPG. Par exemple, Vidal et al. (1979) chez le lapin spinal et Forssberg et al. (1980) chez le chat spinal ont montré que les pattes postérieures de ces animaux se déplaçant sur deux tapis roulants séparés, sont capables d'ajuster leur rythme locomoteur à la vitesse respective du tapis sur lequel elle se déplace, suggérant l'existence d'un CPG pour chacun des membres (Grillner 1981). D'autre part, le groupe de Smith a montré l'existence de patrons locomoteurs différent selon que le chat intact marche en avant, en arrière, en montant ou en descendant une pente (Buford et al. 1990 ;

Buford et Smith 1990 ; Carlson-Kutha et al. 1998 ; Perell et al. 1993 ; Smith et al. 1998 ; Trank et Smith 1996). Par exemple, l'ensemble des articulations de la patte est en extension lors d'une marche en avant, en arrière ou en montant une pente, par contre cette synergie disparaît lors de la descente d'une pente. L'extension de la hanche est remplacée par une flexion de cette articulation durant la phase d'appuie. Ces observations corroborent l'hypothèse avancée par Grillner et Wallen (1985 ; Grillner 1981) qui suggérait que le CPG serait organisé en unités modulaires, chacune contrôlant une articulation de la patte. Cette organisation modulaire est également en accord avec les études de grattage chez le chat spinal (Stein et Smith 1997), qui ont montré un patron moteur différent selon que la patte devait gratter un point en avant ou en arrière de la patte. Une telle organisation modulaire fournirait également la flexibilité nécessaire pour coordonner les changements spécifiques d'activité dans chacune des articulations de la patte durant la marche et lors de l'enjambement d'un obstacle (Drew 1991a,c).

Lennard (1985) en montrant que les afférences proprioceptives contrairement aux afférences cutanées sont capables d'agir sur le rythme locomoteur de la tortue, a proposé l'existence de deux populations distinctes d'interneurones qui contrôleraient respectivement le patron musculaire et le rythme du cycle de marche (Lennard 1985 ; Lennard et Hermanson 1985 ; Loeb 1990). L'existence de ces deux populations permettrait d'expliquer l'indépendance observée entre le patron et le rythme du cycle locomoteur après une déafférentation de la patte chez le chat spinal (Koshland et Smith 1989) ainsi que les effets différentiels évoqués par le feed-back des afférences sur le patron d'activité et le rythme du cycle respiratoire chez le chat et la tortue (Feldman et al. 1988, 1989). Ce concept permettrait également d'expliquer les effets différentiels

évoqués par les structures supraspinales et les afférences périphériques sur le patron et le rythme locomoteur d'un chat marchant librement sur un tapis roulant (Drew 1991a,c ; Drew et al. 1996 ; Kalaska et Drew 1993).

Modulation de la marche par les afférences périphériques et les voies descendantes supraspinales

La moelle épinière isolée du cerveau et de la périphérie est donc capable de générer intrinsèquement un rythme et un patron locomoteur, mais la marche chez l'animal intact est également modulée par les afférences périphériques et les voies descendantes supraspinales.

Afférences périphériques durant la marche

Les afférences périphériques fournissent des informations sur l'activité des muscles via les afférences proprioceptives et sur les interactions avec l'environnement via les afférences cutanées.

Afférences périphériques proprioceptives

Les afférences proprioceptives en encodant le degré d'étirement des muscles semblent en particulier être impliquées dans l'entraînement de la marche, dans les

transitions entre les phases d'appui et de balancement, ainsi que dans les mécanismes adaptatifs à la suite de perturbations de l'environnement.

Plusieurs expériences chez le chat paralysé en locomotion fictive ont montré que les afférences proprioceptives des muscles de la patte semblent entraîner le rythme et le patron locomoteur. La position articulaire de la hanche module le rythme locomoteur durant la locomotion fictive (Andersson et Grillner 1983; Kriellaars et al. 1994; Pearson et Rossignol 1991). Lorsque la hanche est fléchie, la rythmicité des bouffées diminue, alors que lorsque la hanche est étendue la rythmicité des bouffées augmente. Ces effets ne sont pas limités aux afférences proprioceptives de la hanche, une oscillation sinusoïdale du tendon de la cheville augmente et entraîne aussi le rythme locomoteur chez le chat paralysé en locomotion fictive (Conway et al. 1987). Une stimulation électrique des afférences proprioceptives d'un extenseur de la cheville entraîne également le rythme locomoteur chez le chat décérébré (Pearson et al. 1992). En plus de moduler le rythme, la position de la hanche module également le patron locomoteur (Andersson et Grillner 1981). Une extension de la hanche raccourcie la durée de la bouffée dans les extenseurs, alors qu'une flexion de la hanche prolonge la durée de la bouffée dans les fléchisseurs. Enfin, de récentes études ont montré que le patron et le rythme locomoteur sont modulés de manière discrète en fonction de la phase du cycle de marche par les afférences proprioceptives (Saltiel et Rossignol 2004a,b). Il semble donc que les afférences proprioceptives modulent le rythme et le patron de la marche.

En plus d'entraîner le rythme et le patron, les afférences proprioceptives semblent également moduler les transitions entre les phases de balancement et d'appui. Plus précisément, lors de la phase d'appui la stimulation des afférences proprioceptives

des muscles fléchisseurs semble initier la phase de balancement en avance alors que celle des muscles extenseurs semble prolonger la phase d'appui. La phase de balancement est initiée aussitôt que le fléchisseur (Iliopsoas) ou l'articulation de la hanche sont suffisamment étirés chez le chat spinal chronique (Grillner et Rossignol 1978) ainsi que chez le chat paralysé en locomotion fictive (Andersson et Grillner 1981, 1983). Tout comme l'étirement de la hanche, la vibration ou la stimulation électrique des afférences proprioceptives des fléchisseurs de la cheville raccourcie la phase d'appui et initie en avance une nouvelle phase de balancement chez le chat décérébré (Hiebert et al. 1996). Par contre, la phase d'appui est prolongée lorsqu'une tension est maintenue dans les extenseurs de la cheville chez le chat décérébré (Duysens et Pearson 1980). La stimulation électrique des afférences proprioceptives des extenseurs de la cheville prolonge également la phase d'appui et retarde le début d'une nouvelle phase de balancement en locomotion fictive chez le chat paralysé en locomotion fictive (Conway et al. 1987; Guertin et al. 1995), décérébré (Pearson et al. 1992; Whelan et al. 1995a,b) ainsi qu'intact (Whelan et Pearson 1997).

En modulant la transition entre les phases de balancement et d'appui, les afférences proprioceptives permettraient d'adapter le rythme locomoteur à des perturbations de l'environnement, telle que la perte soudaine de support de poids lors d'un délestage par exemple. Un délestage artificiel des extenseurs de la cheville, à l'aide d'une trappe qui se dérobe juste avant le contact de la patte au sol lors de la marche, entraîne une perte de support du poids du corps, qui est corrigée par une nouvelle phase de balancement en avance sur le cycle de marche chez le chat intact (Gorassini et al. 1994), ainsi que chez le chat spinal chronique (Hiebert et al. 1994). Cette correction de

perte de support de poids, c'est à dire cette flexion corrective est absente lors de la stimulation des afférences proprioceptives des extenseurs de la cheville, qui prolonge la durée de la phase d'extension et bloque l'initiation du balancement chez le chat décérébré (Hiebert et al. 1995).

En résumé, les afférences proprioceptives semblent donc agir de façon importante sur le rythme et le patron locomoteur, lors des transitions entre les phases d'appui et de balancement et également lors de corrections.

Afférences périphériques cutanées

Contrairement aux afférences proprioceptives, le rôle des afférences cutanées dans le contrôle de la marche a longtemps été négligé, car ces afférences semblent avoir moins d'influence sur le rythme locomoteur (Duysens 1977a,b ; Lennard 1985 ; Schomburg et al. 1998 ; *voir section Circuits interneuronaux spinaux ou `` Générateur Central de Patron`` (CPG)*). Néanmoins, bien qu'elles n'agissent pas sur le rythme locomoteur chez l'animal intact, les afférences cutanées sont capables de moduler le patron (amplitude et durée des activités musculaires) locomoteur en évoquant des synergies spécifiques qui dépendent de la phase du cycle de marche, ainsi que du lieu de stimulation sur la peau de la patte.

Une perturbation mécanique inattendue sur le dos de la patte antérieure (Drew et Rossignol 1985, 1987) ou postérieure (Wand et al. 1980 ; Forssberg 1979) chez le chat entraîne des changements dans la trajectoire de la patte pour éviter et contourner la perturbation. Lors de la phase de balancement, les ajustements sont organisés en un

retrait de la patte pour éviter la perturbation et un enjambement pour passer au-dessus de l'obstacle fictif. Lors de la phase d'appui, la perturbation raccourcie la durée de la phase d'extension et initie en avance une nouvelle phase de balancement. Ces changements dans la trajectoire de la patte sont dus à des modifications dans l'activité musculaire qui contribue à retirer la patte et enjamber la perturbation. Ces changements cinématiques et musculaires sont uniquement d'origine cutanée, car ils sont absents après une anesthésie locale de la peau de la patte.

Pour préciser la nature des réponses électromyographiques durant la marche, des stimulations électriques ont été appliquées au niveau de la peau ou des nerfs cutanés (Drew et Rossignol 1987 ; Wand et al. 1980). La stimulation électrique induit un patron cinématique et musculaire semblable à celui évoqué par la stimulation mécanique, avec des réponses évoquées excitatrices et maximales dans les muscles fléchisseurs et extenseurs lors de la phase de balancement, et des réponses diminuées ou inhibées dans les extenseurs durant la phase d'appui. Ces réponses dépendant de la phase du cycle de marche ont également été rapportées chez l'homme (Duysens et al. 1996a,b ; Tax et al. 1995 ; Van Wezel et al. 1997). Elles semblent résulter de l'interaction des informations provenant des afférences cutanées et du rythme locomoteur intrinsèque (CPG), puisqu'elles sont présentes chez le chat intact marchant librement (Forssberg 1979 ; Wand et al. 1980), le chat spinal chronique (Forssberg et al. 1975, 1977) ainsi que chez le chat paralysé durant une locomotion fictive (Andersson et al. 1978 ; Andersson et Grillner 1981 ; Hishinuma et Yamagushi 1989 ; Seki et Yamaguchi 1997).

Les réflexes évoqués par les afférences cutanées semblent également dépendre du site de stimulation sur la peau. Bien que les travaux de Sherrington (1910) aient

montré que la stimulation cutanée évoquait un réflexe de flexion stéréotypé de la patte quelque soit le lieu de stimulation sur la peau, Hagbarth dans les années 1960 a remis en question ce concept de réflexe de flexion stéréotypé en démontrant l'importance du site de stimulation cutanée. Il a montré que les muscles extenseurs étaient excités par une stimulation de la peau les recouvrant, mais inhibés par une stimulation de l'ensemble de la patte, alors que les fléchisseurs étaient excités par une stimulation cutanée de l'ensemble de la patte, mais inhibés par la stimulation de la peau recouvrant le muscle extenseur antagoniste (Hagbarth 1952), ce qui a conduit Hagbarth a avancer le concept de ``signe local``. Cette spécificité du site de stimulation a également été rapportée lors d'une stimulation des afférences cutanées lors de la marche chez l'humain (Duysens et al. 1996a,b ; Tax et al. 1995 ; Van Wezel et al. 1997), chez le chat marchant librement (Abraham et al. 1985 ; Duysens et Loeb 1980 ; Duysens et Stein 1978 ; Forssberg 1979 ; Pratt et al. 1991 ; Wand et al. 1980), le chat spinal chronique (Forssberg et al. 1975, 1977) et le chat décérébré (Duysens et Pearson 1976) ainsi que chez le chat paralysé lors d'une locomotion fictive (Burke 1999 ; Degtyarenko et al. 1996, 1998 ; Moschovakis et al. 1991). Ces dernières études en locomotion fictive suggèrent d'ailleurs l'existence de circuits spinaux spécifiques dépendant des afférences cutanées impliquées et de la phase du cycle de marche. Par exemple, la stimulation du nerf Péronier Superficiel innervant le dos de la patte postérieure facilite les muscles impliqués dans une rétraction de la patte durant la phase de flexion ce qui permettrait d'éviter un objet fictif, mais a peu d'effet lors de la phase d'appui. Par contre, la stimulation du nerf Plantaire Médian innervant la surface plantaire de la patte facilite en fin de phase de flexion ou lors de la phase d'appui les muscles impliqués dans une flexion ventrale du bout de la patte, ce qui

pourrait potentiellement contribuer au placement de la patte au sol chez le chat intact. Ces résultats suggèrent donc que les afférence cutanées utilisent des circuits spinaux spécifiques qui sont modulés par l'état du CPG.

Les afférences cutanées semblent ainsi ajuster le patron locomoteur en fonction du site de stimulation sur la peau et de la phase du cycle de marche, mais sont-elles primordiales au contrôle de la marche? Les études de lésions permettent de répondre à cette question. La plupart des études de privation des afférences cutanées par anesthésie des coussinets (Engberg 1964a ; Forssberg et al. 1977 ; Wand et al. 1980) ou neurectomies partielles de la patte (Duysens et Stein 1978) suggèrent comme Sherrington (1910) que les afférences cutanées ne sont pas strictement nécessaires à la marche. Bouyer et Rossignol (2003a) ont néanmoins montré qu'une dénervation cutanée complète du bout de la patte entraîne des déficits important dans le contrôle de la marche, tels qu'un placement anormale de la patte lors d'une marche sur les barreaux d'une échelle horizontale, ainsi qu'un traînement sur le dos de la patte lors d'une marche sur tapis roulant. Ces déficits suggèrent donc que les afférences cutanées contribuent activement au contrôle de la locomotion. Cependant, alors que la marche sur échelle horizontale n'est jamais récupérée, le traînement sur le dos de la patte est rapidement compensé en quelques jours par une augmentation dans le niveau d'activité des muscles fléchisseurs du genou et de la cheville. Par contre, une section complète de la moelle épinière lombaire chez ces chats abolit toute récupération ou compensation fonctionnelle de la marche sur tapis roulant, ce qui suggère l'importance des informations cutanées dans le contrôle du rythme de la marche.

Les études de stimulations mécaniques et électriques de la peau ou des nerfs cutanés suggèrent que les afférences cutanées contribuent activement à la régulation du patron locomoteur et dans une certaine mesure au rythme locomoteur.

Les afférences périphériques fournissent donc des informations sur la position et la vitesse de déplacement des membres lors de la marche, ainsi que sur la nature des contraintes rencontrées dans l'environnement. Ces afférences participent à différents degrés au contrôle de la marche, en régulant le rythme et le patron locomoteur.

Les voies descendantes supraspinales

En plus des afférences périphériques qui fournissent des informations sur l'activité des muscles et sur les interactions avec l'environnement, différentes structures supraspinales projettent dans la moelle épinière et contribuent à réguler différents aspects de la locomotion. Kuypers dans les années 1960 a proposé de catégoriser les voies descendantes en deux systèmes sur la base de leur projection neuroanatomique dans la moelle épinière et des effets à la suite de lésions de ces voies chez le chat (1963) et le primate (Lawrence et Kuypers 1968a,b). Le système médian constitué principalement par les voies réticulo- et vestibulospinales, projette dans la partie ventromédiale de la moelle épinière et innerve les motoneurones contrôlant la musculature proximale et axiale. La section spécifique de ce système chez le chat affecte le contrôle de la posture, mais n'a aucun effet dans le contrôle des parties distales des

pattes. Par contre le système latéral, constitué par les voies cortico- et rubrospinales, projette dans la partie latérale de la moelle épinière et innerve principalement la musculature distale. La section spécifique de ce système chez le chat, affecte le placement des extrémités des pattes sur les barreaux d'une grille ou lors d'un reflex de retrait, mais n'affecte pas le contrôle de la posture. Ces deux systèmes semblent donc complémentaires au niveau comportemental ; le système médian contrôlant les aspects posturaux, le système latéral contrôlant les extrémités de la patte lors de placements. Une telle dichotomie a été rapportée dans le contrôle de la locomotion chez le chat, qui sera abordé dans les prochaines sections. La voie corticospinale, sujet de la thèse, sera décrite plus en détail dans une prochaine section (*Contribution du cortex moteur durant la locomotion*).

Système médian

La lésion complète du système médian au niveau thoracique de la moelle épinière conduit à des déficits dans le support de poids, une diminution dans l'activité des extenseurs et dans la stabilité latérale, ainsi que dans la coordination inter-membre lors de la marche chez le chat (Bem et al. 1995 ; Brustein et Rossignol 1998 ; Gorska et al. 1990, 1993). Une lésion d'une des deux voies réticulo- ou vestibulospinales ou d'un des noyaux du pont conduit à des déficits plus spécifiques.

Les lésions bilatérales des labyrinthes affectent le contrôle de la posture en entraînant un déséquilibre médiolatéral du corps ainsi qu'une diminution du tonus dans les muscles extenseurs chez le chat (Inglis et Macpherson 1995 ; Macpherson et Inglis

1993 ; Thomson et al. 1991). Cette perte de tonus dans les muscles extenseurs a également été rapportée lors de la locomotion après destruction des noyaux vestibulaires latéraux chez le chat décérébré (Orlovsky 1972a ; Yu et Eidelberg 1981). Ces observations sont aussi en accord avec les études de microstimulations du noyau vestibulaire qui ont montré une augmentation des réponses dans les muscles extenseurs durant la phase d'appui sans affecter les muscles fléchisseurs lors de la phase de balancement (Orlovsky 1972a). Les études d'enregistrements unitaires ont par ailleurs montré une augmentation de l'activité des cellules vestibulospinales durant la phase d'extension de la patte postérieure chez le chat (Orlovsky 1972b) et de la patte antérieure chez le cochon d'inde (Marlinsky 1992). Plus récemment, Matsuyama et Drew (2000a,b) ont montré que les cellules vestibulospinales modulaient leur activité en phase avec les muscles extenseurs ipsilatéraux dans la patte postérieure. Ils ont également montré que l'activité des cellules vestibulospinales augmentait alors que le patron de décharge ne changeait pas lorsque le chat devait marcher sur un tapis roulant incliné, ce qui a conduit les auteurs à proposer que le noyau vestibulaire jouerait principalement un rôle dans l'ajustement de la posture, en augmentant le gain des extenseurs lors de la marche.

Concernant la voie réticulospinale, une section de cette voie (Gorska et al. 1993; Mori 1987), une inactivation chimique (Luccarini et al. 1990a,b) ou encore une lésion (Gorska et al. 1995) des noyaux réticulés pontiques conduit à des déficits (à plus ou moins longs termes selon le type d'altération) dans le contrôle de la posture et de la coordination inter-membre entre la patte antérieure et postérieure chez le chat. Ces résultats sont supportés anatomiquement par la nature des projections pontiques et

médullaires qui diffusent sur les segments cervicaux et lombaires (Matsuyama et al. 1999 ; Peterson et al. 1975) et bilatéralement (Matsuyama et al. 1988, 1997). D'un point de vue fonctionnel, les études de microstimulation de la région pontique de la formation réticulée ont montré des diminutions et augmentations dans le tonus postural chez le chat se tenant debout (Mori 1987, 1989 ; Drew et Rossignol 1990a,b), tandis que la stimulation de la région médullaire évoque des combinaisons de réponses dans les fléchisseurs et extenseurs entre les quatre membres, qui sont intégrées dans le cycle locomoteur (Degtyarenko et al. 1993 ; Drew 1991b ; Drew et Rossignol 1984, 1986 ; Orlovsky 1972a ; Perreault et al. 1994). Les études d'enregistrements unitaires de la formation réticulée pontique et médullaire ont également montré une modulation de l'activité des cellules réticulospinales en phase avec l'activité des muscles fléchisseurs et extenseurs des quatre membres durant la marche (Drew et Rossignol 1986 ; Orlovsky 1970 ; Shimamura et Kogure 1983) durant la marche sur un tapis roulant incliné dans le plan antéro-postérieur ou médio-latéral (Matsuyama et Drew 2000a,b) ainsi que lors de l'enjambement d'un obstacle (Prentice et Drew 2001). Ces changements de patron et de niveau d'activité des cellules réticulospinales pourraient donc ajuster la coordination inter-membre pour assurer une posture adéquate lors de mouvements fins. Ce rôle de coordinateur est d'ailleurs appuyé par l'existence de fortes projections vestibulaires (Wilson et Peterson 1981) et corticales (Matsuyama et Drew 1997 ; Rho et al. 1997) sur la formation réticulée qui lui permettrait d'ajuster la posture aux contraintes de l'environnement.

Système latéral

Des lésions complètes du système latéral, interrompant les voies cortico- et rubrospinales au niveau de la moelle spinale lombaire, conduisent à un traînement de la patte lors de la marche sur tapis roulant qui est rapidement compensé (Jiang et Drew 1996). Cependant les mouvements nécessitant un contrôle plus précis sont grandement altérés, le chat n'étant plus capable d'enjamber adéquatement un obstacle attaché au tapis sans le frapper avec les pattes postérieures.

Contrairement au cortex moteur qui a été intensément étudié (*voir la prochaine section*), le rôle du noyau rouge dans le contrôle de la marche a été moins étudié. Les premiers travaux avaient rapporté qu'une lésion d'un noyau rouge induit de faibles déficits lors de la marche sur terrain plat laissant suggérer un rôle négligeable dans le contrôle de la marche (Ingram et Ralson 1932). Cependant, de récents travaux ont montré que la lésion d'un noyau rouge conduit à une asymétrie lors de la marche au sol qui persiste au-delà de plusieurs semaines (Muir et Wishaw 2000). Ces observations sont en accord d'une part avec les études de microstimulation qui ont montré une facilitation dans les fléchisseurs contralatéraux durant la marche (Degtyarenko et al. 1993 ; Orlovsky 1972a ; Rho et al. 1999) et d'autre part avec les études d'enregistrements unitaires qui ont montré une modulation de l'activité des cellules rubrales lors de la phase de flexion durant la marche (Orlovsky 1972c) et le grattage (Arshavsky et al. 1978 1988) suggérant une contribution dans les mouvements rythmiques. Plus récemment, Lavoie et Drew (2002) ont montré que plusieurs cellules du noyau rouge présentaient deux pics d'activité lors de l'enjambement d'un obstacle,

qui pourraient contribuer à la phase de transport et à la phase de placement de la patte lors de l'enjambement d'un obstacle, suggérant une implication dans la coordination intra-membre. De plus, la synchronisation de l'activité de certaines cellules rubrales lors du balancement de la patte ipsi- ou contralatéral suggère une implication dans la coordination inter-membre.

Concernant le cortex moteur, les études d'enregistrements unitaires lors de l'enjambement d'obstacle ont montré que contrairement aux cellules réticulaires et rubrales, les cellules du cortex moteur semblent seulement contribuer soit à la phase de transport, soit à la phase de placement de la patte contralatérale lors de l'enjambement d'un obstacle (Drew 1988 1991a,b 1993; Drew et al. 2002, *voir la prochaine section*), suggérant une activité spécifique dans la coordination intra-membre.

La formation réticulée semble donc contrôler les ajustements posturaux, le noyau rouge semble ajuster la coordination inter-membre, enfin le cortex moteur semble réguler plus spécifiquement un muscle ou un groupe de muscles autour d'une articulation de la patte. Ces différences fonctionnelles lors de l'enjambement d'un obstacle pourraient être attribuées à l'émergence de chacune de ces structures au cours de l'évolution (Lavoie 2001 ; Lavoie et Drew 2002), passant d'une coordination inter-membre à une coordination intra-membre sur l'échelle phylogénétique.

Contribution du cortex moteur dans la locomotion

Dans les sections précédentes nous avons vu que la locomotion est sous le contrôle de plusieurs structures supraspinales et des afférences périphériques. Dans cette section, nous aborderons plus spécifiquement les évidences portant sur la contribution du cortex moteur dans le contrôle de la patte antérieure et celles concernant le contrôle cortical de la patte postérieure.

Le contrôle cortical de la patte antérieure

De nombreuses évidences de lésions, d'enregistrements unitaires et de stimulations électriques suggèrent que le cortex moteur joue un rôle primordial dans le contrôle volontaire du membre antérieur lors de la marche.

Une lésion de l'aire 4 du cortex moteur (Chambers et Liu 1957 ; Liddell et Phillips 1944) ou de la voie pyramidale (Eidelberg et Yu 1981 ; Laursen et Wiesendanger 1966 ; Liddel et Phillips 1944) ont peu d'influence sur la locomotion au sol ; par contre elles altèrent le contrôle de la patte antérieure lors de mouvements fins. Par exemple, en absence de contrôle corticospinal le chat n'est plus capable de poser correctement sa patte sur les barreaux d'une échelle horizontale, de la placer sur une poutre étroite en hauteur ou encore d'enjamber correctement un obstacle (Adkins et al. 1971 ; Beloozerova et Sirota 1993 ; Chambers et Liu 1957 ; Liddel et Phillips 1944 ; Nieoullon et Gahery 1978).

Les études d'enregistrements unitaires dans le cortex moteur ont montré que les neurones projetant à travers la voie pyramidale (PTNs) vers la moelle épinière augmentaient leur taux de décharge lorsque les chats devaient placer correctement leur patte sur les barreaux d'une échelle horizontale (Amos et al. 1987, 1989a,b, 1990 ; Armstrong 1986 ; Marple-Horvat et al. 1993, 1996 ; Marple-Horvat et Armstrong 1999) ou lors de l'enjambement d'un obstacle (Drew 1988 1991a,b 1993; Beloozerova et Sirota 1993). Plus particulièrement, les travaux de Drew ont démontré que ces neurones pouvaient moduler leur taux de décharge en phase avec l'activité spécifique de certains groupes de muscles recrutés lors de l'enjambement d'un obstacle attaché à un tapis roulant (Drew 1988 1991a,b, 1993 ; Drew et al. 2002). Plus précisément, certains neurones augmentaient leur taux de décharge en phase avec une augmentation de l'activité des muscles impliqués lors de la phase de flexion, d'autres lors du transfert de la patte au-dessus de l'obstacle ou encore d'autres lors du placement de la patte au sol au-delà de l'obstacle (Drew 1988, 1991a,b, 1993 ; Drew et al. 2002; Kably et Drew 1998b). Cette modulation spécifique de l'activité corticale en phase avec l'activité de certains groupes de muscles suggère un contrôle spécifique du cortex moteur sur la patte. Ces neurones pyramidaux envoient également une copie éfférente de la commande motrice dans la formation réticulée pontique et médullaire (Kably et Drew 1998a,b) via de fortes projections cortico-réticulaires (Matsuyama et Drew 1997 ; Rho et al. 1997), ce qui pourrait réguler les ajustements posturaux de la formation réticulée et les ajustements plus subtils du cortex moteur lors de l'enjambement d'un obstacle.

Enfin, les études de microstimulations intracorticale (ICMS) ont montré que selon la force et la durée de la stimulation, le cortex moteur pouvait changer le patron

d'activité musculaire ou le rythme du cycle de marche lors de la locomotion de la patte antérieure. Ainsi, lors de la phase de balancement, un court train de stimuli augmente l'amplitude et la durée d'activité des muscles fléchisseurs, alors qu'il a peu d'effets sur les extenseurs. Par contre durant la phase d'appui, le même court train diminue les réponses dans les extenseurs, mais à peu d'effets sur les fléchisseurs. Ces effets dépendent donc de la phase du cycle de marche. D'autre part, un long train de stimulus (200ms) peut moduler le rythme du cycle locomoteur en raccourcissant la phase d'extension et en initiant une nouvelle phase de flexion lorsque la stimulation est appliquée durant la phase d'appui (Armstrong et Drew 1985 ; Orlovsky 1972a ; Rho et al. 1999 ;).

Les neurones corticaux projettent anatomiquement de manière extensive et divergente dans les segments spinaux (Futami et al. 1979 ; Scheibel et Scheibel 1966 ; Shinoda et al. 1976, 1986 ; Shinoda et Yamaguchi 1978). Comme nous l'avons décrit dans la section précédente (*locomotion générale*), la moelle épinière pourrait être organisée en générateurs de patrons unitaires (CPG) qui contrôlerait l'activité musculaire autour de chaque articulation (Grillner 1981). Par conséquent, les neurones corticaux individuels pourraient influencer les générateurs de patrons unitaires, en activant les muscles autour de différentes articulations lors de la marche (Drew 1991a,c ; Drew et al. 1996, 2002 ; Kalaska et Drew 1993).

Ces résultats de lésions, d'enregistrements unitaires et de microstimulations ont démontré à quel point le cortex moteur contribuait au contrôle moteur de la patte

antérieure lors de la locomotion chez le chat, et en particulier lors de situations requérant un contrôle fin tels la trajectoire du membre ou le placement du bout de la patte.

Le contrôle cortical de la patte postérieure

Alors qu'une abondante littérature existe sur le contrôle cortical de la patte antérieure, peu d'évidence existe concernant le rôle du cortex moteur dans le contrôle de la patte postérieure lors de la locomotion, bien qu'il semble clair, en particulier chez l'homme, que le cortex moteur joue un rôle important. Les accidents cérébraux vasculaires du cortex moteur (Knutsson et Richards 1979) ou l'interruption de la voie corticospinale chez l'humain (Holmes 1915; Nathan et al. 1990) conduisent à de sévères incapacités et même à des paralysies de la jambe. Des études de stimulations trans-cranienne magnétique et électrique (Capaday et al. 1999 ; Petersen et al. 1998, 2001 ; Schubert et al. 1997), de tomographies par émission de positons (Fukuyama et al. 1997 ; Hanakawa et al. 1999) et de spectroscopies en infra rouge (Miyai et al. 2001) suggèrent que le cortex moteur contribue au contrôle de la marche. Bien que les études chez le primate non humain (Bucy et al 1966 ; Lawrence et Kuypers 1968a,b ; Vilensky et al. 1997), chez le chat (Chambers et Liu 1957 ; Eidelberg et Yu 1981 ; Jiang et Drew 1996 ; Laursen et Wiesendanger 1966) et le rat (Metz et al. 1998 ; Muir et Wishaw 1999 ; Schucht et al. 2002) ont montré que ces animaux pouvaient récupérer ou compenser très rapidement une locomotion fonctionnelle de la patte antérieure ainsi que de la patte postérieure à la suite de lésions corticales ou de sections de la voie corticospinale. Cette compensation se limitant aux comportements basiques ne nécessitant pas un contrôle fin

de la trajectoire du membre ou du placement de la partie distale de la patte, qui sont nécessaires lors de situations requérant une modification de la posture.

De plus, les études électrophysiologiques du laboratoire de Drew qui ont examiné la décharge des neurones de la représentation de la patte postérieure du cortex moteur chez le chat laissent penser à l'existence d'un contrôle cortical de la locomotion. L'activité unitaire de la représentation corticale de la patte postérieure du chat est modulée de manière aussi spécifique que celle de la représentation de la patte antérieure (Drew et al. 2002 ; Kably et Drew 1998b ; Widajewicz et al. 1994). Certains groupes de neurones corticaux augmentent leur taux de décharge en phase avec certain groupe de muscles requis lors de l'enjambement ou lors du placement de la patte au-delà de l'obstacle. Chez le primate, bien qu'il n'existe pas d'évidence électrophysiologique directe concernant l'implication du cortex moteur dans la locomotion, quelques études suggèrent un contrôle cortical de la jambe lors de mouvements rythmiques (Neafsey 1980 ; Sahrman et al. 1984 ; Tanji et Wise 1981 ; Wise et Tanji 1981b). Neafsey (1980) a identifié deux populations de neurones corticaux qui moduleraient différemment leur activité durant la phase de flexion et d'extension lors d'un pédalage chez le primate. Ces évidences d'enregistrements unitaires chez le chat et le primate suggèrent donc une contribution du cortex moteur dans le contrôle de la locomotion du membre postérieur.

Cependant, cette contribution semble controversée. Par exemple, bien que plusieurs études de microstimulation intra-corticale (ICMS) ont été capables d'évoquer des mouvements proximaux versus distaux depuis différents sites de la représentation de la patte postérieure du cortex moteur chez le singe (Hatanaka et al. 2001 ; Wise et Tanji 1981a) et le rat (Donoghue et Wise 1982 ; Neafsey et al. 1986), une importante étude

chez le chat a rapporté que le cortex moteur agirait sur le membre postérieur dans son ensemble (Nieoullon et Rispal-Padel 1976). Ce manque de spécificité n'est cependant pas en accord avec les études cellulaires discutées précédemment (Drew et al. 2002; Kably et Drew 1998b ; Widajewicz et al. 1994) qui suggèrent une contribution spécifique du cortex moteur lors de la locomotion du membre postérieur.

D'autre part, pour compenser les perturbations extérieures tels que les accidents du terrain, le comportement locomoteur doit être capable d'intégrer à la fois des informations sensorielles et des informations des centres supraspinaux pour modifier le rythme locomoteur aux nouvelles contraintes de l'environnement. Bien qu'il existe quelques évidences avançant que le cortex moteur est capable d'allonger ou raccourcir la durée du cycle de marche et donc de réinitialiser le rythme locomoteur dans les préparations réduites (Degtyarenko 1993 ; Leblond et al. 2001 ; Orlovsky 1972a), leurs résultats sont contradictoires et ne reflètent pas les observations faites dans le contrôle du membre antérieur chez l'animal intact (Rho et al. 1999). En effet, alors que Degtyarenko (1993) et Orlovsky (1972a) ont reporté un racccourcissement de la phase d'appuie et l'initiation d'une nouvelle phase de balancement en avance comme c'est le cas dans le contrôle du membre antérieur chez l'animal intact (Rho et al. 1999), Leblond et al. (2001) ont rapporté un raccourcissement de la phase de balancement et l'initiation d'une nouvelle phase d'extension. De plus, la stimulation transcrânienne magnétique (TMS) n'est pas capable de modifier le rythme du cycle de marche chez l'homme (Capaday et al. 1999) suggérant une absence de contrôle du cortex moteur sur le rythme locomoteur du membre postérieur.

Sur la base de ces observations, nous testerons deux hypothèses selon lesquelles le cortex moteur peut modifier l'amplitude et la durée d'activité musculaire lors de la locomotion du membre postérieur et cela de manière spécifique (Hypothèse 1) ; et qu'il peut modifier le rythme locomoteur du membre postérieur de manière similaire à celui du membre antérieur (Hypothèse 2). Si cela est vrai, cela suggèrera que le cortex moteur contrôle la locomotion du membre postérieur de manière aussi spécifique que le membre antérieur chez le chat. Cette étude sera l'objet de notre premier papier (Bretzner et Drew 2005a).

Interactions entre le cortex moteur et les afférences cutanées

Comme les sections précédentes le suggèrent, la locomotion est modulée par différentes structures supraspinales et divers signaux provenant de la périphérie qui modulent et informent de la réalisation de ces mouvements. Parmi les structures supraspinales, le cortex moteur semble contribuer en particulier à la régulation de la marche en modifiant de manière anticipée le contrôle de la marche sur la base des informations visuelles (Drew 1991a,c) et en étant capable de modifier le rythme du cycle de marche (Armstrong et Drew 1985 ; Bretzner et Drew 2005a ; Rho et al. 1999). Parmi les signaux provenant de la périphérie, le feed-back cutané fournit également une importante information produisant des réponses réflexes intégrées dans le cycle de marche, qui permettent à l'animal de maintenir un rythme locomoteur normal. Enfin, de récentes études (Bouyer et Rossignol 2003a,b) ont montré que les informations cutanées contribuent également à la régulation du rythme locomoteur de base car des déficits importants surviennent à la suite d'une dénervation cutanée chronique du bout de la patte. Cependant, alors que de nombreuses évidences supportent l'idée que le feed-back cutané pourrait moduler l'activité corticale, peu d'informations suggèrent que l'activité corticale pourrait moduler les réflexes cutanés durant la marche.

Feed-back cutané sur l'activité du cortex moteur

De nombreuses évidences suggèrent que le feed-back cutané module l'activité du cortex moteur durant la locomotion. La stimulation du nerf innervant la surface plantaire

du bout de la patte antérieure augmente l'amplitude des réponses corticales durant la phase d'appui chez le rat et le chat marchant sur un tapis roulant (Chapin et Woodward 1986 ; Palmer et al. 1985) ainsi que chez le chat marchant sur une échelle horizontale (Marple-Horvat et Armstrong 1999). Ces changements corticaux suggèrent que le cortex moteur pourrait intégrer les informations cutanées pour ajuster un contrôle fin lors de la locomotion. D'autre part, une altération du système nerveux périphérique par lésion ou conditionnement des afférences périphériques semble également induire des changements corticaux mais sans induire de changements spinaux dans l'efficacité corticospinale. Une amputation (Cohen et al. 1991a,b ; Ridding et Rothwell 1995) ou un blocage ischémique de la jambe (Brasil-Neto et al. 1992) ainsi que le conditionnement au niveau des afférences périphériques de la jambe durant la locomotion chez l'homme (Knasch et al. 2003 ; Ridding et al. 2000) augmentent significativement l'efficacité corticospinale évoquée par TMS dans les muscles proximaux au site d'altération, mais sans évoquer aucun changement dans les réponses évoquées par la stimulation transcrânienne électrique (TES) ou par des électrodes placées au niveau du pont. Il faut savoir que la TMS, en activant les neurones corticospinaux transynaptiquement, stimule la voie corticospinale, alors que la TES en activant directement les axones des neurones corticospinaux au niveau du pédoncule cérébral, stimule seulement les réseaux interneuronaux spinaux de la voie corticospinale (Hallet 2000 ; Rothwell 1997). Ces résultats suggèrent donc que la stimulation ou l'altération des afférences cutanées induisent des changements dans l'activité et l'excitabilité corticale sans affecter l'excitabilité de la moelle épinière.

Activité du cortex moteur sur les réflexes cutanés

Les travaux précurseurs de Lundberg (1962) chez le chat anesthésié suggèrent que les informations provenant du cortex moteur et des afférences périphériques convergent dans la moelle épinière. La stimulation de la voie corticospinale facilite les effets excitateurs des afférences cutanées dans les motoneurones fléchisseurs, alors qu'elle facilite les effets inhibiteurs de ces afférences dans les motoneurones extenseurs (Lundberg 1964 ; Lundberg et Voorhoeve 1962). Néanmoins, quelques expériences ont rapporté une facilitation corticospinale des effets excitateurs des afférences cutanées dans les muscles et les motoneurones extenseurs (Engberg 1964a,b ; Fleshman et al. 1988 ; Hagbarth 1952 ; Pinter et al. 1982). La voie corticospinale facilite également la dépolarisation de plusieurs afférences primaires cutanées (Andersen et al. 1964 ; Anderson et al. 1964 ; Carpenter et al. 1962, 1963 ; Fetz 1968 ; Marchiafava et Pompeiano 1964 ; Morrison et Pompeiano 1965 ; Rudomin et al. 2004 ; Rudomin et Schmidt 1999), ce qui suggèrent que le cortex moteur pourrait moduler les réflexes spinaux par une action via les interneurones ou les neurones des racines dorsales, ce qui a été confirmé en montrant une convergence des inputs corticaux et cutanés dans les interneurones lombaires (Lundberg 1964 ; Lundberg et al. 1962) et dans les neurones des racines dorsales (Anderson et al. 1964 ; Fetz 1968 ; Morrison et Pompeiano 1965).

D'un point de vue anatomique, cette convergence des inputs corticaux et cutanés devra être étudiée plus spécifiquement lors de préparations aiguës afin d'identifier les réseaux de neurones impliqués dans cette convergence. En effet, alors qu'il existe une abondante littérature portant sur le substrat anatomique de cette convergence dans le

segment cervical, peu d'information existe sur le segment lombaire. Par exemple dans le segment cervical, les réseaux corticospinaux et curtanés convergent dans les neurones propriospinaux (Illert et al. 1975b, 1977 ; Alstermark et al. 1984a,b ; Sasaki et al. 1996) ainsi que dans les interneurones inter-segmentaires de la voie corticospinale (Illert et al. 1975a, 1976 ; Hultborn 1976 ; Hongo et al. 1989a,b ; Kitazawa et al. 1993 ; Sasaki et al. 1996). Cette absence d'information peut sembler paradoxale compte tenu de l'abondante littérature portant sur le substrat anatomique de la convergence des réseaux supraspinaux et proprioceptifs dans le segment lombaire, mais elle nécessitera d'être étudiée et clarifiée dans le futur.

D'un point de vue fonctionnel, quelques études chez l'homme ont montré une convergence des inputs corticaux et cutanés au repos (Nielsen et al. 1997 ; Wolfe et Hayes 1995) et lors de la phase de balancement du cycle de marche (Pijnappels et al. 1998). Cependant ces études se sont limitées à deux muscles et au réflexe d'un seul nerf cutané, le Sural. De plus, un certain nombre d'évidences suggèrent que la TMS est une méthode très indirecte pour mesurer l'efficacité corticospinale de la représentation de la jambe chez l'homme (Rothwell 2003 ; Petersen et al. 2003). En effet, on ne sait pas encore avec certitude le mode d'activation de la stimulation magnétique, indirectement via des cellules corticales qui activerait des cellules de la voie corticospinale ou directement au soma ou au premier nœud de Ranvier des axones des cellules corticospinales. Par ailleurs, la TMS semble activer seulement les couches superficielles du cortex moteur, la représentation corticale de la jambe, en s'enfonçant dans la bande du cortex crucié, ne serait donc pas activée adéquatement.

Sur la base de ces études, nous testerons l'hypothèse de recherche selon laquelle le cortex moteur peut augmenter ou diminuer de manière spécifique l'amplitude des réponses musculaires évoquées par différents réflexes cutanés lors de la locomotion du membre postérieur chez le chat. Si elle s'avère vraie, cela suggérera que le cortex moteur module de manière spécifique les réflexes cutanés lors de la marche. Cette étude sera l'objet de notre second papier (Bretzner et Drew 2005b).

Plasticité du système locomoteur

La longue histoire des neurosciences a désigné la moelle épinière comme le site des réflexes et des mouvements automatiques, donnant au cerveau le rôle de contrôler ces réflexes spinaux via les interneurones (Sherrington 1910). Dans cette organisation, seul le cerveau est capable de mécanismes adaptatifs sur l'échelle de l'individu, la moelle épinière ne pouvant être le lieu que de mécanismes adaptatifs sur l'échelle phylogénétique. Il faudra attendre plus récemment pour découvrir que la moelle épinière est capable de mécanismes adaptatifs, par exemple à la suite d'un entraînement ou d'une altération du système périphérique. Par ailleurs, cette plasticité spinale ne semble pas toujours apparaître seule mais parfois simultanément à une plasticité supraspinale.

Récupération fonctionnelle après une section complète de la moelle épinière

Il existe de nombreuses évidences comportementales et neurophysiologiques que l'entraînement améliore la récupération fonctionnelle de la marche après une section complète de la moelle épinière chez le chat. De tels chats sont capables de récupérer une marche automatique avec une activité musculaire rythmée, lorsqu'ils sont entraînés à marcher sur un tapis roulant (Barbeau et Rossignol 1987 ; Bélanger et al. 1996 ; de Leon et al. 1998a ; Rossignol 2000). Cette amélioration de la marche persiste jusqu'à 12 semaines après arrêt de l'entraînement, puis décline lentement avec le temps (de Leon et al. 1999). D'autre part, la récupération fonctionnelle dépend également du type d'entraînement, un chat spinal entraîné à se tenir droit supportera mieux son poids qu'un

chat entraîné à marcher qui récupérera mieux la marche (de Leon et al. 1998b). Ces différentes évidences montrent que l'entraînement améliore et maintient la récupération d'habilités fonctionnelles chez le chat, telle que la marche. Elles montrent également que la moelle épinière est un site de plasticité.

Récupération locomotrice : Plasticité spinale

L'entraînement améliore la récupération, voire la compensation fonctionnelle de la marche chez les chats spinaux, mais il modifie également le gain des réflexes spinaux chez l'animal intact. Les travaux de Pearson ont étudié de manière très scrupuleuse et détaillée les mécanismes de plasticité spinale lors d'une compensation fonctionnelle de la marche à la suite d'une altération d'un nerf moteur extenseur chez le chat intact.

Une section (Misiaszek et Pearson 2002 ; Pearson et al. 1999 ; Pearson et Misiaszek 2000 ; Whelan et Pearson 1997) ou ablation chimique par injection de botulinum toxin (Pearson et al. 2003) des nerfs moteurs innervant les muscles extenseurs de la cheville (gastrocnemius lateral, soleus et plantaris), qui sont des synergistes du gastrocnemius medial (GM), conduit à une faiblesse dans l'extension de la cheville lors de la locomotion chez le chat. Cette faiblesse dans l'extension est caractérisée par une augmentation marquée dans la flexion de la cheville produit par le poids du corps durant la partie initiale de la phase d'appui (phase E2) de la marche. Cependant les chats récupèrent progressivement en une à deux semaines un contrôle normal de leur cheville en augmentant le niveau d'activité du muscle extenseur restant, le gastrocnemius medial. Cette récupération dépend de l'utilisation de la cheville, en effet, lorsque les animaux sont restreints par des attelles, la récupération fonctionnelle de

la cheville et l'augmentation du niveau d'activité du GM sont retardées (Pearson et al. 1999). La récupération s'accompagne également d'une augmentation de l'influence des afférences de groupes I du muscle extenseur GM sur l'amplitude et la durée des bouffées d'activité de ce muscle (Pearson et Misiaszek 2000 ; Whelan et Pearson 1997). De plus, l'injection intra-péritonéale de fortes doses de Pyridoxine, (vitamine B6) qui agit spécifiquement sur les afférences sensorielles de gros calibres (Windebanket al. 1985 ; Xu et al. 1989), diminue significativement la récupération fonctionnelle de la cheville et l'amplitude de la bouffée d'activité du muscle extenseur GM (Pearson et al. 2003). Ces différentes études suggèrent donc l'importance de l'intégrité des afférences proprioceptives et de l'entraînement dans la récupération fonctionnelle de la marche à la suite d'une dénervation motrice des muscles extenseurs de la cheville chez le chat. Cette plasticité qui semble a priori spinale pourrait également être supraspinale.

Récupération locomotrice : Plasticités supraspinales ou spinales

La récupération fonctionnelle de la marche à la suite de diverses altérations du système nerveux périphérique pourrait être due à des mécanismes supraspinaux autant que spinaux. À ma connaissance, Goldberger et al. dans les années 1980, ont été les premiers à essayer de discriminer les sites supraspinaux des sites spinaux nécessaires à la récupération fonctionnelle de la marche après une altération du système nerveux périphérique. La récupération de la marche après une section des racines dorsales en combinaison avec une hémisection de la moelle épinière chez le chat laisse suggérer une plasticité spinale (Goldberger 1977 ; Goldberger et Murray 1974). Néanmoins cette

compensation pourrait également provenir d'une plasticité supraspinale via la demi moelle épinière non lésée. Les récentes études du laboratoire de Rossignol ont poursuivi cette démarche, en combinant des dénervations motrices ou cutanées de la patte postérieure à des sections complètes de la moelle épinière, identifiant ainsi clairement le lieu de plasticité (Bouyer et al. 2001 ; Bouyer et Rossignol 2003a,b ; Carrier et al. 1997).

Pour déterminer le site de plasticité responsable de la récupération fonctionnelle de la marche à la suite d'une dénervation motrice des extenseurs de la cheville, modèle développé par Pearson (*décrit dans la section précédente*), Bouyer et al. (2001) ont étudié la récupération de la marche à la suite d'une neurectomie des extenseurs chez des chats spinaux. Il est bien connu que les chats après une section complète de la moelle épinière sont capables de récupérer une marche automatique, lorsqu'ils sont entraînés sur tapis roulant (Bélanger et al. 1996 ; Barbeau et Rossignol 1987 ; Rossignol 2000). Une section des nerfs moteurs extenseurs de la cheville a donc été pratiquée chez des chats spinaux ayant récupéré la marche sur tapis roulant. À leur grande surprise, la récupération fonctionnelle de la patte dénervée (i.e. la cinématique et l'activité musculaire des extenseurs) chez les chats spinaux dénervés suivait le même décours temporel que ceux des chats simplement dénervés. Ces évidences suggèrent, par conséquent, que cette récupération fonctionnelle serait due à des mécanismes spinaux.

Un autre modèle de dénervation motrice développé dans le laboratoire de Rossignol a montré que la récupération de la marche peut être le fait d'une plasticité spinale ou supraspinale selon que la moelle épinière est isolée ou non. Carrier et al. (1997) ont démontré que les chats récupèrent rapidement une marche normale à la suite d'une dénervation unilatérale des nerfs moteurs innervant les fléchisseurs de la

cheville (Tibialis Anterior et Extensor Digitorum longus), en augmentant le niveau d'activité des fléchisseurs de la hanche et du genou. À la suite d'une section complète de la moelle épinière lombaire, ces chats dénervés présentent un patron locomoteur asymétrique et désorganisé avec une prédominance de flexion du genou et une absence de placement plantaire du pied, contrairement aux chats intacts qui regagnent un patron locomoteur régulier et symétrique après une section complète de la moelle épinière. Ces évidences suggèrent donc une compensation d'origine supraspinale et spinale. Par contre, une dénervation unilatérale à la suite d'une section de la moelle épinière n'altère ni la symétrie du patron locomoteur, ni n'entraîne d'hyperflexion du genou, ce qui suggèrent une plasticité spinale. Il semble donc que les chats récupèrent la marche à la suite d'une dénervation motrice via une compensation supraspinale et spinale, qui disparaît à la suite d'une section de la moelle épinière, alors que les chats spinaux récupèrent une marche à la suite de la dénervation via une compensation spinale.

De manière similaire, alors que les chats spinaux après une dénervation motrice des extenseurs récupèrent la marche (Bouyer et al. 2001), il est fort probable que les chats ayant récupérés à la suite d'une dénervation motrice des extenseurs (Misiaszek et Pearson 2002 ; Pearson et al. 1999 ; Pearson et Misiaszek 2000 ; Whelan et Pearson 1997), ne compenseraient pas après une section complète de la moelle épinière.

Cette plasticité supraspinale ou spinale selon que la moelle épinière est isolée ou non n'est pas limitée aux dénervations motrices puisqu'elle a également été rapportée dans un modèle de dénervation sensorielle. À la suite de dénervations cutanées bilatérales, les chats sont incapables de poser correctement le bout de leur patte sur les barreaux d'une échelle horizontale, mais ils récupèrent rapidement le traînement du bout

de leur patte lors de la marche au sol ou sur tapis roulant, en augmentant le niveau d'activité des fléchisseurs du genou et de la cheville (Bouyer et Rossignol 2003a). Cette récupération fonctionnelle est cependant annulée après une section complète de la moelle épinière (Bouyer et Rossignol 2003b) ou diminuée et ralentie chez les chats ayant une lésion électrolytique de la représentation de la patte postérieure du cortex moteur (Bouyer et al. 2000), suggérant donc une compensation d'origine corticospinale. Par contre, les chats spinaux soumis à une dénervation cutanée bilatérale partielle (Bouyer et Rossignol 2003b) sont capables de marcher correctement aussi longtemps qu'ils leur restent le nerf cutané innervant le bout distal de la patte. Cependant, après retrait de cette dernière afférence cutanée, ces chats spinaux sont incapables d'assurer un placement normal du bout de la patte ou un support normal de leur poids (Bouyer et Rossignol 2003b), suggérant que cette plasticité spinale à la suite d'altération du système nerveux périphérique chez les chats spinaux est limitée et nécessite au moins une afférence cutanée.

Ces études de récupération fonctionnelle suggèrent que les voies descendantes supraspinales chez l'animal intact compensent une altération du système nerveux périphérique, puisqu'il n'y a plus compensation après section de la moelle épinière. Par contre la moelle épinière en absence de contrôle descendant du cerveau peut être le lieu d'une plasticité et peut compenser les altérations du système nerveux périphérique mais dans certaines limites, puisqu'il n'y a plus de compensation après une dénervation cutanée complète de la patte.

Conditionnement supraspinal de réflexes spinaux

Les études portant sur les structures supraspinales ont longtemps négligé le rôle de mécanismes spinaux, et vice versa. Cependant de récents travaux portant sur le conditionnement des réflexes spinaux et en particulier du réflexe H ont démontré que l'acquisition et le maintien de ces réflexes seraient sous le contrôle des centres supérieurs telle que la voie descendante corticospinale (Wolpaw et Tennissen 2001). Lors de la stimulation d'un nerf moteur, une réponse primaire motrice, M, est évoquée orthodromiquement dans le muscle, ainsi qu'une réponse secondaire antidromique, H, qui passe par les réseaux de la moelle épinière. En ajustant périodiquement l'intensité de stimulation du nerf pour évoquer une réponse M constante, l'amplitude du réflexe H peut être diminuée ou augmentée selon la présence d'une récompense attendue chez le rat et le primate (Chen et Wolpaw 1995 ; Wolpaw 1987 ; Wolpaw et al. 1983, 1993 ; Wolpaw et Lee 1989 ; Wolpaw et Tennissen 2001), ainsi que chez l'humain (Evatt et al. 1989 ; Wolf et Segal 1990, 1996). De nombreuses évidences d'enregistrements intracellulaires suggèrent que la moelle épinière serait le lieu de ce conditionnement et plus précisément que chacun des conditionnements du réflexe H serait associé à des réseaux interneuronaux spinaux spécifiques (Carp et Wolpaw 1994, 1995 ; Feng-Chen et Wolpaw 1996 ; Wolpaw 1997, 2001).

Cependant une section de la voie corticospinale empêche toute acquisition du conditionnement du réflexe H (Chen et al. 2002 ; Chen et Wolpaw 1997, 2002). De plus, elle empêche également le maintien du conditionnement à la baisse du réflexe H mais pas celui du conditionnement à la hausse (Chen et al. 2003). L'acquisition d'un des

conditionnements du réflexe H est également impossible chez l'homme atteint d'accidents cérébrovasculaires du cortex moteur (Wolf et al. 1995 ; Wolf et Segal 1990, 1996).

Ces dernières évidences suggèrent donc, que la moelle épinière peut être le lieu de mécanismes de plasticité mais sous contrôle de la voie descendante corticospinale.

Plasticité corticale à la suite d'une altération du système nerveux périphérique

L'étude de la plasticité, tout comme celle du développement, nous renseigne sur les mécanismes et la dynamique du système nerveux central et périphérique. Comment le cerveau et la moelle épinière interagissent au niveau fonctionnel, systémique ou cellulaire pour compenser ou récupérer une perception ou une fonction motrice à la suite d'un dommage central ou périphérique. Les premières études portant sur la plasticité corticale se sont intéressées aux considérables capacités de réorganisation survenant dans les représentations du cortex somatosensoriel à la suite d'une altération du système nerveux périphérique (Kalaska et Pomeranz 1979, 1982). Une amputation d'un simple doigt conduit à une réorganisation des représentations des doigts adjacent en envahissant le territoire qui occupait les doigts amputés chez le primate (Merzenich et al. 1984). De manière similaire, l'amputation de la main, du bras ou de l'avant bras induit également une rapide invasion des représentations de la partie proximale au site d'amputation dans le territoire normalement dévolu à la partie amputée (Florence et Kaas 1995 ; Pons et al. 1991). Certaines études ont même rapporté une invasion des représentations de la face et du tronc dans le territoire anciennement dévolu à la main après une amputation, chez le primate (Pons et al., 1991) ainsi que chez l'homme (Elbert et al. 1994). Ces changements qui surviennent rapidement, semblent par ailleurs persister longtemps au-delà de plusieurs années. Il semble donc exister une énorme réorganisation au sein du cortex somatosensoriel à la suite d'une altération périphérique. Comparativement, le cortex moteur présente une réorganisation plus limitée dans ses représentations à la suite

d'une altération périphérique. Par ailleurs, cette réorganisation dans le cortex moteur s'accompagne aussi de changements dans l'excitabilité de la voie corticospinale, c'est à dire dans son efficacité sur l'activité musculaire. Dans les sections suivantes, j'aborderai donc les phénomènes et les mécanismes cellulaires sous-tendant la réorganisation des représentations du cortex moteur ainsi que les modifications d'excitabilité de la voie corticospinale à la suite d'une altération du système nerveux périphérique.

Changements dans les représentations du cortex moteur

Phénomène

Bien que le cortex moteur soit capable de se réorganiser à la suite d'une altération périphérique, les changements sont plus limités comparativement à ceux survenant dans le cortex somatosensoriel. Chez les rats néonataux amputés du membre antérieur, la stimulation à bas seuil dans la représentation corticale anciennement dévolue à la patte, évoque des mouvements dans la face, plus particulièrement dans les vibrisses et, moins fréquemment, dans le moignon de l'épaule (Donoghue et Sanes 1987, 1988). Chez les rats adultes, la section du nerf facial innervant la musculature des vibrisses est suivie en quelques heures par une expansion des représentations corticales innervant la musculature péri-oculaire et celle innervant la patte antérieure (Donoghue et al. 1990 ; Sanes et al. 1988, 1990). Par contre, l'amputation de la patte antérieure chez le rat adulte entraîne une expansion de la représentation du moignon de l'épaule dans le territoire déafférenté, mais aucun changement dans la représentation des vibrisses ou de

la face (Sanes et al. 1990). Ces résultats lors de l'amputation d'un membre ont également été rapportés dans d'autres espèces de primates, juvénile et adulte, ainsi que chez l'homme.

L'amputation du membre antérieur ou postérieur chez le primate entraîne à long terme un envahissement de la représentation corticale du moignon (épaule ou hanche) dans le territoire déafférenté, (Qi et al. 2000 ; Wu et Kaas 1999), en absence de changements dans la représentation corticale de la face ou du tronc dans le cas des amputations du membre antérieur (Qi et al. 2000 ; Schieber et Deuel 1997). Une expansion similaire des représentations corticales des muscles proximaux au site d'amputation dans les territoires déafférentés a également été observée chez l'homme (Chen et al. 1998 ; Karl et al. 2001 ; Ojemann et Spibergeld 1995). Il en est de même lors d'un blocage ischémique du bras ou de la jambe à l'aide d'un garrot placé autour du bras ou de la jambe, qui constitue en quelque sorte une amputation transitoire (Brasil-Neto et al. 1993).

Il semble donc que l'amputation ou le blocage ischémique du membre antérieur ou postérieur entraîne une expansion des représentations corticales des muscles proximaux au site d'altération dans les territoires déafférentés chez le sujet juvénile ainsi que l'adulte, mais sans changements dans les autres représentations adjacentes (visage et tronc par exemple dans le cas d'amputation du membre antérieur). Cette réorganisation à la suite d'une amputation ou d'un blocage ischémique est cependant beaucoup plus limitée comparativement à celle survenant chez le rat néo-natal ou à la suite d'une section du nerf facial moteur chez le rat adulte, suggérant des capacités de plasticité différentes. Elle est également plus ``limitée`` à celle survenant dans le cortex

somatosensoriel, où le territoire déafférenté est envahi par les représentations de la face et du tronc dans le cas des amputations du membre antérieur.

Différences dans la réorganisation du cortex moteur et cortex somatosensoriel après amputation

Les différences rapportées dans la réorganisation du cortex moteur versus celle du cortex somatosensoriel à la suite d'une amputation semblent résulter également des différences dans leur voie anatomique respective. Le système ascendant sensoriel présente une grande divergence et plusieurs relais jusqu'au cortex somatosensoriel. Les changements synaptiques dans le noyau ventro-latéral du thalamus (Jones et Pons 1998 ; Pollin et Albe-Fessard 1979), dans les noyaux du pont (Florence et Kaas 1995 ; Jain et al. 2000 ; Jones et Pons 1998) ainsi que dans les noyaux de la colonne dorsale (Florence et Kaas 1995 ; Jain et al. 2000 ; Kalaska et Pomeranz 1982), dans lesquels les distances entre les représentations du bras, du tronc et de la face sont beaucoup plus réduites que dans le cortex somatosensoriel pourraient contribuer à l'invasion de plusieurs représentations corticales dans le territoire déafférenté (Florence et al. 1996 ; Florence et Kaas 1995). La réorganisation dans chacun de ces centres du système ascendant sensoriel serait alors amplifiée par la réorganisation des centres subséquents et conduirait aux changements observés dans le cortex somatosensoriel. À l'inverse, le cortex moteur présente une connectivité plus directe depuis les neurones pyramidaux aux motoneurones via une série d'interneurones spinaux, dont le nombre varie selon l'espèce animale. La réorganisation dans le cortex moteur est donc beaucoup plus

limitée comparativement à celle du cortex somatosensoriel, avec des changements qui ont essentiellement lieu au niveau du cortex moteur et de la moelle épinière.

Mécanismes cellulaires

Cette réorganisation dans les représentations corticales du cortex moteur à la suite d'une altération périphérique pourrait résulter de changements qui n'ont lieu qu'au niveau cortical. En effet, il existe de nombreuses évidences chez l'animal que les changements survenant rapidement dans les représentations corticales pourraient résulter d'une levée de l'inhibition des interneurones corticaux GABAérgiques qui conduiraient alors à une expansion des représentations corticales.

L'injection de Bicuculline, un antagoniste GABAergique-A, dans le cortex moteur conduit à des changements dans les représentations corticales, semblables à ceux survenant à la suite d'une section du nerf facial chez le rat (Jacobs et Donoghue 1991 ; Sanes et Donoghue 2000). Cette réorganisation corticale en présence d'un antagoniste GABAergique suggère donc que les réarrangements survenant dans les représentations corticales à la suite d'une altération du système nerveux périphérique pourraient résulter d'une levée de l'inhibition, qui en retour éveillerait des connexions horizontales intracorticales anciennement silencieuses. La diminution de densité des récepteurs GABAa (Hendry et al. 1990), la réduction de l'expression du neurotransmetteur GABA (Hendry et Jones 1986, 1988), ainsi que la réduction de l'expression de l'enzyme de synthèse du GABA (Akhtar et Land 1991 ; Hendry et Jones 1986, 1988 ; Welker et al.

1989) rapportées dans divers modèles de privation sensorielle chez l'animal corroborent l'existence de cette levée de l'inhibition GABAergique après altération périphérique.

Ces connexions horizontales nouvellement actives expliqueraient alors l'expansion et la rétraction des représentations corticales dans les quelques heures qui suivent une altération du système nerveux périphérique chez l'animal (Donoghue et al. 1990 ; Sanes et al. 1990). En effet, la section du nerf facial chez le rat entraîne une réorganisation dans les représentations corticales des vibrisses et de la patte antérieure qui sont reliées par des connexions horizontales, alors que les représentations corticales des vibrisses et de la patte antérieure ne possédant peu ou pas de connexions horizontales ne présentent pas de réorganisation (Huntley 1997).

Des évidences indirectes suggèrent également que la réorganisation corticale survenant à la suite d'une amputation ou d'un blocage ischémique chez l'homme résulte d'une levée de l'inhibition GABAergique. Les mesures indirectes de l'amplitude des réponses évoquées par TMS et de l'inhibition intracorticale chez l'homme sont modifiées sous l'effet de drogues. Lorsqu'une stimulation au-dessus du seuil est précédée par une stimulation conditionnante en dessous du seuil, l'amplitude de la réponse évoquée dépend de l'intervalle entre le test et le conditionnement (Kujirai et al. 1993) et représente alors une mesure de l'inhibition ou facilitation intracorticale selon l'intervalle interstimulus. L'inhibition intracorticale apparaît habituellement dans l'intervalle interstimulus 1-5 ms, alors que la facilitation intracorticale apparaît dans l'intervalle interstimulus entre 6-20 ms. À la suite d'une amputation ou d'un blocage ischémique d'un membre, l'inhibition intracorticale diminue significativement dans la représentation corticale des muscles proximaux au site d'altération dans la jambe (Chen

et al. 1998) et dans le bras (Dettmers et al. 1999 ; Schwenkreis et al. 2000 ; Ziemann et al. 1998a,b, 2002). Cette inhibition intracorticale semble être d'origine GABAergique, car la prise de Lorazepam, un agoniste des récepteurs GABAa augmente l'inhibition intracorticale chez le sujet sain (Di Lazzaro et al. 2000 ; Ziemann et al. 1996) et bloque la diminution de l'inhibition intracorticale et l'augmentation de la représentation corticale des muscles proximaux au site de blocage ischémique du bras (Ziemann et al. 1998a,b ; 2001).

Ces diverses évidences directes et indirectes suggèrent donc que la réorganisation des représentations corticales résulterait de la diminution de l'inhibition intra-corticale et de l'éveil de connexions intra-corticales horizontales anciennement silencieuses.

Augmentation de l'efficacité corticospinale

Alors qu'il existe une abondante littérature portant sur la réorganisation des représentations du cortex moteur et somatosensoriel à la suite d'une altération périphérique dans différentes espèces, seules les études chez l'homme ont investigué de manière intensive les changements d'excitabilité de la voie corticospinale (efficacité corticospinale) après une altération périphérique. L'augmentation dans l'amplitude des mouvements ou des réponses musculaires évoquées par la stimulation du cortex moteur après altération périphérique suggère une diminution dans le seuil d'excitabilité de la

voie corticospinale, qui pourrait avoir lieu au niveau du cortex moteur ou de la moelle épinière.

Phénomène

De nombreuses évidences suggèrent des changements dans l'efficacité corticospinale à la suite de l'amputation d'un bras ou d'une jambe chez l'homme. Par exemple, Chen et al. (1998) ont montré que l'amplitude des réponses musculaires évoquées à une même intensité de stimulation est souvent plus élevée et que le seuil moteur (pour évoquer 50 % de l'amplitude maximale des réponses) est souvent plus bas dans le cortex moteur qui contrôle les muscles proximaux au site d'amputation de la jambe que dans le cortex non déafférenté, suggérant une augmentation d'excitabilité de la voie corticospinale. Pour déterminer si ces changements étaient dus à des changements corticaux versus spinaux, les seuils moteurs évoqués par les stimulations trans-craniennes magnétiques et électriques ont été comparés entre les côtés amputé et intact. Avec la TMS qui active les neurones corticospinaux transynaptiquement, le seuil était plus bas du côté amputé, alors qu'avec la TES qui active directement les axones des neurones corticospinaux au niveau du pédoncule cérébral (Hallet 2000 ; Rothwell 1997), les seuils étaient similaires des deux côtés, suggérant un changement d'excitabilité au niveau cortical. De plus, les seuils moteurs d'excitabilité spinale testés à l'aide de stimulation électrique spinale et du réflexe H ne montraient aucune différence entre les côtés amputé et intact, confirmant une absence de changement dans l'excitabilité spinale. Ces différentes études montrent donc une diminution du seuil moteur et une

augmentation dans l'amplitude des réponses motrices évoquées par une stimulation magnétique du cortex moteur contrôlant les muscles proximaux de la jambe amputée, ce qui suggèrent essentiellement un changement d'excitabilité au niveau cortical.

Bien que de nombreuses études aient rapporté une augmentation dans l'amplitude des réponses motrices évoquées chez des amputés d'une jambe (Chen et al. 1998 ; Fuhr et al. 1992 ; Hall et al. 1990) ou d'un bras (Cohen et al. 1991a,b ; Dettmers et al. 1999 ; Hall et al. 1990 ; Kew et al. 1994 ; Roricht et al. 1999a,b 2001), des différences ont été observées dans le seuil moteur. Roricht et al. (1999b) ont rapporté une diminution dans le seuil moteur chez les amputés de l'avant bras, mais non dans les amputés de la partie plus proximale du bras par rapport au côté intact. Néanmoins, d'autres études chez les amputés du bras ont rapporté une absence de changements dans le seuil moteur entre les côtés intact et amputé (Capaday et al. 2000 ; Kew et al. 1994 ; Schwenkreis et al. 2000).

Chez le primate amputé depuis longtemps, le seuil moteur est augmenté (Schieber et Deuel 1997) ou encore au même niveau (Qi et al. 2000 ; Wu et Kaas 1999) dans les muscles proximaux du côté amputé par rapport au côté intact. Par contre chez le rat, le seuil moteur mesuré avant et après amputation de la patte antérieure, semble diminuer (Sanes et al 1990). Ces résultats qui semblent contradictoires, pourraient s'expliquer par une plasticité dépendante de l'utilisation, qui dépendrait du site d'amputation et du degré d'utilisation du membre amputé. En effet, lors d'une amputation distale, les sujets continueraient à utiliser leur membre amputé pour prendre un objet chez l'homme ou encore se déplacer chez le rat, par contre une amputation proximale limiterait l'utilisation du membre en particulier chez l'homme.

Néanmoins, ces changements d'efficacité corticospinale chez les primates non humains et les sujets amputés ne reflètent pas forcément une réalité car ils résultent de la comparaison des efficacités entre les côtés amputé et intact, et non pas des changements d'efficacité de la même voie corticospinale avant et après amputation. Le blocage ischémique du bras ou de la jambe, à l'aide d'un garrot placé autour du membre résout en partie ce problème en créant une amputation transitoire qui permet de mesurer les changements d'efficacité corticospinale avant et après le blocage ischémique du membre. Brasil-Neto et al. (1992) ont démontré une augmentation progressive dans l'amplitude des réponses motrices évoquées (MEPs) par TMS dans un muscle extenseur, le vastus lateralis, proximal au blocage ischémique de la jambe. Cette augmentation dans l'amplitude des réponses motrices évoquées par TMS retourne après le retrait du garrot, au niveau de base observé avant le blocage. Bien que l'amplitude des MEPs ait été augmentée par la TMS, aucun changement significatif n'a été observé dans le seuil moteur pendant et après le blocage ischémique par rapport au contrôle avant blocage. Des observations similaires ont été relevées lors d'un blocage ischémique du bras (Brasil-Neto et al. 1992, 1993 ; Ridding et Rothwell 1995, 1997 ; Ziemann et al. 1998a). De plus, la TES, la stimulation électrique spinale et le réflexe H n'évoquaient aucun changement dans l'amplitude des MEPs pendant le blocage de la jambe (Brasil-Neto et al. 1992), suggérant une absence de changement d'excitabilité spinale. Cette augmentation rapide dans l'amplitude des MEPs évoqués seulement par TMS dans les muscles proximaux au site de blocage ischémique de la jambe ou du bras suggère donc un changement à court terme dans l'excitabilité corticale.

Des changements similaires ont également été rapportés dans quelques études lors d'anesthésie de nerfs moteurs ou mixtes chez l'homme avec une augmentation dans l'amplitude des MEPs par la TMS dans les muscles proximaux au site d'altération, ainsi qu'une diminution dans les muscles en dessous du territoire anesthésié lors d'une anesthésie focale d'un nerf mixte de la main (Murphy et al. 2003 ; Rossi et al. 1998).

En résumé, la plupart des études de plasticité de la voie corticospinale rapportent une augmentation dans l'amplitude des réponses motrices évoquées par le cortex moteur dans les muscles proximaux au site d'altération périphérique et une absence de changements dans l'amplitude des réponses évoquées par la moelle épinière, ce qui suggère une augmentation dans l'excitabilité corticale. Plus précisément, dans le cas de dénervation ou anesthésie de nerfs, l'amplitude des réponses évoquées par le cortex moteur dans les muscles distaux ou sous jacents à la lésion diminue. Néanmoins, de nombreuses contradictions persistent concernant les changements dans le seuil moteur évoqué par la stimulation du cortex moteur après altération périphérique.

Mécanismes cellulaires

L'augmentation de l'efficacité corticospinale à la suite d'une altération du système nerveux périphérique pourrait résulter simplement de l'activation d'un plus grand bassin de neurones corticospinaux, puisque la levée de l'inhibition GABAergique et l'éveil de nouvelles connexions intra-corticales horizontales anciennement silencieuses augmentent la taille des représentations corticales. Néanmoins, cette

augmentation de l'efficacité synaptique, et en particulier la diminution du seuil moteur, pourrait également résulter d'un mécanisme de potentialisation à long terme (LTP) via les récepteurs NMDA qui augmenterait l'efficacité synaptique de la voie corticospinale depuis un même groupe de neurones corticospinaux.

Les mécanismes de potentialisation ou dépression des connexions synaptiques ont été étudiés par des stimulations électriques dans des tranches de cortex moteur du rat. Une stimulation électrique à haute fréquence augmente la force d'une connexion en induisant une potentialisation alors qu'à basse fréquence elle diminue la force en induisant une dépression (Hess et Donoghue 1996). Plus précisément, une stimulation électrique tétanique dans les couches II-III ou V du cortex moteur induit une potentialisation dans les connexions intracorticales horizontales ou dans les connexions intracorticales verticales (Aroniadou et Keller 1993 ; Hess et al. 1994). La manipulation pharmacologique a montré que les mécanismes de potentialisation et dépression dépendaient des récepteurs NMDA (Hess et al. 1994 ; Keller 1993). L'utilisation d'antagonistes des récepteurs NMDA et non-NMDA a démontré que cette potentialisation nécessite l'intégrité des récepteurs NMDA et du glutamate. D'autre part, cette potentialisation semble également nécessiter la levée de l'inhibition, car elle est difficile à induire en absence d'un antagoniste du récepteur GABAa dans le cortex moteur (Hess et al. 1994, 1996 ; Hess et Donoghue 1994).

Quelques évidences indirectes provenant des études chez l'homme suggèrent des changements d'efficacité synaptique lors d'une altération périphérique. Ziemann et al. (1998b) ont montré, que l'ingestion de Lorazepam, un agoniste des récepteurs GABAa,

empêche l'augmentation de l'efficacité corticospinale et la diminution de l'inhibition intracorticale survenant à la suite d'un blocage ischémique du bras. De plus, ils ont également montré que l'ingestion seule de Dextromethorphan, un antagoniste non compétitif du NMDA (récepteur du Glutamate), empêche la diminution de cette inhibition intracorticale. Ces résultats suggèrent donc une diminution de l'activité des interneurones GABAergiques corticaux qui désinhibent les neurones corticospinaux et parallèlement une potentialisation des connexions intracorticales horizontales et verticales via les récepteurs NMDA qui exciteraient les neurones corticospinaux.

Ces mécanismes de potentialisation et dépression permettraient donc d'augmenter ou diminuer l'efficacité synaptique des nouvelles connexions intracorticales réveillées par la levée de l'inhibition GABAergique intracorticale. Bien qu'il n'existe pas d'évidence claire, ces mécanismes de potentialisation et dépression pourraient également modifier l'efficacité synaptique entre les neurones corticaux et les interneurones et motoneurones spinaux à la suite d'une altération du système nerveux périphériques.

Plasticité corticale et spinale à la suite d'une dénervation cutanée chronique du bout de la patte chez le chat

Il existe une abondante littérature sur la réorganisation des représentations corticales après différents types d'altération transitoire ou chronique du système nerveux périphérique chez différentes espèces. Cependant les informations portant sur les

changements d'excitabilité corticale et spinale, qui proviennent majoritairement des études chez l'homme, sont discutables selon le type d'altération périphérique étudié.

L'amputation ne constitue pas un modèle adéquat, car les changements rapportés dans le niveau d'excitabilité corticale résultent souvent de la comparaison du côté amputé versus non-amputé dans le meilleur des cas, quant ils ne résultent pas de la comparaison avec un groupe de sujets sains dans les études chez l'homme et le primate.

Le blocage ischémique du bras ou de la jambe, bien qu'il permette une mesure plus objective du changement d'excitabilité de la voie corticospinale, ne constitue qu'une amputation artificielle transitoire, puisque les effets du blocage, c'est à dire la désensibilisation, se font sentir au bout de 20 à 25 minutes et sont réellement efficace sur une durée de 5 minutes maximum (Commentaires et expérience personnelle du Dr Allan Smith).

Bien que les amputations et les blocages ischémiques d'un membre chez l'homme soient loin de notre modèle de dénervation cutanée chronique du bout de la patte chez le chat, les résultats de ces expériences suggèrent des changements d'excitabilité corticale en absence de changements spinaux.

Nous testerons l'hypothèse de recherche, selon laquelle la dénervation cutanée chronique du bout de la patte chez le chat entraîne une augmentation du niveau d'excitabilité corticale et une absence de changements dans l'excitabilité de la moelle épinière. Si cette hypothèse est confirmée, elle suggèrera que le cortex moteur contribue à la récupération fonctionnelle de la marche à la suite d'une dénervation cutanée

chronique du bout de la patte. Cette étude sera l'objet de notre troisième papier (Bretzner et Drew 2005c).

Objet de la thèse et résumés

L'objet de cette thèse est d'étudier la contribution et la plasticité du cortex moteur et des afférences cutanées lors de la marche du membre postérieur chez le chat.

Le premier chapitre de cette thèse s'intéresse à la contribution du cortex moteur durant la marche du membre postérieur. Cette étude testera deux hypothèses. H(1) : le cortex moteur peut modifier l'amplitude et la durée d'activité musculaire du membre postérieur et cela de manière spécifique. H(2) le cortex moteur peut modifier le rythme locomoteur du membre postérieur de manière semblable à celui du membre antérieur. Cette première étude constituera un premier article qui sera soumis au Journal of Physiology (Bretzner et Drew 2005a).

Le deuxième chapitre de cette thèse s'intéresse à la modulation corticale des réflexes cutanés durant la phase de balancement du cycle de marche. Nous testerons l'hypothèse selon laquelle le cortex moteur est capable de modifier l'amplitude des réflexes cutanés lors de la marche du membre postérieur, et cela de manière spécifique. Cette deuxième étude constituera un deuxième article qui sera soumis au Journal of Neurophysiology (Bretzner et Drew 2005b).

Le troisième chapitre de cette thèse s'intéresse à la contribution du cortex moteur et des réseaux interneuronaux spinaux dans la récupération fonctionnelle de la marche à la suite d'une dénervation cutanée chronique du bout de la patte postérieure. Nous

testerons l'hypothèse selon laquelle la dénervation cutanée chronique du bout de la patte chez le chat entraîne une augmentation du niveau d'excitabilité corticospinale et spinale. Cette troisième étude constituera un troisième article qui sera soumis au Journal of Neurophysiology (Bretzner et Drew 2005c).

En tant que premier auteur de ces trois manuscrits, j'ai contribué aux travaux (préparation du matériel d'enregistrement et de stimulation, chirurgies, recueillement et analyse des données), à l'évolution des hypothèses initiales de recherche, ainsi qu'aux versions originales et avancées de ces trois articles scientifiques.

Article # 1

Contribution of the motor cortex to the structure and the timing of hindlimb locomotion in the cat: a microstimulation study

by

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Abstract

We used microstimulation to examine the contribution of the motor cortex to the structure and timing of the hindlimb step cycle during locomotion in the intact cat. Stimulation was applied to the hindlimb representation of the motor cortex in a total of 34 sites in 3 cats using either standard glass-insulated microelectrodes (16 sites in 1 cat) or chronically implanted microwire electrodes (18 sites in 2 cats). Stimulation at just supra-threshold intensities with the cat at rest produced multi-joint movements at most sites (21/34, 62%) but also evoked responses restricted to a single joint, normally the ankle, at the other 13/34 (38%) sites. Stimulation during locomotion normally evoked larger responses than the same stimulation at rest and frequently activated additional muscles. Stimulation at all 34 sites evoked phase dependent responses in which stimulation in swing produced transient increases in activity in flexor muscles while stimulation during stance produced transient decreases in activity in extensor muscles. Stimulation with long (200 ms) trains of stimuli in swing produced an increased level of activity and duration of flexor muscles without producing changes in cycle duration. In contrast, stimulation during stance decreased the duration of the extensor muscle activity and initiated a new and premature period of swing, resetting the step cycle. The results show that the motor cortex is capable of exerting independent control over hindlimb activity during locomotion that is similar to that seen for the forelimb.

Introduction

There is abundant evidence from lesion (Adkins et al. 1971; Chambers and Liu 1957; Liddell and Phillips 1944; Kuypers 1963), single unit recording (Amos et al. 1990; Armstrong 1986; Beloozerova and Sirota 1993; Drew 1988, 1993) and intracortical microstimulation (ICMS) (Armstrong and Drew 1985b; Rho et al. 1999) studies that the motor cortex makes an important contribution to the control of the forelimb during locomotion in cats, particularly in situations that require a fine control over paw placement or limb trajectory.

Less is known concerning the nature of the cortical contribution to the control of the hindlimb during locomotion, although it is clear, especially in humans, that the integrity of the motor cortex is important. For example, motor cortical infarct (Knutsson and Richards 1979) or interruption of the corticospinal pathway (Nathan 1994) each leads to severe paresis or even paralysis of the hindlimb, making walking difficult or impossible. Moreover, transcranial electrical and magnetic stimulation (Capaday et al. 1999; Petersen et al. 1998, 2001; Schubert et al. 1997) and imaging (Fukuyama et al. 1997; Hanakawa et al. 1999; Malouin et al. 2003; Miyai et al. 2001) studies, all support a contribution of the motor cortex to human locomotion.

Damage to the corticospinal system in non-human primates, cats and rats (Bucy et al. 1966; Chambers and Liu 1957; Eidelberg and Yu 1981; Jiang and Drew 1996; Laursen and Wiesendanger 1966; Lawrence and Kuypers 1968; Metz et al. 1998; Muir and Wishaw 1999; Schucht et al. 2002; Vilensky et al. 1997) produces only transient deficits in hindlimb locomotor behaviour over a flat surface. Under more challenging circumstances,

however, the importance of the motor cortex for ensuring appropriate hindlimb function is evident (Drew et al. 1996; 2002; Schucht et al. 2002). In addition, the few single unit recording studies that have examined discharge characteristics of neurons in the hindlimb representation of the motor cortex have shown that neurons increase their discharge rates during tasks in which cats are required to step over obstacles attached to a treadmill belt (Drew et al. 2002; Widajewicz et al. 1994) as well as during rhythmical or discrete voluntary movements of the hindlimb in the primate (Neafsey 1980; Sahrmann et al. 1984).

While there is evidence that the motor cortex contributes to the control of hindlimb locomotion, the nature of that contribution remains unclear, particularly with respect to its relative strength and specificity. For instance, although several studies suggest a differential contribution to the control of the proximal and distal limb in non-human primates (Hatanaka et al. 2001; Wise and Tanji 1981;) and rats (Donoghue and Wise 1982; Neafsey et al. 1986), one of the major studies in the cat reports that the motor cortex exerts a global influence on the hindlimb in its entirety (Nieoullon and Rispal-Padel 1976). Moreover, although stimulation of the motor cortex is able to reset the locomotor rhythm in reduced preparations (Degtyarenko 1993; Leblond et al. 2001; Orlovsky 1972), there is little indication whether this is so in the intact animal or whether the nature of the resetting is similar to that observed during stimulation of the forelimb representation of the motor cortex (Rho et al. 1999).

Clarification of these issues is important as most locomotor studies concentrate on the hindlimb because of the greater accessibility of lumbar spinal circuits (Rossignol 1996). Similarly most studies on reflex pathways, even in the intact animal, concentrate on the hindlimb (Pearson et al. 1999; Rossignol et al. 1988; Wolpaw et al. 1993). Moreover, the

fact that the hindlimbs and the forelimbs are used in a very similar manner in quadrupedal locomotion provides an opportunity for a comparative examination of the relative contribution to the different limbs that is not available in other tasks in other species, and especially in primates. We therefore used ICMS to examine the contribution of the motor cortex on the structure and timing of the pattern of electromyographic (EMG) activity observed in the hindlimbs during treadmill locomotion. The results suggest that in all important aspects the cortical contribution to regulation of the hindlimb during locomotion is similar to that for the forelimbs.

Methods

Care and training

Experiments were carried out on five male cats (weights 4.2-5.5 kg) trained to walk at a comfortable and constant speed (*circa* 0.35-0.45 m/s) on a treadmill. Cats were carefully selected on the basis of their willingness to walk for uninterrupted periods of ~ 20 minutes.

Surgical Procedures

In two cats (MC23 and 24), microwire electrodes were chronically implanted into the hindlimb representation of the motor cortex. Anesthesia was induced with a mix of ketamine hydrochloride (30 mg/kg im) and acepromazine maleate and was maintained with additional doses of ketamine hydrochloride (5 mg/kg iv) as needed to maintain a constant heart rate and a lack of corneal reflex. Microwire electrodes (Tri-ML insulated stainless steel: 25 μm diameter) attached to a miniature connector (Neuralynx: EIB27) were manually inserted, one at a time, into the posterior bank of the cruciate sulcus that contains the hindlimb representation of the motor cortex (Armstrong and Drew, 1984b; Nieoullon and Rispal-Padel, 1976; Widajewicz et al., 1994). Appropriate positioning of the microwires was facilitated by recording neuronal activity and applying ICMS as the wires were inserted. The cortex was covered with a hemostatic material (Sterispon) and the microwire connector was attached to the cat's cranium with dental acrylic. Penicillin (Novopharm) (40000 UI/kg iv) and analgesics: buprenorphine hydrochloride (5 μg/kg) were provided at the beginning and at the end of each surgery, and for at least 48 hours

following surgeries. Antibiotics (cephadroxil: 100-200 mg/day) were administrated daily for the duration of the experiment.

One to two weeks after recovery from the initial surgery, the cats were anesthetized with Isoflurane (2-3% with oxygen) to complete the surgical procedures. At this time, a recording chamber was positioned over the motor cortex (Drew 1988, 1993; Widajewicz et al. 1994) in one additional cat (MC25) to allow comparison of the responses evoked with tungsten microelectrodes with those evoked by cortical microwires. In 4 cats (MC24-27), microwires were implanted in the pyramidal tract at P7 (Drew, 1993) to allow comparison with the responses evoked by ICMS. In all cats (MC23-27), multiple pairs of Teflon-insulated, braided stainless steel wires were implanted into selected muscles of the fore- and hindlimbs to record EMG activity. In the forelimb, electrodes were implanted bilaterally into the cleidobrachialis (ClB), protactor of the shoulder and flexor of the elbow and into the triceps brachii, lateral head (TriL), extensor of the elbow. In the hindlimb, electrodes were also implanted bilaterally into: extensor digitorum brevis (EDB), dorsiflexor of the hindpaw digits; extensor digitorum longus (EDL), dorsiflexor of the digits and flexor of the foot; lateral and medial heads of the gastrocnemius (GL and GM), extensors of the ankle; anterior head of the sartorius (Srt), a hip flexor; semitendinosus (St), a knee flexor; tibialis anterior (TA), an ankle flexor; and vastus lateralis (VL), a knee extensor. In some cats, electrodes were additionally implanted into the following muscles contralateral to the cortical stimulation sites: flexor digitorum longus (FDL) (MC23-24), ventroflexor of the digits and extensor of the foot; and soleus (Sol) (MC25-27), extensor of the ankle. Recovery and post-operative procedures were as before.

All surgical and experimental procedures followed the recommendations of the

Canadian Council for the Protection of Animals and were approved by the local ethics committee.

Protocol

In the cat with a recording chamber, the electrode was slowly advanced into layer V of the motor cortex which was identified by the presence of neurons that were antidromically activated by pyramidal tract stimulation (Armstrong and Drew, 1984a). Intra-cortical microstimulation (cathodal current, 11 pulses at 330 Hz, pulse duration 0.2 ms, ≤ 35μA) was then applied while the cat was held gently in a prone position to test the threshold and the nature of the evoked responses in the contralateral hindlimb. Evoked EMG responses were digitized on-line at a frequency of 5 kHz for 25 ms before and 150 ms after the onset of the stimulus train. EMGs were band-pass filtered between 100 Hz and 3 kHz.

The animal was then placed onto the treadmill to assess the effects of microstimulation during locomotion. Trains of stimuli (identical to those used for stimulation at rest) were delivered at a range of current strengths of 5 to 35 μA (5 μA steps) at a delay of 50 ms following the onset of activity in the sartorius (i.e. at swing onset). Subsequently trains of stimuli at 25 μA were applied at different times throughout the step cycle. Ten to fifteen repetitions were made at each delay in the following order: 50-150-300-500-700-900-0-100-200-400-600-800 and 1,000 ms after the onset of the activity in the Srt. All responses were recorded and digitized on-line as above. In addition, a continuous record of the EMG activity during locomotion was also digitized at 1 kHz. Lastly, longer trains of stimuli (200 ms duration) were delivered to determine the cortical

contribution to the cycle timing. Trains of stimuli were applied in every fifth step cycle at each delay (5 repetitions at each stimulus delay) using the same order as detailed above.

Penetrations were made in a grid pattern separated by a minimum of 0.5 mm in both the anteroposterior and mediolateral planes. To aid histological reconstruction, small electrolytic lesions (20 μA, DC cathodal current) were made in layer V in selected penetrations.

A similar recording protocol was used for cats with microwires chronically implanted in the motor cortex and in the pyramid. For some of these cortical microwires, it was possible to record antidromically activated cells in layer V. However, as these wires could not be adjusted to ensure that they were in the optimal location, we did not use a constant intensity for these experiments but rather adjusted the strength of the stimulus to a level that evoked clear responses in flexor muscles during the swing phase of locomotion.

Data analysis

Data were analyzed as previously described (Rho et al., 1999). In brief, the data obtained with the cat at rest were computer-rectified and averaged. The onset and offset of cortically evoked EMG responses were determined manually using the interval of confidence ($P<0.01$) of the standard error of the mean (SE) of the prestimulus period as a guideline. The amplitude of the net evoked responses was computed by subtracting the area of the prestimulus EMG responses (for an identical period of time) from the selected evoked EMG responses. The latency, duration and amplitude of the net evoked responses were computed and plotted.

For the data in which short trains of stimuli were applied during locomotion, control

and stimulated cycles were manually identified in reference to the onset of the Srt. The step cycle was subdivided into ten equal phases (groups) synchronized on the onset of the sartorius. The responses evoked by stimuli in each phase were averaged and plotted on a display monitor. The average activity from a similar time period taken from unstimulated cycles was superimposed on this display (Drew and Rossignol, 1984). The onset and offset of the response was determined manually using the interval of confidence ($P<0.01$) of the standard error of the mean of the control activity during the identical phase of the unstimulated cycles as a guideline. Evoked responses were included in the analysis if their latency was $\leq$ 50ms and their duration exceeded 5ms. The initial change in activity, regardless of sign, is referred to as a primary response. Additional changes, following the primary response, are referred to as secondary responses. At phases of the step cycle at which longer latency responses were evoked in the absence of an initial response, we continue to refer to this response as a secondary response. For example, in Fig. 2B, a short latency increase in activity is evoked in EDB in late stance while only the longer latency decrease in activity is observed in early stance: the latter is, therefore, referred to as a secondary response.

For the statistical analysis, the net amplitude of the evoked responses was computed from the individual traces. For this analysis we used the latencies measured from the averages to define a window for the response evoked in each EMG and for each group. The magnitude of the responses was then calculated from each single trial using these windows: a similar period was measured from the control cycles. Subtracting the control value from that of the stimulated cycle provided the net amplitude. These values were then averaged within each group to provide the mean amplitude as well as the standard deviation (SD) of

the mean.

For the experiments in which long trains of stimuli were applied during locomotion (MC24-27), the onset and offset of selected flexors and extensors was measured during the stimulated step cycle and during the two steps before and after that cycle. The phase at which the stimulation was applied was computed as described above, and the stimuli were grouped into 10 equal phases. We then calculated the average duration of each of the stimulated step cycles, as well as the duration and integrated amplitude of the EMG bursts occurring before, during and after the stimulus. Step cycles and EMG bursts occurring before the stimulus were used as controls.

Histology

At the end of the experimental manipulations, the cats were deeply anaesthetized with sodium pentobarbitol (Somnotol 40 mg/kg) and perfused *per cardium*. The brains were sectioned and stained with cresyl violet.

Results

Database

Short trains of intracortical microstimulation (ICMS) were applied throughout the hindlimb representation of the motor cortex using either microwires (n=18 sites) or glass-insulated tungsten electrodes (n=16 sites). Stimulation with the cat held gently on the experimenter's lap and with the hindlimb unsupported evoked brief, twitch movements involving one or more joints of the hindlimb at all 34 sites (Table I). At just supra-threshold current intensities, the most common effect was a brief movement of two joints (18/34 sites: 53%) normally the knee and the ankle. In a relatively high proportion of sites (13/34: 38%) stimulation at low intensities evoked responses around a single joint, most frequently the ankle (10/13: 77%). At the other sites (3/34, 9%), the stimulation evoked movement of all three joints (hip, knee and ankle).

Histology

Most of the stimulated sites were located within the caudal bank of the cruciate sulcus. Fig. 1A illustrates tracings of two histological sections taken from cat MC25 showing electrode penetrations terminating in layer V within the caudal bank of the cruciate sulcus. The approximate location of all 16 sites at which stimulation was applied in this cat are illustrated in the pseudo-3D representation of Fig 1B (see Widajewicz et al. 1994). Similar regions of the motor cortex were stimulated in cats MC23 and MC24, as illustrated in Figs. 1C and D.

Short trains of ICMS during locomotion

Phase-dependent nature of the responses

Figure 2 illustrates representative examples of the effects evoked by ICMS at different phases of the locomotor cycle via a microwire (current strength 35 μA: Fig. 2A and 2B) and a tungsten microelectrode (current strength 25 μA: Fig. 2C). Each of the microelectrodes was verified to be in layer V of the cortex by the presence of neurones discharging antidromically to stimulation of the pyramidal tract. Stimulation through the cortical electrodes during the swing phase (Figs. 2B-C: top) evoked short latency primary responses in the flexor muscles, St and TA and was without effect in the EDB and the GM. In contrast, during the early and late stance phases, primary responses were absent or smaller in St and TA and the stimulation produced a clear decrease in the level of activity of the GM that was more pronounced in late stance; there was also a long-latency, secondary decrease in activity in the TA in the example in Fig. 2C (early stance). The responses in the EDB were more complex. Clear primary increases in activity were seen in late stance in the example in Fig. 2B and in early stance in the example in Fig. 2C; there was also a weak increase in the level of activity in the EDB in early stance in the example in Fig. 2B (not significant) that was followed by a secondary decrease.

The phase-dependant nature of the EMG responses evoked in these four muscles, as well as in several others recorded in the same experiments, is illustrated in Figure 3. Maximal responses in the hip, knee and ankle flexor muscles, St, TA, EDL and Srt were observed during the swing phase (phases 0.1 to 0. 3) and at the end of stance (phases 0.9 - 1.0) (Fig. 3A-C). In the paw dorsiflexor, EDB, increased responses were maximal at the end

of swing (phase 3) and again in late stance (phases 0.8-1.0) (Fig. 3D). Secondary, longer latency decreases in EMG activity were evoked in EDB, EDL and Srt during the swing phase. In extensor muscles, primary increases in activity were observed in the GM and GL at the end of the swing phase (Fig. 3E), and decreases in activity were observed during stance in all four recorded extensor muscles (Fig. 3E-F).

Figure 4 illustrates that the responses evoked by the stimulation were reproducible from cat to cat and were independent of the method used to stimulate the motor cortex. This was especially clear for the primary responses. As for the example illustrated in Fig. 3, primary increases in the flexor muscles St, TA, EDL and Srt were always present during the swing phase and at the end of stance (≥ 91% of cortical sites; Table II). The responses in EDB were more variable. In MC23 and MC24 they were observed during both swing and stance while in MC25 they were predominant in late swing. This difference in response is probably related to the difference in the pattern of activity in the EDB as indicated by the rectangles above the graphs. In the illustrated extensor GM, as well as in all other extensor muscles (FDL, VL, GL, Sol), the predominant effect was a decrease in activity during the stance phase. Increases in activity were seen in some sites at the end of swing and occasionally, as in MC25, during early stance..

Latencies

The latencies of the responses evoked at different phases of the step cycle are summarized in Table II. In the flexor muscles, primary increased responses were always evoked at short latency (average values ≤ 16 ms) during the swing phase. When responses were evoked in flexor muscles during stance, responses were always smaller and at longer

latency than those evoked during swing.

In the extensor muscles, the primary increased responses evoked during swing were at longer latencies (≥ 21 ms) than those evoked in flexor muscles. During stance, the averaged latency of the primary decrease in activity ranged from 19.7 ms in FDL to 31.8 ms in Sol.

Responses in both flexor and extensor muscles were generally evoked at longer latency at rest than during locomotion.

Effects of current strength

Figure 5 illustrates the effect of modifying the current strength on the cortically evoked EMG responses during the swing phase (Group 1) of the step cycle. In the illustrated example, stimulation of the motor cortex through a tungsten electrode at the standard intensity of 25 μA (Fig. 5A: *middle*) activated muscles acting around all of the major joints of the hindlimb. At 10 μA similar activation patterns were evoked in the three illustrated flexor muscles but there was no longer a significant response in the VL. At 35 μA, the amplitudes of the evoked responses were increased in all four illustrated muscles. As shown in Fig. 5C (*left*), at this site all of the recorded flexor muscles were recruited at the lowest strengths used, while extensors were recruited at slightly higher intensities. Subsequently, as stimulus strength was increased there was a relatively monotonic increase in the amplitude of the evoked responses in all of the muscles over the limited range of intensities that we employed.

Similar effects were seen for stimulation through microwire electrodes. For example, the responses evoked in the example illustrated in Fig. 5B showed a very similar

pattern of activity to that described for stimulation through the tungsten microelectrode, even though the stimulus intensity was relatively greater. As illustrated in the graphs of Fig. 5C (*middle and right*), the relationship between stimulus intensity and response magnitude for the microwire electrodes was similar to that observed for the tungsten microelectrode (Fig. 5C: *left*). There was a tendency for the responses evoked through the microwires to reach a plateau at the highest intensities, likely explained by the greater range of intensities used with the microwires.

The percentage of recorded, contralateral flexor and extensor muscles recruited at different intensities of ICMS during the swing phase of the step cycle is summarised in Fig. 5D for the 3 cats. In cat MC25, in which ICMS was applied through tungsten electrodes, stimulation at 15 μA recruited almost 75% of the total complement of flexor muscles while stimulation at 25 μA recruited 95%. In other words, stimulation at 25 μA was sufficient to recruit nearly all of the flexor muscles that we recorded in all 16 sites that were stimulated. Similar results were observed for the microwire stimulation in cat MC23 in which 75% of flexor muscles were recruited at 25 μA and there was only a slight increase after this (note that some flexor muscles in some sites were not activated even at 150 μA). The results from cat MC24 were slightly different in that there was a relatively large jump in the frequency of flexor muscles recruited at 50 μA (57%) to the proportion recruited at 75 μA (82%). The differences between these two cats was mainly caused by the fact that very few responses were evoked in the Srt from any of the stimulated sites in cat MC24.

Specificity of the responses evoked by ICMS

Although a number of cortical sites produced movement only at a single joint, most

sites produced movement at multiple joints (Table I). Moreover, even in sites in which movement was produced at a single joint, the stimulation frequently activated multiple muscles. However, even though several muscles were activated, the relative amplitude of the responses evoked in different muscles varied according to the stimulated site. For instance, Fig. 6 illustrates the responses evoked in four hindlimb flexor muscles by stimulation of three different sites (columns 6A-C respectively) distributed within the hindlimb representation of the motor cortex from the same cat (MC25). At rest, stimulation at 25 μA at cortical site A evoked a flexion of the hip, at site B the same stimulation evoked a strong knee flexion, while at site C it evoked a weak flexion of the ankle. At all 3 sites threshold was between 10 - 15 μA. These mechanical effects were largely reflected in the evoked responses recorded from the illustrated EMGs. Stimulation of site A evoked a response only in the Srt while stimulation at site B evoked strong responses in the St, together with relatively weaker responses in Srt and TA. Stimulation at site C evoked no response in the St but weak responses in Srt, TA and EDB; the latter muscle was not activated from either of the other two sites when the cat was not walking.

Stimulation of these same sites during locomotion evoked responses that were not as clearly related to the mechanical effects that we identified in the resting cat. While stimulation at site A still activated Srt, it also evoked responses in the St and the TA. At site B, where the responses in St were relatively much larger than in Srt and TA at rest, the responses during early swing were of almost the same magnitude. At site C, stimulation in early swing evoked a weak response in St that was not observed when the cat was at rest and had no effect on EDB. In contrast, stimulation in early stance, when the EDB muscle was active, evoked a strong response in the EDB at site C, as well as in site B, although not

at site A.

Thus, while at rest, the mechanical effects of the microstimulation were relatively well reflected in the relative magnitudes of the evoked responses, locomotion produced changes in excitability that tended to mask these differences.

Short trains of stimuli in the pyramidal tract during locomotion

The responses evoked by stimulation of the pyramidal tract (PT) are plotted in Fig. 7. In general, stimulation of the PT evoked phase-dependant responses in all muscles that were similar to those evoked from the more discrete stimulation of individual cortical sites (compare with Fig. 4). Responses in flexor muscles were largest during swing and responses in extensors were increased at the end of swing and decreased during stance. The only discrepancy that was observed was the lack of any response in St during the swing phase of locomotion from stimulation of the PT in cat MC24. The reason for this discrepancy is not known.

Stimulation of the motor cortex and the pyramidal tract with long trains.

Long trains of stimuli during different phases of the locomotor cycle were applied in 12/16 cortical sites in cat MC25 and 5/8 cortical sites in cat MC24. During swing, the stimulation invariably produced a hyperflexion of the contralateral hindlimb, while the same stimulation during stance was either without a visible behavioural effect or produced a curtailment of stance and initiated a premature flexion of the hindlimb. In the representative example illustrated in Fig. 8A, stimulation during swing and late stance produced increases in the amplitude and the duration of both Srt (Fig. 8D) and St but had

no effect on the overall duration of the step cycle at these times (Figs. 8B and C). The same stimulation during stance (Fig. 8A: *middle and bottom*) led to a significant decrease in the duration of the extensor muscle, GM (Fig. 8E), and the initiation of a new burst of activity in the St and the Srt. This caused a significant decrease in the duration of the step cycle (to ~70% of control values) which was reset by the stimulation (compare subsequent cycles in Fig. 8A: *middle and bottom*).

Similar effects on the duration of the step cycle and of selected EMGs were seen from other sites in this cat (Fig. 9A) as well as in the other cat, MC24 (Fig. 9B). Stimulation of the motor cortex in swing invariably produced an increase in the duration of Srt (Fig. 9A) that ranged up to 179% of the control (Table III). Similar increases were seen in the other flexor muscles (not illustrated). During stance, the stimulation, when effective, produced a decrease in the duration of the GM of up to 30% (i.e. decreased to 70% of control duration, Table III). Stimulation in 1 site only produced a significant increase in cycle duration during swing but stimulation in 12/17 sites produced a significant decrease in cycle duration ranging from 74 -90% of control (mean 85% of control) in stance. Similar, although slightly weaker, effects were also seen from stimulation of the PT (Fig. 9C and Table III).

Discussion

This study details the effects of stimulation of the hindlimb representation of the motor cortex on locomotion in the intact cat. The results show that the motor cortex has a similar capacity to modulate locomotor activity in the hindlimb as in the forelimb. In particular, short trains of stimuli evoked phase-dependent responses in all recorded muscles while longer trains of stimuli reset the hindlimb step cycle. The results also support the view that the motor cortex is able to differentially regulate activity around different joints of the hindlimb as opposed to exerting a global influence over the hindlimb as a whole.

General Considerations

Most of these experiments were performed with the microwire electrodes used in our current studies of the long-term plastic changes in corticospinal efficacy that occur following cutaneous denervation of the hindpaw (Bretzner and Drew, 2003). As with most experiments in which microwires are used, one of the limitations of this method is the inability to modify the location of the electrode once it has been implanted. In contrast to experiments using moveable microelectrodes it is, therefore, not possible to displace the electrode to ensure that the tip is located in the optimal region for eliciting motor activity, namely within layer V (Asanuma 1975; Asanuma and Sakata 1967; Porter and Lemon, 1993). Nevertheless, some of the microwire electrodes used in this study were positively identified to be within layer V by the presence of antidromic activity produced by stimulation of the pyramidal tract and most others were identified as being in the gray matter by the presence of multiunit neuronal activity. Moreover, there was no qualitative

difference between the results obtained with stimulation of these microwire electrodes and those obtained from the experiments with a moveable tungsten microelectrode. The more elevated thresholds obtained for the microwire electrodes are to be expected based on the inability to specifically localise them and the larger exposed areas of these electrodes compared to glass-insulated tungsten microelectrodes (Armstrong and Drew 1985a). The following discussion therefore makes no distinction between the results obtained using the two different types of electrode.

Phase-dependence of the evoked responses

Stimulation of the hindlimb representation of the motor cortex led to clear phase-dependent responses in which stimulation during swing evoked increased activity in flexor muscles, but had relatively minor effects in extensor muscles, while stimulation in stance had little effect on flexor muscles and generally decreased activity in the extensor muscles. The major exceptions to this generalization were the increased responses that were frequently seen in the extensor muscles at the end of swing and the onset of stance and the complex pattern of activity in the EDB. Indeed, responses in the latter muscle varied in different cats and seemed to depend on the exact pattern of activity in the muscle during locomotion (Fig. 4). This pattern of activity is very similar to that observed in the Extensor Digitorum Communis muscle in the forelimb (Rho et al. 1999) and suggests that the pattern of activity, and excitability, of some of the distal muscles is more flexible than that of more proximal muscles.

Overall, the pattern of responses that was observed in hindlimb flexor and extensor muscles in this study was similar to that detailed for the forelimb in previous studies

(Armstrong and Drew 1985b) suggesting that the cortex has a similar capacity for modulating the activity of hindlimb muscles during locomotion as for the forelimbs. It has previously been suggested that most of the phase-dependence from cortical stimulation is the result of the rhythmic changes in excitability of the interneuronal networks in the spinal cord that are activated by the descending corticospinal volley (Rho et al. 1999). None the less, there is also the possibility that changes in cortical excitability produced by step related changes in discharge activity, may also influence the magnitude of the evoked responses (Petersen et al. 1998). However, the finding that the responses evoked by stimulating the PT, which should be relatively uninfluenced by changes in cortical excitability, were almost identical to those obtained from stimulating the motor cortex supports the contention that the phase-dependency is determined more by the state and excitability of the spinal interneuronal pathways than on the excitability of the cortex itself. This does not imply that changes in cortical excitability do not contribute to this process, only that the excitability changes in spinal circuits are sufficient to produce the phase-dependent response.

Reset of the step cycle

In order to compensate for unexpected changes in the environment, animals must be able to modify the timing of the step cycle in order to lengthen or shorten stride and, in extreme conditions, they must be able to interrupt, or reset, the step cycle and start a new one. Previous studies have shown that stimulation of the forelimb representation of the motor cortex is able to produce such changes in the forelimb in intact animals (Armstrong and Drew 1985; Rho et al. 1999). The present results show that the hindlimb representation

of the motor cortex has a similar, although somewhat reduced, capacity to reset the hindlimb cycle. In previous studies in the lab in which we stimulated the forelimb representation of the motor cortex, stimulation during the stance phase of the step cycle could shorten the cycle by up to 38% (mean 65%). Indeed, in that study, 17/32 of the sites produced a reduction of the step cycle that was greater than 25% while in the present study the greatest reduction that we observed was 26 % and the mean reduction was 15%. Although it is possible that this reduced capacity for modifying the cycle might be due to the limited number of sites that we examined, examination of the data in Fig. 9 and comparison with Fig. 10 in Rho et al. (1999) suggests an overall reduction in the capacity of the motor cortex to modify hindlimb cycle duration. It is possible that the further reduction in hindlimb cycle duration that would be needed to ensure appropriate coordination between the fore- and hindlimbs might be contingent on simultaneous activation of the forelimb regions of the motor cortex.

The results obtained from this cortical stimulation differ from some of those obtained in reduced cat preparations in which, by definition, only the PT could be stimulated. Although our results agree with those obtained in thalamic and mesencephalic cats walking on a treadmill (Orlovsky, 1972) as well as those obtained in the fictive preparation by Degtyarenko et al. (1993), they are somewhat different from some of those reported by Leblond et al. (2001). In particular, while stimulation during the swing phase in the intact cat always evoked increased flexor activity in our study, as it did in the studies by Orlovsky (1972) and Degtyarenko et al. (1993), Leblond et al. (2001) found stimulation sites that curtailed the flexion phase and initiated an extension. The reason for these differences is not clear. However, it is not likely to be a difference between stimulation of

the motor cortex and of the PT as stimulation of the PT in the intact cat produced similar results to those obtained from cortical stimulation. Moreover, Orlovsky and Degtyarenko also obtained their results from stimulating the PT. One possibility is that the location of the electrodes in the PT might be a factor. Leblond et al. (2001) specifically looked for areas producing an extensor bias. In our study, the PT electrodes were placed stereotaxically and fixed in place. However, we emphasize that similar results were obtained from all 17 electrodes in 2 cats and that none of these sites caused a curtailment of flexor activity. It is also possible that the lack of any phasic peripheral afferent feedback makes the spinal neuronal networks more open to perturbation from central sources. For example, stimulation of the reticular formation in the intact (Drew 1991) or decerebrate, walking (Drew and Rossignol 1984) cat has much less influence over step cycle timing that the same stimulation does during fictive locomotion (Perreault et al. 1994). Similar effects can also be seen from red nucleus stimulation which does not change cycle timing in the intact cat (Rho et al. 1999) but which does during fictive locomotion (Degtyarenko et al. 1993).

Specificity of the responses

Nieoullon and Rispal-Padel (1976) previously reported that stimulation of the hindlimb representation of the cat motor cortex evokes global effects in which the whole hindlimb is flexed, even at threshold levels. This is in contrast to the forelimb in which threshold stimulation more frequently produces movement around a single joint (Armstrong and Drew 1984; Nieoullon and Rispal Padel 1976). We also found that microstimulation at threshold levels frequently activated movement around 2 or more joints. However, we

also found several sites at which threshold stimulation activated only a single joint, much as is the situation with stimulation of the forelimb representation of the motor cortex. Even so, the proportion of sites eliciting activity around a single joint (38%) was reduced compared to the 60% reported for the forelimb by Armstrong and Drew (1984b), even though the latter were stimulating at a constant strength of 35 μA. Nevertheless, the results do show that the motor cortex can exert differential control over the hindlimb as in other species (see Introduction) and as for the forelimb. The differences between the results obtained here possible reflect differences in stimulus intensity (with respect to the study of Nieoullon and Rispal-Padel 1976) and differences in the regions of the hindlimb representation that were stimulated (mostly in the bank of the cruciate sulcus in this study compared to mostly superficially in the study of Armstrong and Drew 1984b). Indeed, most of the sites producing a movement around a single joint were found quite deep within the cruciate sulcus and, in most cases, single joint responses were restricted to the ankle and occasionally the knee. The more specific nature of the responses in this study is also in agreement with the results from the unit recording study of Widajewicz et al. (1994) showing neurones discharging in phase with the activity of different groups of muscles.

Some of the more overt specificity was lost during locomotion as the increased excitability of the spinal circuits resulted in increased recruitment compared to that seen in the resting cat (see. Fig. 6). Even in this situation, however, there were clear differences in the relative amplitude of the responses evoked in different muscles. This is seen in Fig. 6 which shows that in some sites the relative amplitude in muscles such as the TA and the Srt was relatively larger than that in St, and in others relatively smaller.

Nevertheless, it is clear that most sites will influence the activity of many muscles

acting around several joints. It is unlikely that this result is caused by excessive stimulus intensities as multiple responses were evoked from most cortical sites even at strengths of 10-15 μA and increasing stimulus intensity resulted in relatively low supplementary recruitment (see Fig. 5D). Given the relatively low spread of current that might be expected at these low intensities (Asanuma et al. 1968), even allowing for trans-synaptic activation, the results suggest that a restricted area of cortex will activate a large number of hindlimb muscles. This again is similar to the result obtained from stimulation of the forelimb representation of the motor cortex (Armstrong and Drew 1985a,b).

General Conclusions

It is clear that in the process of evolution, the motor cortex has become more and more associated with the control of the forelimb and particularly of the wrist and hand (Heffner and Masterton 1975; Kuypers 1981; Lawrence and Kuypers 1968; Nudo and Masterton 1990; Phillips 1986). Although this process is at its clearest in humans and non-human primates, there is also evidence showing that the corticospinal projections to the lumbar enlargements are smaller than those to the cervical enlargements in most species and, in some species are even absent (Heffner and Masterton 1975). In agreement with this, in most mammals, including the cat, the hindlimb representation of the motor cortex is normally smaller than that of the forelimb. Despite the difference in the overall magnitude of the projection, however, the results in this paper suggest that the nature of the contribution of the motor cortex to the control of the hindlimbs is qualitatively the same as that for the forelimbs. This is especially true for its capacity to modify the level of activity in a wide selection of muscles acting around different joints of the hindlimb and, moreover,

of its capacity to differentially modulate activity around these joints. The results also show that the motor cortex maintains a similar capacity to modulate cycle timing of the hindlimb as it does for the forelimb although there might be a quantitative difference in the strength of this capacity. As we have previously discussed (Widajewicz et al. 1994) this argues for an ability of the motor cortex to control the hindlimbs independently of the forelimbs. Overall, the results presented in this manuscript argue in favour of a substantial contribution of the corticospinal system to the regulation of locomotion in cats. It is highly likely that a similar contribution is made to the control of human locomotion, particularly in light of the devastating effects of motor cortical lesion on human locomotion.

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Figure Legends

Fig. 1: Histological reconstructions for the three cats used in this study. A: Tracings of two parasagittal sections from cat MC25 showing the reconstructions of 1 penetration in each section. The thinner, inner line illustrates the location of layer V. B: pseudo-3D representation of the motor cortex showing the approximate location of each of the 16 sites used in this animal. The arrows indicate the location of the sections illustrated in A while the squares indicate the location of the two stimulation sites indicated in A. C: representative tracings taken from cat MC24. D: location of all stimulated sites in MC23 (filled circles) and MC24 (open circles); arrows indicate location of the sections shown in C, squares indicate the location of the two stimulation sites. Abreviations: T: threshold.

Fig. 2: Representative examples of the responses evoked by short trains of stimuli applied to the motor cortex during locomotion via an implanted microwire (A,B) and by stimulation via a conventional glass-insulated tungsten microelectrode (C). A: Untreated data showing the EMG activity in selected muscles when stimuli were applied in swing and in early and late stance through the implanted microwire (current strength 35 μA). B: Average EMG responses, evoked by the implanted microwire, are illustrated by thick lines during swing (Gp1, n=57) and early (Gp4, n=13) and late (Gp 8, n=15) stance. Values in brackets indicate the average phase of the cycle at which the stimulus was applied. Average background EMG activity is illustrated by thin lines at the same phases in unstimulated cycles (n=424); dotted lines indicate the 0.01 confidence level of the standard error of the mean for the control data. Dotted line at time 0 in this and all other similar figures indicates

the time of stimulus application. C: Average EMG responses, evoked by the glass-insulated tungsten microelectrode (current strength 25 μA), at similar phases of the step cycle. Filled areas in B,C indicate the net evoked EMG responses that we measured as primary responses (black) and as secondary responses (grey). Amplitudes are arbitrary but constant for each muscle at each site. All illustrated muscles in this and all other figures are contralateral to the stimulation site. Abbreviations: EDB: Extensor Digitorum Brevis; GM: Gastrocnemius Medialis; Gp: group; Stim: stimulation; St: Semitendinosus; TA: Tibialis Anterior.

Fig. 3: Relative amplitude of the net evoked EMG responses for the muscles recorded in the experiment illustrated in Fig. 2A, B, as well as for other muscles recorded in the same experiment, plotted as a function of the step cycle. Data are plotted as a percentage of the maximal response evoked in each muscle; vertical bars indicate ± 1 standard deviation. Positive values are plotted as a percentage of the largest increase, negative values are plotted as a percentage of the largest decrease. Primary responses are shown as black lines and secondary responses as grey lines. Rectangles above each graph indicate the average period of activity of the recorded muscles calculated from 35-45 step cycles. Abreviations: EDL: Extensor Digitorum Longus; FDL: Flexor digitorum longus; GL: Gastrocnemius Lateralis; Srt: Sartorius; VL: Vastus lateralis.

Fig. 4: Summary of the responses evoked by motor cortical stimulation in three cats MC24 (A: N= 8 sites), MC23 (B: N= 10 sites) and MC25 (C: N= 16 sites) in five representative muscles. As in Fig. 3, data are plotted as a percentage of the maximal response in each muscle for each cortical site. The average duration of the EMGs (gray boxes) is calculated

from a minimum of 40 step cycles in either 4(MC23) or 5 experiments (MC24 and MC25).

Fig. 5: Effects of current strength on the responses evoked by short trains of stimuli applied to the motor cortex during swing via a conventional glass-insulated tungsten microelectrode (A) and via an implanted microwire (B). Scales for each muscle and for each electrode are constant for the different stimulus intensities. C: Amplitude of primary responses as a function of the current strength for the two examples illustrated in A (*left*) and B (*middle*), as well as for a site in cat MC23 (*right*). For cat MC25, the left ordinate (arbitrary units) is used for all flexor muscles while the right is used for the extensors, GL, GM and VL; for cat, MC24, the left ordinate indicates the scale of St, while the right one indicates that of all other muscles. The vertical dotted line in the graphs C indicates the current strength used when stimulation was applied throughout the step cycle in each cat. Scale for the ordinate is in arbitrary units. D: Percentage of flexors (upper panel) and extensors (lower panel) recruited as function of the current strength for the three cats illustrated in 5C.

Fig. 6: Representative examples of the responses evoked at rest *(top)*, swing *(middle)* and early stance *(bottom)* from three different cortical sites (A-C) in cat MC25 producing movement about the hip (A), knee (B) and ankle (C). Arbitrary units at the same scale for each muscle in each column.

Fig. 7: Summary of the responses evoked in representative muscles of the contralateral hindlimb by stimulation of the pyramidal tract in three cats MC24 (A), MC25 (B) and MC26 (C). As in Fig. 4, data are plotted as a percentage of the maximal response in each

muscle for each stimulation site.

Fig. 8: Representative examples of the responses evoked by long trains of stimuli (200ms) applied to a single site in the motor cortex during locomotion in cat MC25. A: Untreated data showing the EMG activity in selected muscles when stimuli, triggered on the onset of Srt (first vertical line), were applied in swing and early and late stance. The time at which the next periods of activity in the Srt would be expected is indicated by the second and third vertical dotted lines. B: Scatter plot illustrating the duration of the step cycle as a function of the phase of stimulation for individual cycles. C: phase plot of the average duration of the step cycle D,E: mean duration of the Srt and GM activity as a function of the phase of stimulation. Horizontal dotted line and gray band in B-E indicate the mean and standard deviation of the unstimulated, control, cycles (N=79). Asterisks in C-E indicate values significantly different from control ($p < 0.01$)

Fig. 9: Comparison of changes in cycle duration (top) and burst duration of representative hindlimb muscles (middle and bottom) evoked by long trains of stimuli applied within the motor cortex in two cats (A,B) and by stimulation of the pyramidal tract (C). All data are plotted as function of the percentage of the average values in unstimulated cycles (horizontal dotted line). Each line represents the results from stimulation at a single site.

Table I

Movements evoked by cortical microstimulation

Cat	1 joint	2 joints	3 joints
MC23 (10 sites)	4	5	1
MC24 (8 sites)	1	5	2
MC25 (16 sites)	8	8	0

The table indicates the number of joints that were moved by cortical microstimulation applied at current intensities that were just supra-threshold in 3 different cats.

Table II. Averaged latencies of EMG responses evoked during locomotion and at rest by stimulation of hindlimb loci

	Short trains during locomotion											
	Swing				Stance				At rest			
Muscle	Onset			(%)	Onset			(%)	Onset			(%)
Semitendinosus (+)	15.7	±	3.5	94.1	35.0	±	6.6	8.8	24.9	±	5.6	94.1
Tibialis Anterior (+)	14.0	±	5.1	97.1	20.5	±	7.8	50.0	26.9	±	6.1	100.0
Extensor Digitorum Longis (+)	13.5	±	1.9	97.1	23.6	±	9.0	58.8	25.4	±	7.0	94.1
Sartorius (+)	12.3	±	5.4	91.2	_		_		21.9	±	7.6	94.1
Extensor Digitorum Brevis (+)	14.7	±	2.1	67.6	20.5	±	6.8	58.8	29.6	±	10.4	31.3
Vastus Lateralis (+)	30.7	±	12.5	29.4	20.8	±	4.6	26.5	27.8	±	7.0	25.0
Vastus Lateralis (-)					22.6	±	4.4	35.3	_		_	_
Flexor Digitorum Longis (+)	25.9	±	6.8	66.7	30.0	±	2.8	11.1	n.a.		n.a.	n.a.
Flexor Digitorum Longis (-)					19.7	±	9.4	66.7	n.a.		n.a.	n.a.
Gastrocnemius Medialis (+)	28.9	±	7.8	29.4	18.9	±	3.7	20.6	43.4	±	13.1	31.3
Gastrocnemius Medialis (-)					22.7	±	8.3	70.6	_		_	_
Gastrocnemius Lateralis (+)	21.1	±	9.1	67.6	16.6	±	3.0	41.2	29.7	±	8.2	68.8
Gastrocnemius Lateralis (-)					25.3	±	10.7	55.9	_		_	_
Soleus (+)	32.0	±	22.6	12.5	17.3	±	1.0	25.0	57.4		_	6.3
Soleus (-)					31.8	±	4.1	68.8				

Mean and standard deviation of the latency of the EMG responses evoked during the swing and stance periods of the step cycle and with the cat at rest. Data for most flexor and extensor (shaded area) muscles are calculated from the minimum value selected during swing (groups 1-3 and 9-10) and stance (within groups 4-8) at each site. Because of the double period of activation of the EDB, the mean was calculated from the minimal value, irrespective of phase. Values are given for both primary increases (+) and decreases (-). % indicates the percentage of occurrence of responses for each muscle during locomotion and at rest from 34 cortical sites of three cats MC23-25. Responses in Sol were evoked from 16 cortical sites in MC25, those in FDL were evoked from 18 cortical sites in MC23-24.

Table III. Amplitude and frequency of the responses produced by long trains (200ms) of stimuli

		Step Cycle	Sartorius		Gastrocnemius	
			Amplitude	Duration	Amplitude	Duration
			Motor Cortex			
Swing	Loci	1/17	11/17	14/17	17/17	12/17
	No of groups	2/51(4)	30/51 (59)	44/51 (86)	46/51 (90)	8/51 (16)
	Range, %	90-109	70-212	81-179	109-373	84-129
	Mean, %	99±13	131±33	131±20	171±58	102±16
Stance	Loci	14/17	12/17	16/17	16/17	16/17
	No of groups	49/85 (58)	20/85 (24)	41/85 (48)	13/72 (18)	57/85 (67)
	Range, %	74-90	89-138	88-179	82-129	70-108
	Mean, %	85±4	118±11	123±17	105±33	87±8
			Pyramid			
Swing	Loci	0/0	2/3	2/3	2/3	3/3
	No of groups		3/9 (33)	5/9 (56)	5/9 (55)	4/9 (44)
	Range, %		112-261	121-147	126-155	105-116
	Mean, %		196±76	133±10	143±13	110±5
Stance	Loci	3/3	2/3	1/3	2/3	3/3
	No of groups	9/15 (60)	3/15 (20)	2/15 (13)	2/15 (13)	6/15 (40)
	Range, %	84-91	33-153	127-139	82-129	85-112
	Mean, %	89±2	101±61	133±8	105±33	95±9

Frequency and magnitude (mean ±SD) of the changes produced in the step cycle and in a representative flexor and extensor muscle of the hindlimb by long trains of stimuli applied to the motor cortex and the pyramidal tract. Loci indicates the number of sites at which significant changes in a given value were observed. No. of groups provides an indication of the frequency with which the stimulus produced a significant response. Range provides the minimum and maximum significant values. Mean is calculated only from the significant values. Amplitude is the total integrated magnitude of the burst of EMG activity.

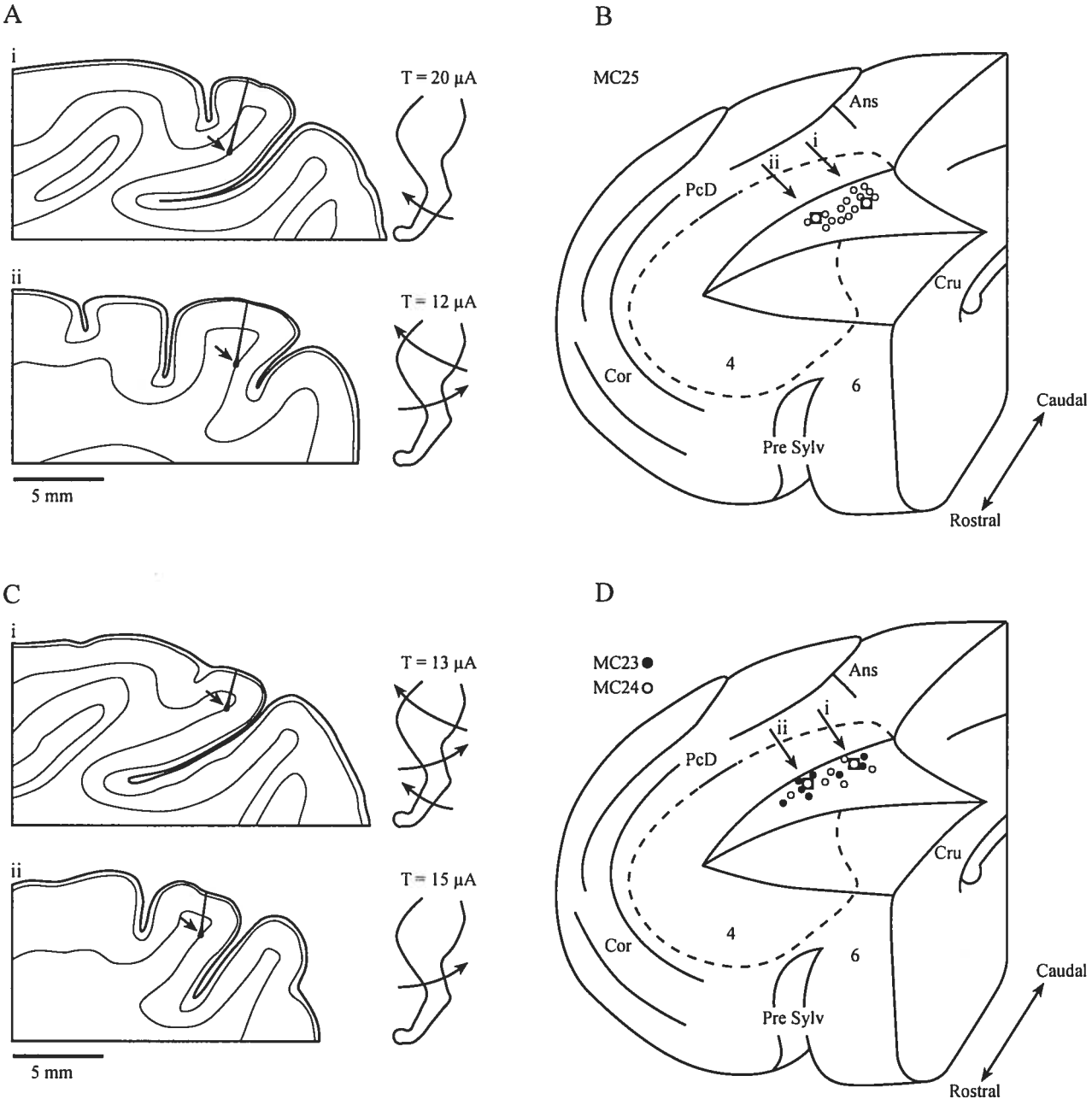
A
i
T = 20 µA
ii
T = 12 µA
5 mm
B
MC25
Ans
i
ii
PcD
Cru
Cor
4
6
Pre Sylv
Caudal
Rostral
C
i
T = 13 µA
ii
T = 15 µA
5 mm
D
MC23●
MC24○
Ans
i
ii
PcD
Cru
Cor
4
6
Pre Sylv
Caudal
Rostral

Figure 1

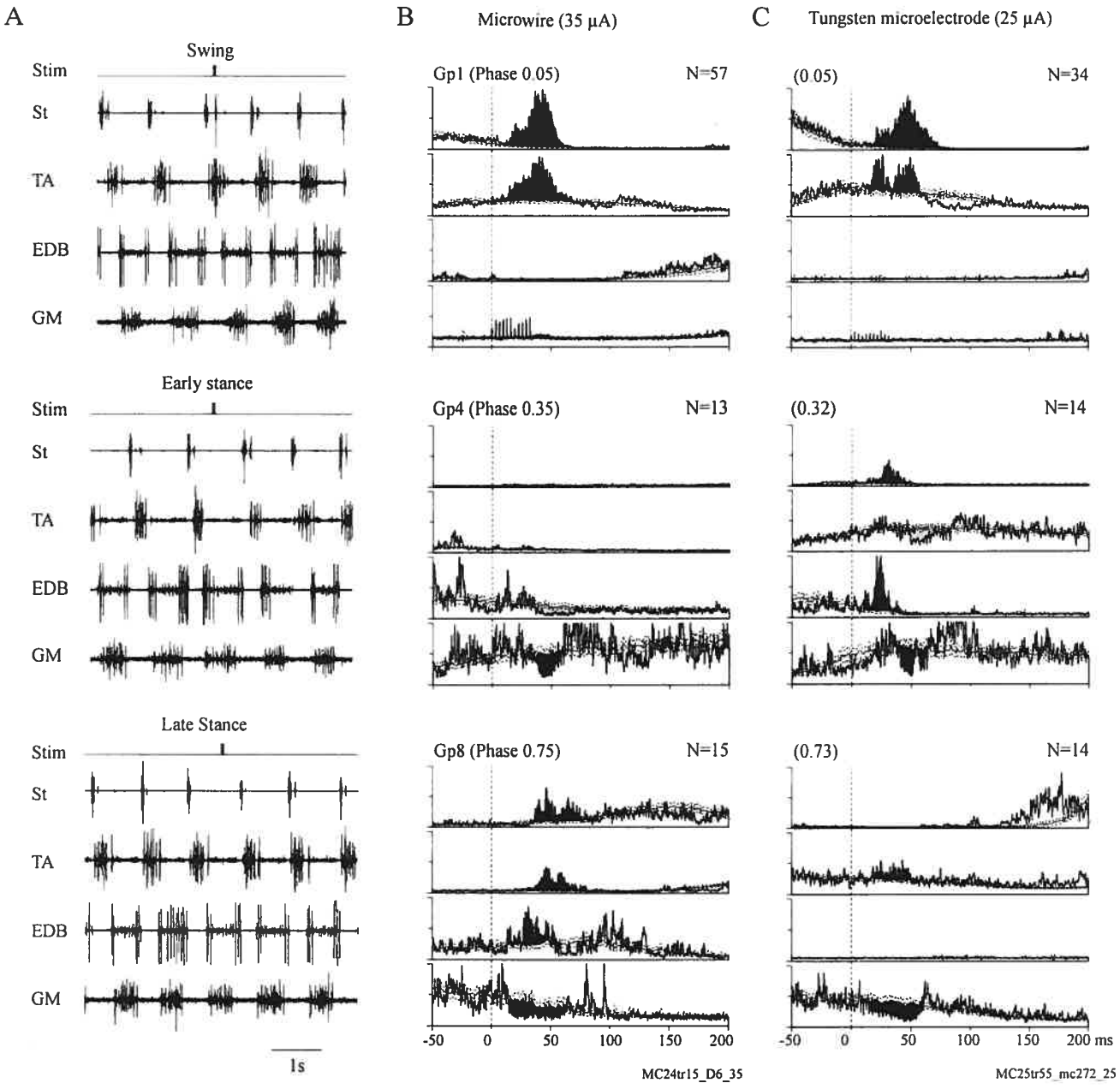
A
B
Microwire (35 µA)
C
Tungsten microelectrode (25 µA)
Swing
Stim
St
TA
EDB
GM
Gp1 (Phase 0.05)
N=57
(0.05)
N=34
Early stance
Gp4 (Phase 0.35)
N=13
(0.32)
N=14
Late Stance
Gp8 (Phase 0.75)
N=15
(0.73)
N=14
1s
-50
0
50
100
150
200
200 ms
MC24tr15_D6_35
MC25tr55_mc272_25

Figure 2

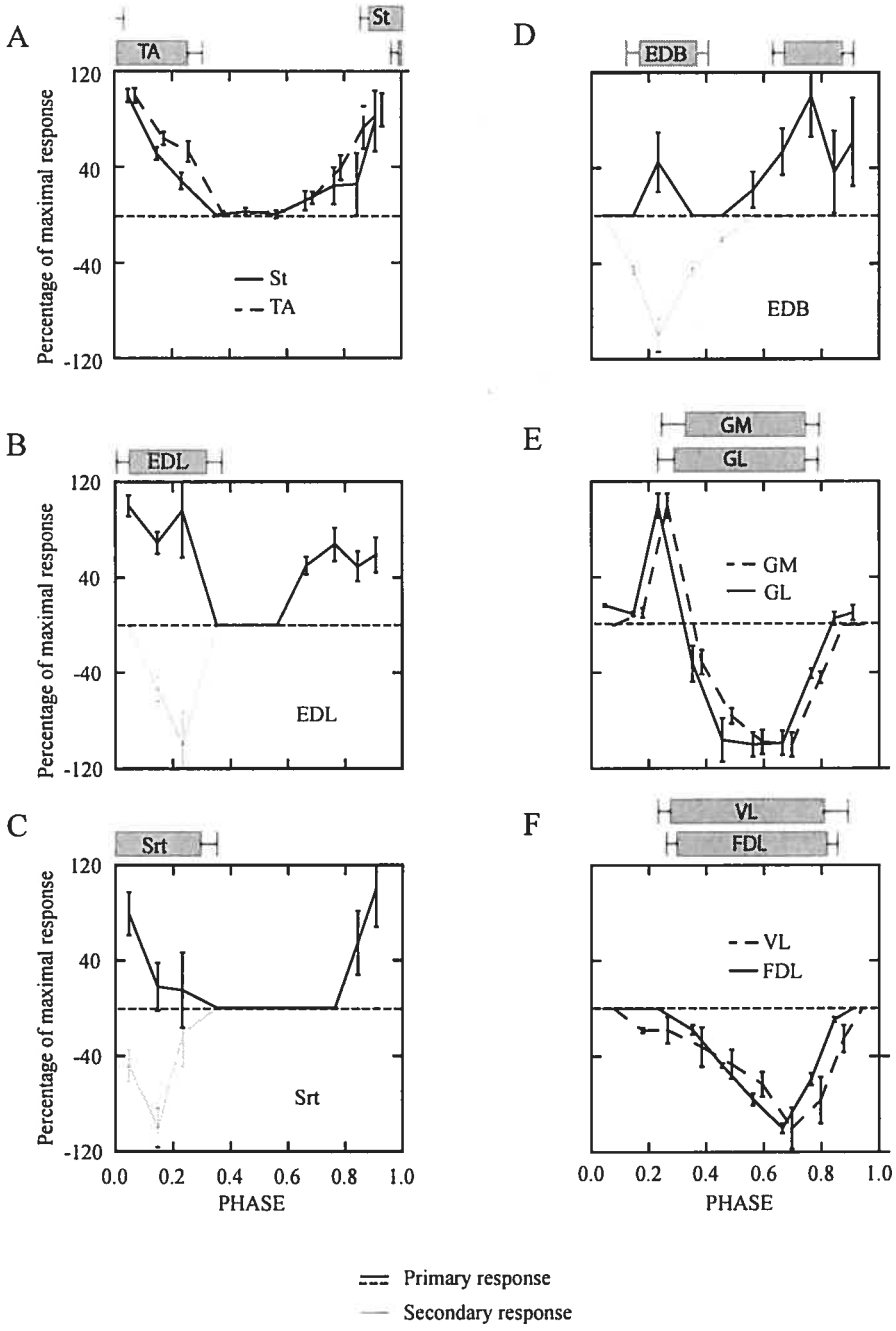
A
TA
St
120
40
-40
-120
Percentage of maximal response
St
TA
D
EDB
EDB
B
EDL
EDL
E
GM
GL
GM
GL
C
Srt
Srt
F
VL
FDL
VL
FDL
0.0 0.2 0.4 0.6 0.8 1.0
PHASE
Primary response
Secondary response

Figure 3

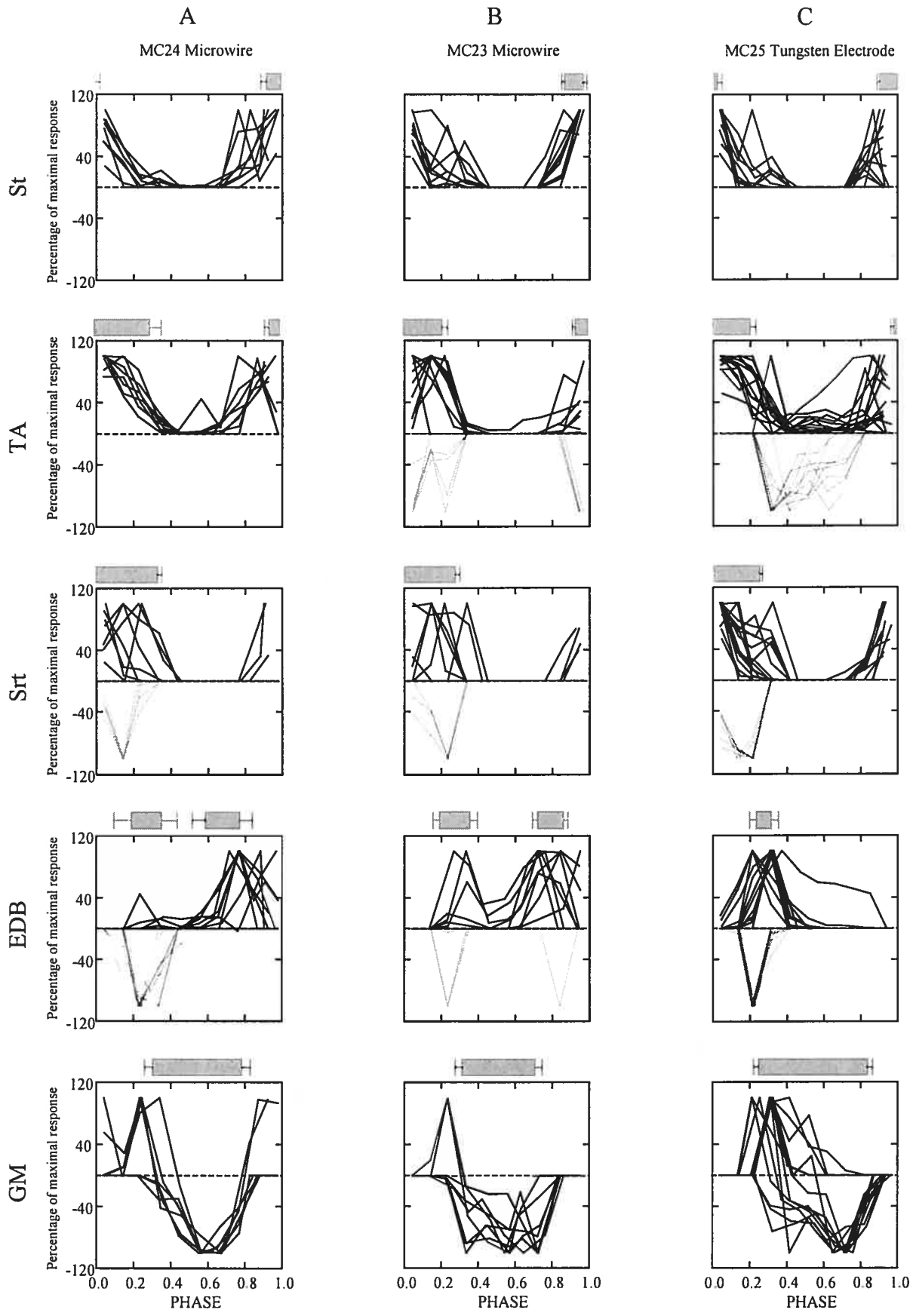
A
B
C
MC24 Microwire
MC23 Microwire
MC25 Tungsten Electrode
St
TA
Srt
EDB
GM
Percentage of maximal response
120
40
-40
-120
0.0
0.2
0.4
0.6
0.8
1.0
PHASE

Figure 4

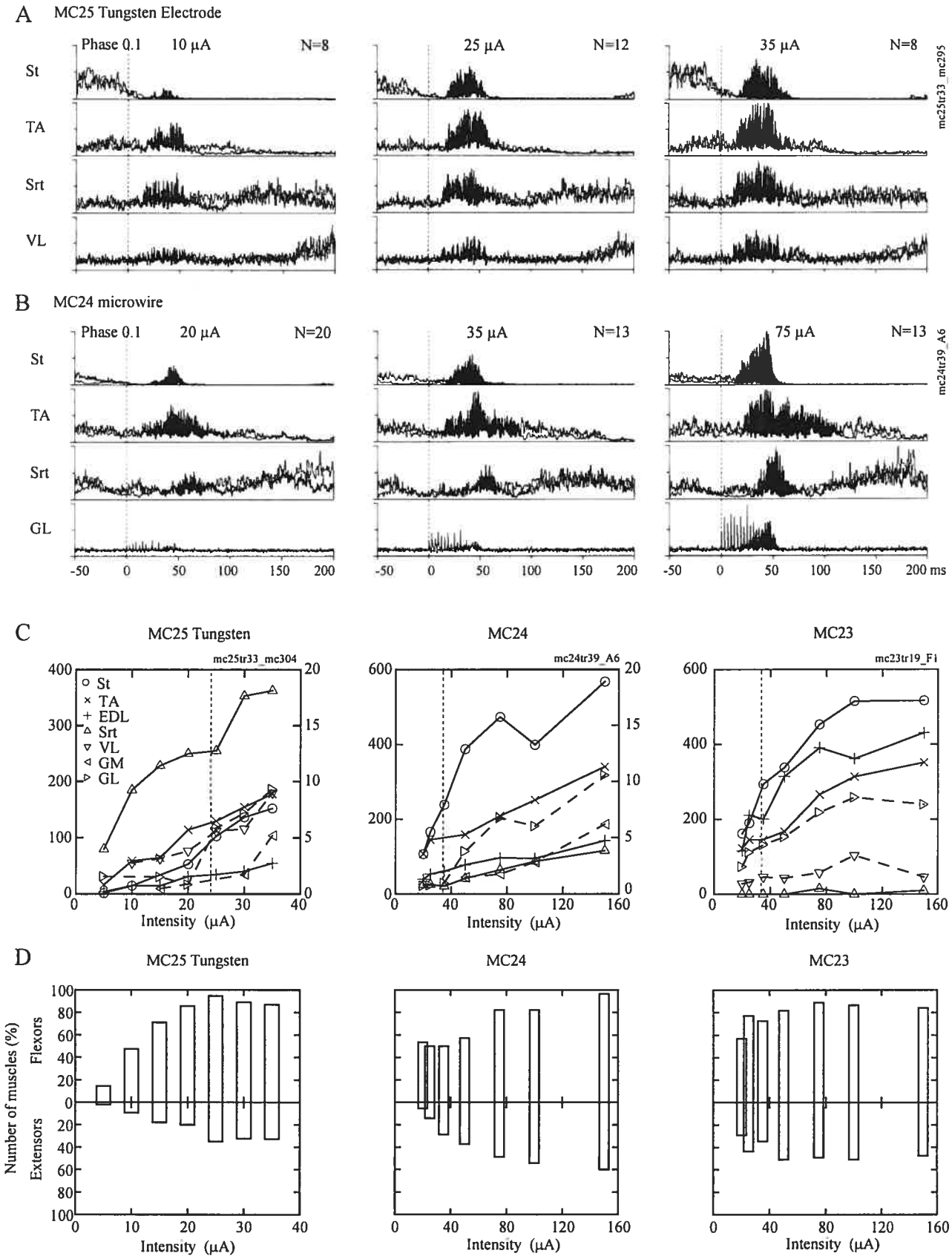
A
MC25 Tungsten Electrode
Phase 0.1
10 µA
N=8
25 µA
N=12
35 µA
N=8
St
TA
Srt
VL
mc25tr33_mc295
B
MC24 microwire
Phase 0.1
20 µA
N=20
35 µA
N=13
75 µA
N=13
St
TA
Srt
GL
mc24tr39_A6
-50 0 50 100 150 200
-50 0 50 100 150 200
-50 0 50 100 150 200 ms
C
MC25 Tungsten
MC24
MC23
mc25tr33_mc304
mc24tr39_A6
mc23tr19_F1
St
TA
EDL
Srt
VL
GM
GL
Intensity (µA)
Intensity (µA)
Intensity (µA)
D
MC25 Tungsten
MC24
MC23
Number of muscles (%)
Flexors
Extensors
Intensity (µA)
Intensity (µA)
Intensity (µA)

Figure 5

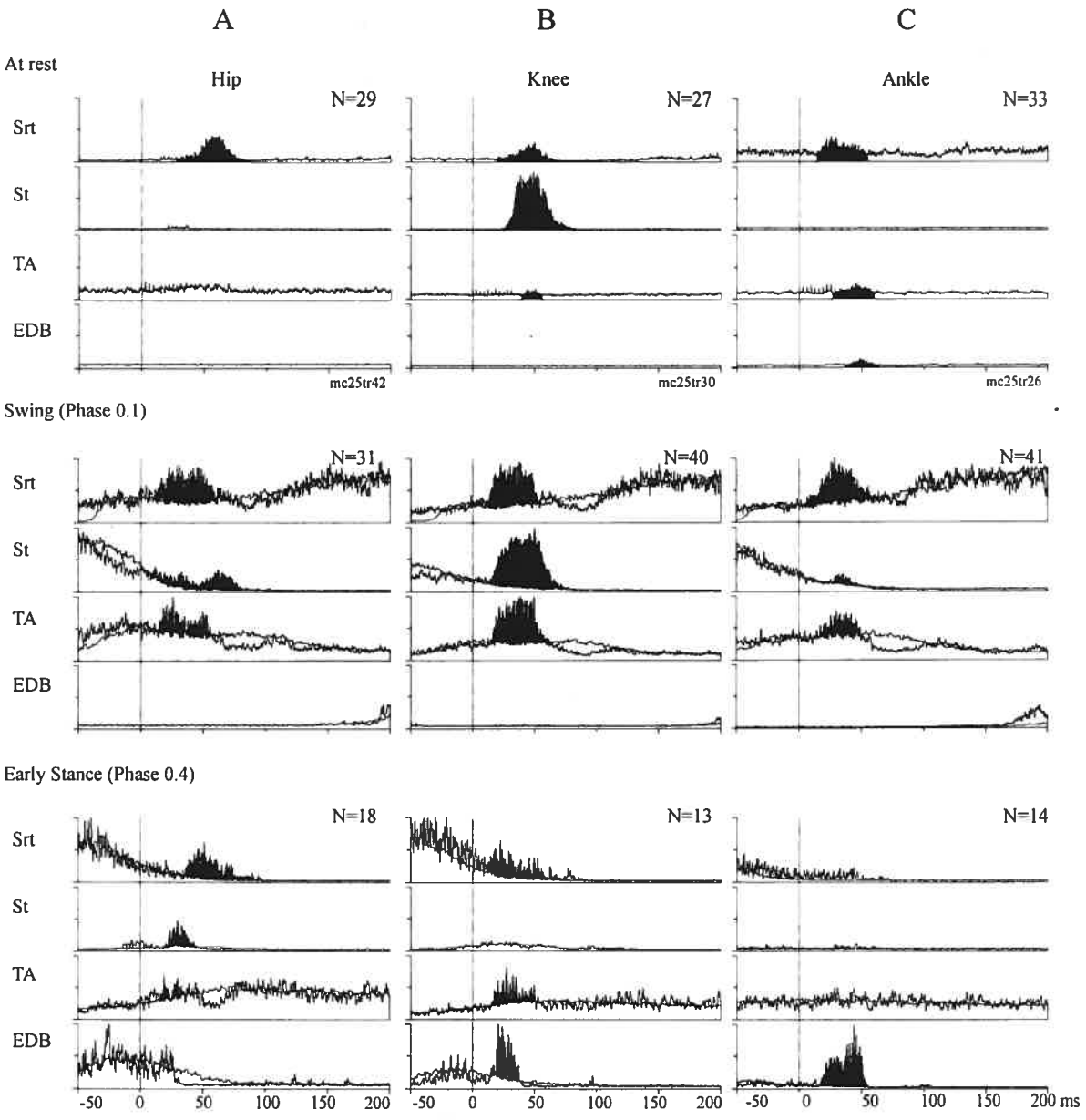
A
B
C
At rest
Hip
Knee
Ankle
N=29
N=27
N=33
Srt
St
TA
EDB
mc25tr42
mc25tr30
mc25tr26
Swing (Phase 0.1)
N=31
N=40
N=41
Srt
St
TA
EDB
Early Stance (Phase 0.4)
N=18
N=13
N=14
Srt
St
TA
EDB
-50 0 50 100 150 200
-50 0 50 100 150 200
-50 0 50 100 150 200 ms

Figure 6

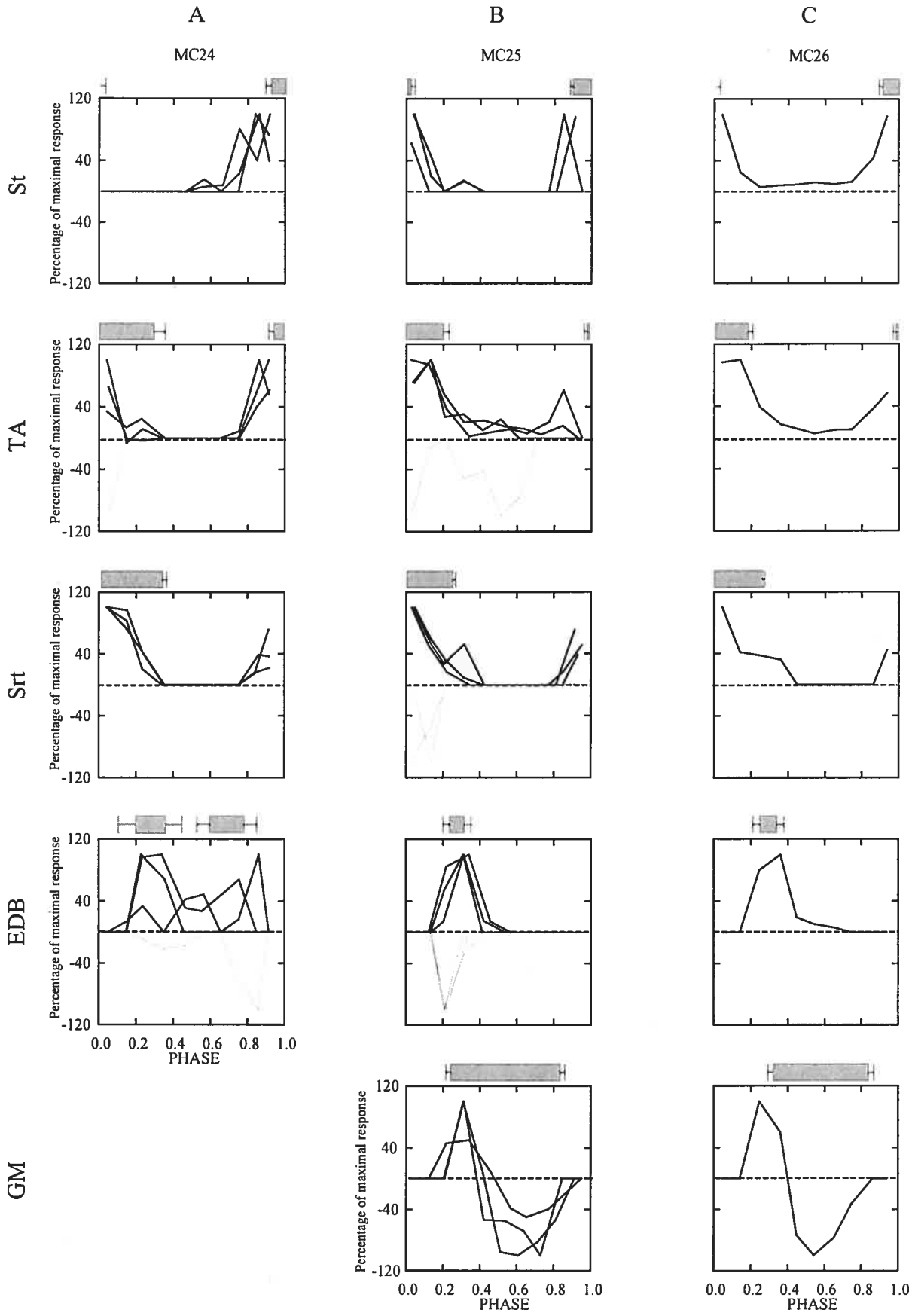
A
B
C
MC24
MC25
MC26
St
TA
Srt
EDB
GM
Percentage of maximal response
120
40
-40
-120
0.0
0.2
0.4
0.6
0.8
1.0
PHASE

Figure 7

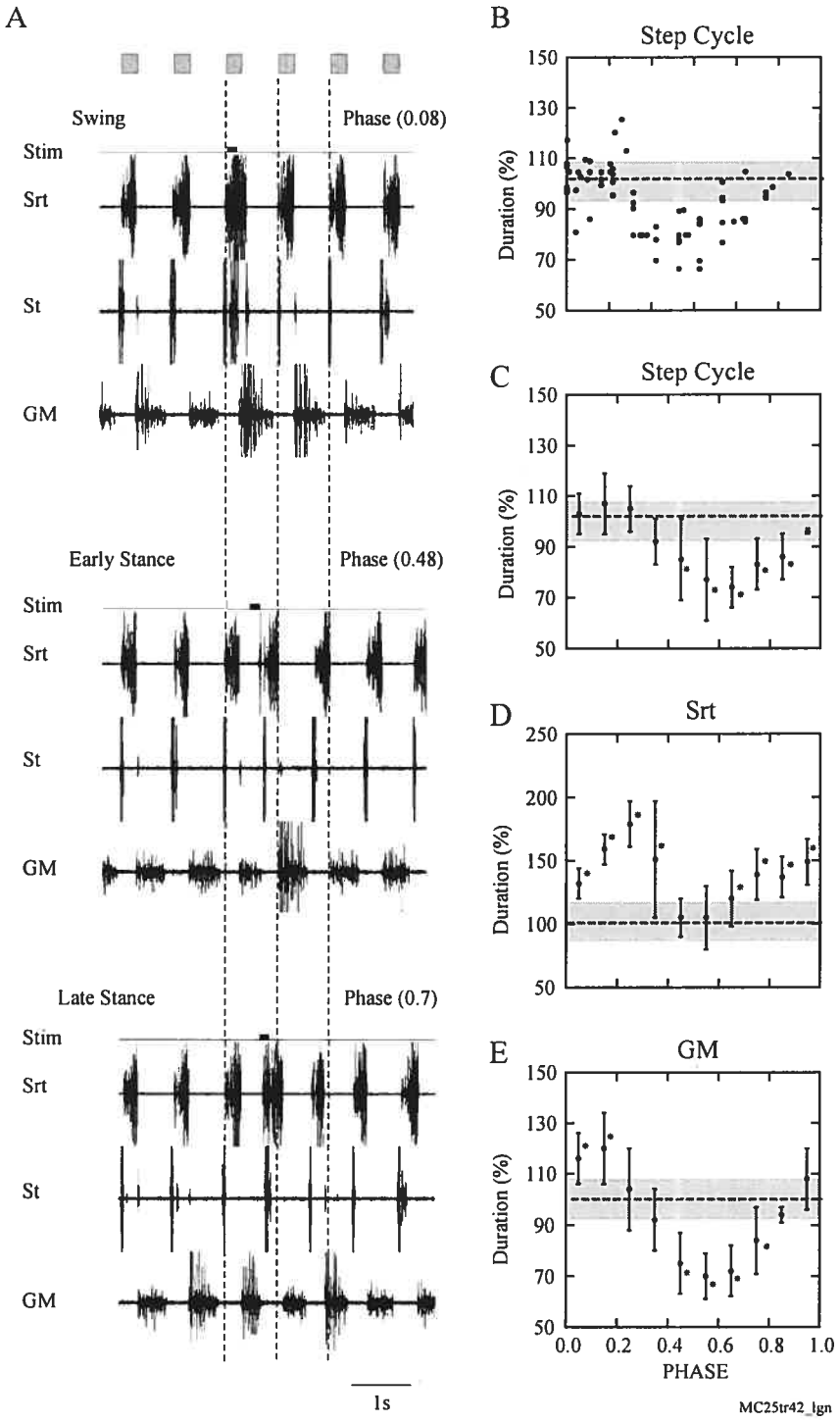
A
Swing
Phase (0.08)
Stim
Srt
St
GM
Early Stance
Phase (0.48)
Stim
Srt
St
GM
Late Stance
Phase (0.7)
Stim
Srt
St
GM
1s
B
Step Cycle
Duration (%)
150
130
110
90
70
50
C
Step Cycle
Duration (%)
150
130
110
90
70
50
D
Srt
Duration (%)
250
200
150
100
50
E
GM
Duration (%)
150
130
110
90
70
50
0.0
0.2
0.4
0.6
0.8
1.0
PHASE
MC25tr42_lgn

Figure 8

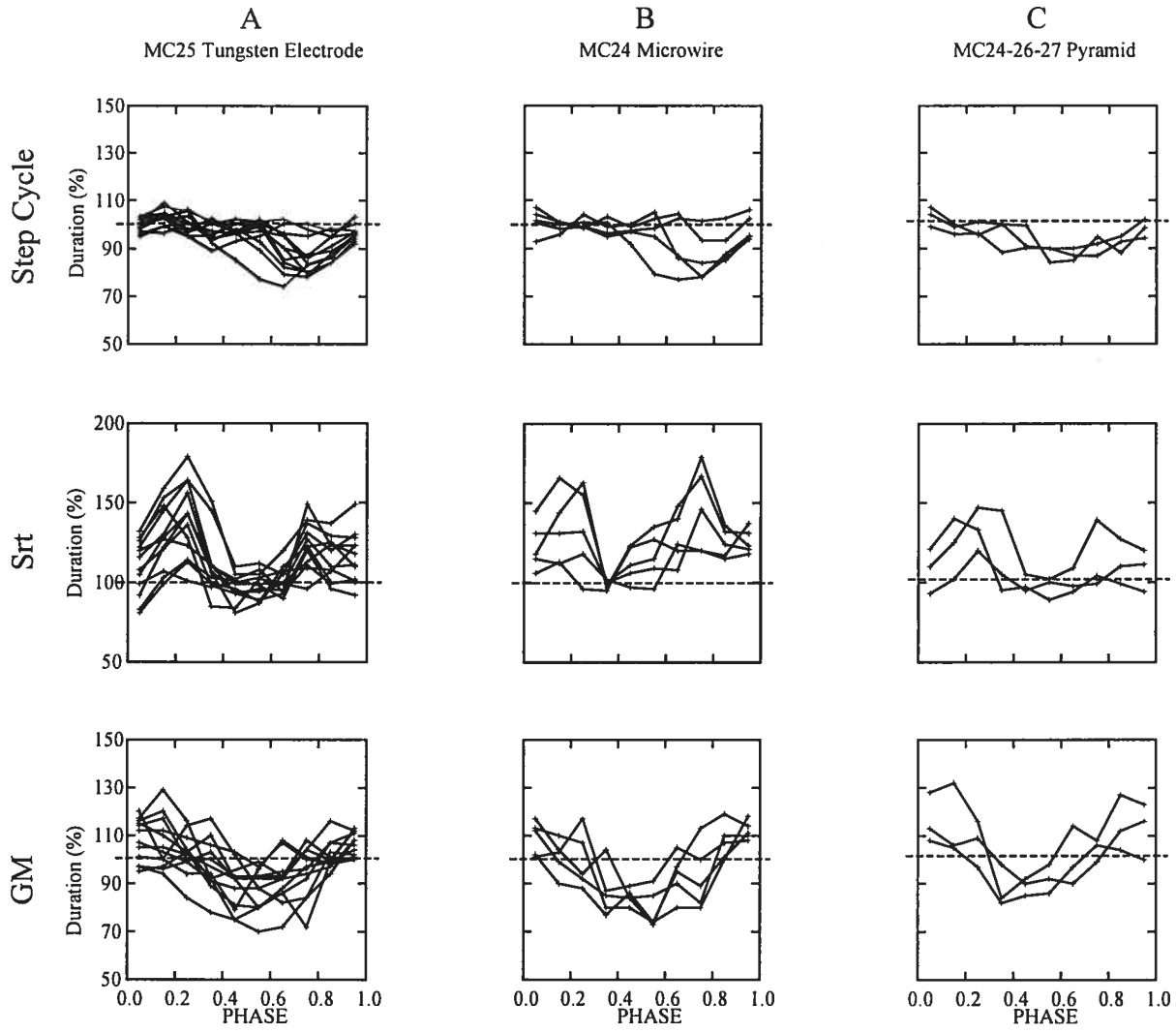
A
MC25 Tungsten Electrode
B
MC24 Microwire
C
MC24-26-27 Pyramid
Step Cycle
Srt
GM
Duration (%)
150
130
110
90
70
50
200
150
100
50
0.0 0.2 0.4 0.6 0.8 1.0
PHASE

Figure 9

Article # 2

Motor cortical modulation of cutaneous reflex responses in the hindlimb of the intact cat

by

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En préparation pour Journal of Neurophysiology

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Tables: 2

Abstract

We have used the technique of spatial facilitation to examine the interactions between the signals conveyed by the corticospinal tract and those of cutaneous afferents in the hindlimb of the intact, walking cat. Microstimulation was applied to 20 cortical sites in the hindlimb representation of the motor cortex and to 3 different cutaneous nerves innervating the hindpaw in 4 cats. Conditioning stimuli to the motor cortex induced both facilitation and depression of cutaneous reflexes evoked by stimulation of nerves in the hindlimb contralateral to the stimulation site. Facilitation was most frequently evoked by conditioning stimuli in the range of 10 - 30 ms before the cutaneous stimulation; depression was normally evoked by shorter and longer conditioning delays. Similar changes were observed after conditioning stimuli to the pyramidal tract, suggesting that the changes were independent of any changes in cortical excitability. Modulation of reflex activity varied according to the muscle under study, the cutaneous nerve used to evoke the reflex and the cortical site used to condition the reflex. Together, these results suggest that there is spatial convergence of corticospinal and cutaneous afferent activity and that this convergence is mediated by distinct subpopulations of spinal interneurones.

Introduction

Most goal-directed voluntary movements require the integration of feed-forward signals from the cortical structures controlling the movement with the feedback signals generated by the peripheral afferent receptors that are activated by that movement. This is particularly true for locomotion in which cutaneous feedback both provides information on the nature of the substrate (Bouyer and Rossignol 2003; Engberg 1964) and signals unexpected perturbations, particularly during the swing phase (Abraham et al. 1985; Duysens and Loeb 1980; Duysens and Stein 1978; Duysens et al. 1980; Forssberg 1979; Loeb 1993; Pratt et al. 1991; Prochazka et al. 1978;Wand et al., 1980).

The results from electrophysiological experiments in reduced preparations suggest that such cutaneous reflex activity is subject to regulation from supraspinal structures, including the motor cortex. For example, in one early series of experiments, Lundberg and Voorhoeve (1962), showed that stimulation of the motor cortex in the anaesthetised cat increased the magnitude of the responses evoked in hindlimb flexor and extensor motoneurones by stimulation of flexor reflex afferents, including low-threshold cutaneous afferents. Later experiments confirmed and extended these observations by showing clear spatial convergence of corticospinal and cutaneous afferent inputs onto motoneurones inervating both the forelimbs (Illert et al. 1976, 1977) and the hindlimbs (Fleshman et al. 1988; Pinter et al. 1982). In both limbs, the minimal corticospinal connection is disynaptic, although cutaneous pathways in the hindlimb are more frequently trisynaptic (Burke 1999).

From a functional viewpoint, however, there is very little information on the effects

of concurrent activation of the corticospinal and cutaneous pathways. Certainly, there is abundant evidence that cutaneous afferent information modulates cortical neuronal activity (Asanuma 1981) and that, during locomotion, this afferent information is itself modulated according to the dynamics of the step cycle (Chapin and Woodward 1986; Marple-Horvat and Armstrong 1999; Palmer et al. 1985). There is also evidence from experiments using Transcranial Magnetic Stimulation (TMS) in humans that cutaneous afferent stimulation may initiate a transcortical reflex loop that participates in the production of the longer latency responses evoked by cutaneous stimulation both during quiet standing (Nielsen et al. 1997; Wolfe and Hayes 1995) as well as during the swing phase of locomotion (Christensen et al. 1999; Pijnappels et al. 1998). However, there is no information about the capacity of corticospinal afferents to modulate the short latency responses evoked during locomotion. Moreover, experiments in humans can give only a global view of the effects of cortical activation on cutaneous reflexes and leave open the question of the extent to which the cortex may specifically modulate reflex pathways.

In the present manuscript, we address these issues by using the technique of spatial facilitation (Lundberg 1964) to examine the effects of intracortical microstimulation on the reflex responses evoked from different cutaneous nerves of the hindlimb in intact, walking cats.

Methods

Care and training

Experiments were carried out on four male cats (weight 4.2-5.5 kg) trained to walk at a comfortable and constant speed (*circa* 0.35-0.46 m/s) on a treadmill. Cats were carefully selected on the basis of their willingness to walk for uninterrupted periods ≥ 20 minutes. These were the same four animals that were used in our previous experiments detailing the contribution of the motor cortex to the structure and the timing of hindlimb locomotion in the cat (Bretzner and Drew 2005).

Surgical Procedures

The surgical procedures used in these experiments are detailed in Bretzner and Drew (2005). In brief, in three cats (MC24, 26 and 27), microwire electrodes (Tri-ML insulated stainless steel: 25 μm diameter) attached to a miniature connector (Neuralynx: EIB27) were manually inserted, one at a time, into the posterior bank of the cruciate sulcus that contains the hindlimb representation of the motor cortex (Armstrong and Drew 1984b; Bretzner and Drew 2005; Nieoullon and Rispal-Padel 1976; Widajewicz et al. 1994). Appropriate positioning of the microwires was facilitated by recording neuronal activity and by stimulating through the wires as they were inserted. The cortex was covered with a haemostatic material (Sterispon) and the microwire connector was attached to the cat's cranium with dental acrylic. In one cat, MC25, a recording chamber was positioned over the motor cortex (Drew 1988, 1993; Widajewicz et al. 1994). Penicillin (Novopharm) (40000 UI/kg iv) and analgesics: buprenorphine hypochloride (5 μg/kg) were provided at

the beginning and at the end of each surgery, and for at least 48 hours following surgeries. Antibiotics (cephadroxil: 100-200 mg/day) were administrated daily for the duration of the experiment.

One to two weeks after recovery from the initial surgery, the cats were anaesthetized with Isoflurane (2 - 3% with oxygen) to complete the surgical procedures. In all cats (MC24-27), multiple pairs of Teflon-insulated, braided stainless steel wires were implanted into selected muscles of the fore- and hindlimbs to record EMG activity during locomotion. These muscles included physiological flexor and extensor muscles acting around all of the major joints of the hindlimb contralateral to the motor cortex stimulated (see Bretzner and Drew 2005 for a list of these muscles and their major functions). Microwires were also implanted in the pyramidal tract at P7 (Drew 1993) to allow comparison with the responses evoked by cortical stimulation. In addition, in all cats (MC24-27), cuff electrodes (Julien and Rossignol 1982) were implanted around the Saphenous, Superficial Peroneal and Tibial Posterior nerves of the left and right hindlimb. Table I summarizes the number of cortical and pyramidal sites stimulated with each cutaneous nerve in all 4 cats.

All surgical and experimental procedures followed the recommendations of the Canadian Council for the Protection of Animals and were approved by the local ethics committee.

Protocol

In initial experiments, we tested the integrity and threshold of all chronically implanted electrodes. For stimulation of the cortex and of the pyramidal tract we used trains

of stimuli (cathodal current, 11 pulses at 330 Hz, pulse duration 0.2 ms) adjusted to produce a small response in the left (contralateral) semitendinosus, St, when the stimulus was applied at the onset of swing (delay 50 ms with respect to the onset of activity in the anterior head of the sartorius, Srt). For stimulation of the cuff electrodes implanted around the nerves of the left hindlimb (contralateral to the motor cortex), we used single pulses (pulse duration of 0.2 ms) and also adjusted the intensity to produce a small response in the St at a delay of 50 ms (with respect to Srt).

A similar procedure was used for stimulation of the nerves in the right hindlimb, so that stimulation occurred at the onset of swing in the left hindlimb. In this case, the evoked EMG responses represent crossed reflexes. In some cases, stimulation of the nerves in the right hindlimb were ineffective in eliciting responses in the left St; in these cases we adjusted the stimulus intensity to evoke responses in either the Srt or the tibialis anterior (TA). In the case of the experiments performed in the cat with the recording chamber, the electrode was advanced to layer V (as verified by antidromic activation of neurones by stimulation of the pyramidal tract) and threshold was then verified as for the implanted cortical wires.

The threshold for each nerve and cortical site was verified at the beginning of each experimental session. Once the current strength was appropriately adjusted, the stimulation of the cortex/pyramid and the cutaneous nerve were combined with the cutaneous stimulation always being delayed with respect to the onset of the cortical stimulation. The cortical stimulation thus served to condition the later cutaneous reflex. The cutaneous stimuli were applied in a pseudo-random order at delays ranging from 0 to 40-70 ms (5 ms steps) relative to the onset of the cortical or pyramidal stimulation (see Fig. 1A, B). Ten to

twenty-five repetitions were applied at each delay. In some cases, control responses evoked by the cortical, pyramidal or cutaneous nerve stimulation alone were recorded at the beginning and end of the experiment to ensure that the small additional delay (with respect to Srt onset) of the cutaneous stimulus had no effect of the results (see Discussion). Because of the time required to perform each series of experiments, we normally performed a maximum of two series of stimuli in any one experimental session; in a few exceptional cases 3 series were performed.

Evoked EMG responses were digitized on-line at a frequency of 5 kHz for ≥ 25 ms before and ≥ 150 ms after the onset of the stimulus train. EMGs were band-pass filtered between 100 Hz and 3 kHz. In addition, a continuous record of the EMG activity during locomotion was also digitized at 1 kHz.

Data analysis

Data were analysed as previously described (Rho et al., 1999; Bretzner and Drew, 2005). The responses evoked by the stimuli were computed-rectified and averaged and displayed on a monitor. The average activity from a similar time period taken from unstimulated cycles was superimposed on this display (Drew and Rossignol 1984). The onset and offset of the responses were determined manually using the interval of confidence ($P<0.01$) of the standard error of the mean of the control activity as a guideline. Evoked responses were included in the analysis if their latency was ≤ 50 ms and their duration exceeded 5 ms.

For each stimulus, we initially examined the averaged responses and determined the latency of the onset and offset of those responses that were greater or less than the interval

of confidence for each of the recorded muscles. We then used these values to calculate the integrated value of the traces within this window for each individual stimulus. A similar operation was performed on the control (unstimulated) traces. We then calculated the net amplitude of the responses by subtracting the values obtained from the unstimulated cycles from those in which stimulation was applied. The values were then averaged to produce a mean response together with the standard deviation (SD) and the standard error (SE) of the mean.

To determine the effect of the conditioning stimuli, we initially calculated the magnitude of the responses evoked by stimulation of the motor cortex (or pyramidal tract) and the nerve alone. These values were then added algebraically and an interval of confidence ($P<0.01$) for the SE of the mean was calculated from these values. This control level of activity is plotted as a gray shaded region on the graphs of Figs. 1-5. Mean values that were greater than the interval of confidence were considered to be evidence of a facilitation of the response and those that were less of a depression.

Histology

At the end of the experimental sessions, the animals were deeply anaesthetized with sodium pentobarbitol (Somnotol) and perfused *per cardium*. The brain was removed and sectioned in the parasagittal plane before being stained with cresyl violet.

Results

The effects of cortical and pyramidal tract conditioning stimuli on the magnitude of cutaneous reflexes evoked from different cutaneous nerves was investigated in four cats (Table I). Conditioning of reflex responses evoked from the left hindlimb by the motor cortex was studied in depth for 3 cutaneous nerves in cat MC25; supplementary data were obtained from two other cats. Conditioning reflex responses in the right hindlimb were obtained in 4 cats, primarily for the saphenous and superficial peroneal nerves. In addition, conditioning stimuli to the pyramidal tract were studied for all 3 nerves from both limbs. All of the cortical sites stimulated were within the caudal bank of medial aspect of the cruciate sulcus and produced twitch responses in hindlimb muscles; these sites are detailed in Bretzner and Drew (2005).

Cortical modulation of cutaneous reflexes in the left hindlimb

Figure 1 illustrates a representative example of the effects of a conditioning stimulus delivered at a site in the right motor cortex on the reflex effects evoked in the St by stimulation of the left Saphenous nerve. Just supra-threshold stimulation of both the motor cortical site and of the saphenous nerve evoked relatively small responses in the St (Fig 1A,C). Simultaneous stimulation of the motor cortex and the Saphenous nerve (delay = 0 ms) evoked a response that was slightly smaller than the algebraic sum of the responses to the individual stimuli (Figs. 1C, F). However, the combination of both stimuli at delays of ≥ 10 ms evoked a response that was more than twice the magnitude (216%) of the algebraic sum of the responses evoked individually. Still greater delays produced

progressively smaller responses and at a delay of 40 ms the response was almost abolished. Note that the response evoked by stimulation of the saphenous nerve alone at a delay of 20 ms (Fig 1D, F) is similar to that produced at a delay of 0 ms (Fig. 1C), demonstrating that the enhanced responses were not simply due to the small change in the relative phase of application of the stimulus. Although the cortical response at this delay was slightly greater than at 0 ms (Fig. 1E), the combined response did not exceed the limits of the interval of confidence based on the initial measures (Fig. 1F).

Figure 2 A-D illustrates the results from stimulation of a different cortical site in the same cat on the reflex effects evoked by the left Saphenous nerve on selected flexor and extensor muscles. As in Fig 1, the strength of each stimulus was adjusted to produce just supra-threshold responses in the St when delivered alone. No noticeable change in amplitude was observed when the motor cortex and the Saphenous nerve were stimulated simultaneously (0 ms delay). However at delays of 15 - 35 ms, reflex amplitude was increased in the St and at delays of 20 - 35 ms there was a facilitation of the reflex responses evoked in the ankle extensor, Gastrocnemius Lateralis (GL). No changes were observed in the amplitude of the TA.

As illustrated in the graphs of Fig 2D, the cortical facilitation of reflex effects in the St reached 400 % of the algebraic sum of the two stimuli applied independently and facilitation of the response in the GL reached almost 300% of control. Similar effects on reflex activity in these three muscles were observed from conditioning stimuli to the pyramidal tract in the same cat, MC25 (Fig 2E). As for the single cortical site, the pyramidal tract stimulation evoked facilitation of the responses evoked in the St and the GL

and was without effect on the TA. In this example, the facilitation of the St was relatively less than that produced by stimulation of the cortical site illustrated in Fig 2D while facilitation of the GL was relatively greater. In addition, the facilitation was observed over a wider range of conditioning delays than was observed for the cortical stimulation. Very similar responses were also observed for stimulation of cortical sites and the pyramidal tract in other cats. Fig 2F, for example, illustrates the effects of a conditioning stimulus applied to the motor cortex via a microwire electrode in cat MC27. As in the other illustrated examples, there was a facilitation of the responses in the St and the GL but no effect on the TA.

Cortical modulation of left Superficial Peroneal and Tibial Posterior reflexes in the left hindlimb

Cortical stimulation also modified the responses evoked from other cutaneous nerves of the left, contralateral, limb. For example, Fig 3A-D shows that stimulation of the same cortical site as illustrated in Fig 2A-D also modulated the responses evoked from the Superficial Peroneal nerve. However, in this case, the reflex responses evoked in both the St and the GL were depressed at delays from 0 -15 ms and weakly facilitated at conditioning delays of 25 -35 ms. The reflex responses in the TA was weakly depressed at all conditioning delays tested. Other cortical sites more strongly facilitated the reflex responses evoked in the St and GL by the superficial peroneal stimulation. Fig. 3E, for example, illustrates the effect of stimulation of a different cortical site from the same cat on these same 3 muscles. In this example, the responses in both the St and the GL were strongly facilitated, as was the response in the TA. Similar effects on the reflex responses

evoked in St and in the biceps femoris (BF) were also seen by stimulation of the cortex in other cats as illustrated in Fig. 3F. However, the conditioning stimuli in this cat produced a depression of the responses in TA, similar to that observed in Fig. 3D. There was no effect on the GL in this cat (not illustrated). Facilitation of the reflex responses in the St, the GL and the BF were also seen from stimulation of the pyramidal tract in both cats MC25 and MC26.

Cortical stimulation preceding the stimulation of the left, contralateral, Tibial Posterior nerve also facilitated the reflex responses. However, in contrast to stimulation of the other cutaneous nerves, stimulation of the Tibial Posterior nerve at just supra-threshold intensities in cat MC25 frequently evoked both short and long latency duration reflexes in several of the recorded muscles. In the example illustrated in Figs. 4A-C, stimulation of the tibial posterior nerve at low intensity evoked either small (St) or no (TA and GL) short latency responses but evoked large longer latency responses in both the St and the GL. Conditioning stimuli to the motor cortex had differential effects on these two responses. This is most clearly observed with a conditioning delay of 25 ms for the St (Fig. 4A) in which it can be clearly observed that the short latency response (first arrow) is strongly facilitated (see Fig. 4D) while the longer latency response is almost abolished (second arrow). Similarly the longer latency response in the GL was also abolished at similar delays. Similar effects on the long latency responses were observed in all 9 sites in cat MC25 in which the effects of conditioning stimuli on the Tibial Posterior nerve were tested and were observed to stimulation of both the motor cortex and the pyramidal tract.

The effects of the conditioning stimuli on the short latency responses evoked by the

Tibial Posterior nerve were more variable than those seen with the other two nerves. However, one of the more frequent observations was a depression of the reflex response at short conditioning delays. This is illustrated for a different site in the same cat in Fig. 4E. Conditioning stimuli at short delays produces a depression in all 3 muscles. However, in contrast to the example of Fig 4D, there is no subsequent facilitation of the TA while there was facilitation of the GL.

Cortical modulation of the reflex effects evoked by stimulation of the cutaneous nerves in the right hindlimb

We also examined whether conditioning stimulation of the motor cortex and pyramidal tract had any effect on the crossed reflex responses that were evoked in the left hindlimb (contralateral to the motor cortical stimulation) by stimulation of cutaneous nerves in the right hindlimb. In this case the stimuli were triggered to arrive at the onset of the swing phase of the left limb, ie. the stimuli were applied during stance of the stimulated, right, limb. As illustrated in Figs. 5A - D, the crossed responses were normally not modulated or, if they were, depression was the normal effect as illustrated for the Srt. In a few cases only, facilitatory responses were occasionally observed in the St (Fig. 5E).

Synthesis

As is clear from the preceding paragraphs, there is clear evidence that conditioning stimuli applied either to the motor cortex or to the pyramidal tract modulates the reflex responses evoked by stimulation of cutaneous nerves, both ipsilateral and contralateral to the site of cortical stimulation. However, the magnitude and the time course of that

modulation varied quite extensively.

Figure 6 illustrates the results obtained by stimulation of different cortical sites in cat MC25 on the reflex responses evoked in 4 different muscles by the 3 cutaneous nerves of the left hindlimb that we studied and serves to illustrate several points. First, the magnitude of the modulation of any given reflex evoked from any one cortical site could vary quite widely. For example, while some cortical sites produced a modulation of > 300% of the reflex responses evoked by the saphenous nerves, others had much less influence on the magnitude. Second, the cortical sites that produced the greatest modulation of the reflexes evoked by the saphenous nerve did not necessarily produce the largest modulation of the reflexes evoked from the other nerves. This is quite clear when inspecting the responses evoked in the St; the sites evoking the largest responses in the saphenous reflex (yellow and brown) were not those evoking the largest responses in response to stimulation of the superficial peroneal nerve. Third, in the most general terms, the nature of the responses evoked in any one muscle in response to all 3 cutaneous nerves was very similar The reflex responses evoked in St and GL, for example, were generally facilitated by stimulation of most cortical sites at delays of 10-30 ms. Conversely, stimulation of very few cortical sites facilitated the responses evoked in the Srt, regardless of the nerve stimulated, despite the fact that clear reflex responses were frequently evoked in the Srt by the cutaneous stimulation alone. The situation was slightly different in the TA in which the reflex responses evoked by the superficial peroneal and tibial posterior nerves but not to the saphenous nerve. This is probably because the just supra-threshold stimulation of the saphenous (determined on the basis of the St) rarely produced clear reflex responses in the TA.

Although the cortical modulation of the reflex responses evoked in any one given muscle by each of the 3 cutaneous nerves tested were broadly similar, there were, nonetheless, quite consistent differences in the pattern of activation. These are summarized in Fig. 7 which illustrates only those responses that exceeded the limits of the interval of confidence of the algebraic sum of the two independent stimuli. Considering data from all sites in all cats, it can be seen that cortical stimulation (gray bars) normally depresses the responses evoked in the St by stimulation of the saphenous nerve at delays of 0 - 10 ms and again at delays ≥ 40 ms but that there was facilitation at delays of 15 - 35 ms (Fig. 7: *top left*). Facilitation, at some conditioning delay, was observed at 70% of the sites stimulated and averaged 190% of the control (Table II). Depression of the response was observed at 60% of the sites and averaged 29% of control. Stimulation of the superficial peroneal nerves and the tibialis posterior also induced a mix of depression and facilitation with the former producing greater depression at longer delays and the latter greater depression at earlier delays. This same pattern of response was also seen in the other 3 muscles illustrated. For example, the responses evoked in the Srt, TA and GL by stimulation of the Tibial Posterior nerve were all depressed by conditioning stimuli applied at delays of less than 15-30 ms. Conversely, the pattern of modulation of the reflex responses evoked in the TA and the GL by the superficial peroneal nerve resembles that observed in the St, consisting of facilitation at delays of 10 - 30 ms and depression at longer conditioning delays.

Conditioning stimuli to the pyramidal tract tended to show similar effects to those obtained from stimulation of individual cortical sites. For example, inspection of Fig 7, illustrates that the pattern of facilitation and depression following pyramidal tract

stimulation (dotted line) follows closely that observed from cortical stimuli (gray bars), particularly in the case of the responses evoked by the saphenous nerve. In addition, the crossed reflex responses evoked in the muscles of the left hindlimb (solid black lines) were also modulated, on a population basis, in a similar manner to the uncrossed reflexes.

That these differences in the nature of the modulation of the responses for the 3 nerves are not simply the result of population averaging is illustrated in Fig. 8 for a site in cat MC25 in which cortical stimulation at a single site was applied during stimulation of each of the 3 nerves. Inspection of this figure clearly shows that responses of the same type as described with respect to Fig. 7 were also observed in individual cases. In particular, the figure emphasizes the depression of the reflex response evoked by the superficial peroneal nerve at long delays but the depression of the response evoked by the Tibialis Posterior only at short delays.

Discussion

Conditioning stimuli to the motor cortex and the pyramidal tract produced strong modulation of the short latency reflex effects evoked by stimulation of several cutaneous nerves of the hindlimb in the cat. This modulation consisted of both facilitation and depression of reflex responses and varied according to the muscle recorded and the nerve and cortical site stimulated. The results suggest a convergence of cortical and cutaneous afferent activity on distinct interneuronal populations in the spinal cord.

General Considerations

We have concentrated in this report specifically on the effects of spatial convergence between the corticospinal tract and cutaneous afferents rather than detailing the responses evoked by each stimulus individually. However, it should be emphasized that the reflex effects evoked in these cats by stimulation of the cutaneous nerves ipsi- and contralateral to the recordings in the left hindlimb were identical to those reported in other studies in intact walking cats, in which stimulation during swing generally facilitates flexor muscle activity and stimulation during stance produces inhibition of extensors (Abraham et al. 1985; Duysens and Loeb 1980; Duysens et al. 1980; Loeb 1993; Pratt et al. 1991; Rossignol et al. 1988). The effects produced by stimulation in the same cortical sites as used in this report have been detailed in a recent manuscript (Bretzner and Drew 2005a) and are analogous in their properties to those that have been described in more detail in the forelimb (Armstrong and Drew 1985; Rho et al 1999). As such, the effects of the spatial convergence that we describe in this report provide a firm basis for discussion of the effects

of cortical and cutaneous convergence.

Cortical modulation of cutaneous reflexes during locomotion

Conditioning stimuli to many cortical sites produced modulation of the responses evoked in muscles by stimulation of one or more of the cutaneous nerves of the same limb. This was particularly true for the responses evoked in the St muscle in which substantial facilitation of the reflex responses were observed in more than 60% of the experiments (irrespective of the nerve stimulated) (Table II). Moreover, similar results to stimulation of the saphenous nerve were observed in all cats. The extent and magnitude of this spatial convergence is compatible with the results from experiments in the anaesthetized preparation in which spatial convergence onto flexor and extensor motoneurones (including St) was observed from stimulation of the motor cortex and the sural nerve (Lundberg and Voorhoeve 1962; Lundberg et al. 1962; Pinter et al. 1982) or of the superficial peroneal nerve (Fleshman et al. 1988). In the current experiments, facilitation was most frequently observed at conditioning delays of 15 -20 ms, although facilitatory responses in some cases could be obtained at shorter delays. This is similar to the delays used by Lundberg and Voorhoeve (1962) and by Fleshman et al. (1988).

Stimulation in most effective sites also produced depression of the responses, in both flexor and extensor muscles, at conditioning delays both shorter and longer than those that produced facilitation. Such a depression of reflex responses was not described in the aforementioned experiments in anaesthetized cats. The reason for this is not clear but it might be related to the more complex and phase-dependent changes in excitability that are to be expected in an intact waking cat. Moreover, depression of reflex excitability can

evidently only be discerned in experiments in which background activity is evoked by stimulation of each site (compare St in Figs. 1 and 2).

Because, in our experiments, stimulation was applied during locomotion, the experimental protocol resulted in the cutaneous stimuli being applied at progressively later times during the swing phase. We therefore have to consider the possibility that apparent changes the amplitude of the reflex responses are simply a result of a phase dependent modulation of the amplitude of the cutaneous reflexes. We controlled for this in several experiments by stimulating the cutaneous nerve alone at the different conditioning delays. As illustrated in Figs. 1D, F, the small change in delay did not result in any appreciable change in the amplitude of the cutaneous reflex. This was true for all 12 sites in which this was tested and it seems most likely that the results obtained were due to spatial convergence of the supraspinal and peripheral inputs.

Specificity according to the muscle recorded

Conditioning stimuli at any one given cortical site frequently modulated reflex amplitude in a number of muscles, including both flexor and extensor muscles. This is not unexpected given the relatively widespread effects that are produced by stimulation of each structure independently. Stimulation of cutaneous nerves during the swing phase of locomotion invariably produces an organised pattern of reflex activity throughout the limb involving primarily flexor muscles but also some extensor muscles (Rossignol et al. 1988). Similarly, stimulation of many cortical sites during the swing phase of locomotion may produce evoked responses in multiple hindlimb muscles, again including both flexors and extensors (Bretzner and Drew 2005). It is, therefore, to be expected that conditioning

cortical stimuli will modulate reflex effects in multiple muscles. What is perhaps more unexpected is that the conditioning stimuli, did not modulate the reflex responses in more muscles and that the effects evoked in any one experiment frequently produced facilitation of one muscle but depression of another (see. e.g. Figs. 3D and F).

The former finding may be related to the fact that we examined the effects of conditioning stimuli at only one phase of the step cycle (the onset of swing). While stimulation of both the motor cortex and the cutaneous nerves at this time of the step cycle is optimal for activation of the St, it is less efficient in producing reflex responses in some other flexor muscle. For example, cortically evoked responses in both TA and Srt are maximal in mid-swing and responses in muscles such as the EDB are optimal at the end of swing (Bretzner and Drew 2005). Conditioning stimuli applied at different times of the step cycle would therefore be likely to modulate differentially the reflex activity in different muscles. In addition, the threshold was determined on the basis of the responses evoked in the St, and in some experiments the stimulation was sub-threshold for responses in muscles such as the TA.

The fact that responses in some muscles were facilitated and those in other muscles were depressed or unchanged, may speak to the specificity of the terminations of cortical and cutaneous pathways on different interneuronal networks in the spinal cord. These differential effects on the different muscles, particularly facilitation vs. depression, infer that the site of the spatial convergence is specific to interneuronal populations regulating the activity of different synergistic groups of muscles.

Specificity according to the nerve stimulated

Conditioning stimuli applied to the motor cortex produced modulation of the reflex responses evoked by each of the 3 nerves that we tested. The nature of the cortical modulation of the reflex responses was broadly similar for each nerve although there were some consistent differences, as seen in Fig. 7. For example, while the reflex responses evoked by the superficial peroneal nerve were generally facilitated at conditioning delays ≤ 25 - 30 ms and depressed by greater conditioning delays, the inverse was seen for the reflex responses evoked by the Tibialis Posterior nerve which were depressed at conditioning delays ≤ 30 ms and facilitated at longer delays. Indeed, it is possible that one should consider the pattern of modulation of the responses evoked by the Tibialis Posterior nerve (inervating the plantar surface of the foot) to be the reciprocal of those evoked by stimulation of the Superficial Peroneal nerve (inervating the dorsum of the paw), at least in the St. The modulation of the responses evoked by the saphenous nerve was different again from both the superficial peroneal and the Tibialis Posterior nerves, consisting primarily of a facilitation at medium delays. That these responses are not purely a population effect is illustrated by Fig. 8 which clearly shows the same effect from stimulation of a single cortical site. Evidence of a specificity according to the site of origin of the reflex response is also provided by the results obtained from stimulation of the cutaneous nerves of the right hindlimb. In this case, the crossed reflex responses were almost always depressed by cortical stimulation, irrespective of the conditioning delay.

These findings again argue for a certain degree of specificity in the interneuronal populations as the modulation evoked in a given muscle from a given cortical site differs according to the cutaneous nerve that is stimulated. In the case of the Superficial Peroneal

nerve and the Tibial Posterior nerve, there is the added possibility that the cortical stimulation acts through reciprocal excitatory and inhibitory pathways.

Specificity according to the cortical site stimulated

The magnitude and temporal profile of the effect of the conditioning stimulation also depended on the cortical site that was stimulated. This can be clearly observed in the summary presented in Fig. 6 for the responses evoked from different cortical sites in cat MC25. While stimulation in some sites strongly facilitated the responses in a given muscle (e.g. St), stimulation in other sites was less effective. Moreover, the magnitude of the modulation evoked in a given muscle from a given cortical site showed wide variability. This variability is almost certainly related to the differences in the termination patterns of the corticospinal neurones activated by the cortical stimulation. As shown in our recent study of the effects of cortical stimulation on EMG activity during locomotion (Bretzner and Drew 2005), some cortical sites produced their strongest effects on knee flexors such as the St while others more strongly activated hip flexors such as the Srt, or ankle flexors, such as the TA. Corticospinal afferents from different regions of the hindlimb representation of the motor cortex, therefore, have differential terminations on the interneuronal populations regulating the activity of muscles acting around different joints of the hindlimb. This diversity in the pattern of the responses evoked from different cortical sites undoubtedly underlies the diversity in the modulation of the reflex patterns observed in the present experiments.

Possible mechanisms

There are two major mechanisms that may explain the modulation of the evoked responses by the conditioning stimuli.

The first is that the cutaneous stimuli might modulate the activity of the cortical neurones, thus modulating cortical excitability, and by this means alter the descending corticospinal volley. This mechanism has been suggested to be, at least in part, responsible for the changes in the long latency cutaneous reflexes evoked by stimulation of cutaneous nerves during human locomotion (Christensen et al. 1999, 2000; Pijnappels et al. 1998). However, we feel that this is unlikely to be a major contributing factor to the results obtained in this study as we obtained similar effects from stimulation of both the motor cortex and of the pyramidal tract. As argued by Nielsen et al. (1997), the magnitude of the descending volley evoked by stimulation of the corticospinal tract should be independent of any changes in corticospinal efficacy. Although our conclusion is different from that of Nielsen and his collaborators, it should be emphasised that we examined the effect of conditioning only on short latency responses while they studied longer latency reflexes. In addition, their conclusions pertained mostly to the earliest parts of the response evoked by TMS, i.e. that part which is most likely to be produced by monosynaptic cortical activation.

The other major possibility, and the one that we favour, is that the modulation is the result of changes in excitability of interneuronal pathways onto which cutaneous and corticospinal pathways converge. Spatial facilitation in such interneuronal pathways would explain all of the observed changes in modulation. Such spatial convergence may be direct, i.e. both afferents converging directly onto the same interneurones or indirect, i.e. each afferent contacting different interneuronal populations that then converge at a later stage.

The present experiments cannot address this issue. It is also possible that the final convergence may occur at the motoneuronal level, although the specificity of the responses evoked by different nerves and from different cortical sites argues against this possibility. Similarly, the difference in the temporal profile of the responses argues against the spatial facilitation being a simple function of non-linear motoneuronal properties (Brownstone et al. 1994) as this might be expected to affect convergent inputs to a given muscle in a similar manner; clearly, this is not always the case. Lastly, there is also the possibility that the modulation of the cutaneous reflexes might be influenced via cortical modulation of presynaptic afferent depolarisation (PAD)(Andersen et al. 1964; Lundberg 1964; Rudomin et al. 2004). Again, our experiments do not address this issue.

Regardless of the exact mechanism, the arguments in the preceding sections and paragraphs strongly suggest a very strong degree of specificity in the organisation of this spatial facilitation. Clearly, the nature of the modulation differs according to the muscle under study, the cutaneous nerve that is used to produce the cutaneous reflex and the exact cortical site that is stimulated. Within each of these categories, different patterns of modulation can be observed if any one factor (muscle, nerve, or cortical site) is changed. This suggests that there must be a highly specific and fractionated organisation of interneuronal pools (and/or PAD pathways) to produce the range of modulatory patterns observed in these experiments. In this respect, our suggestions strongly resemble those made by others who have argued for highly differentiated and specialized interneuronal pathways in the mediation of the reflexes produced by low and high threshold cutaneous reflexes (Burke 1999; Moschovakis et al. 1991; Pratt et al. 1991; Schouenberg et al 2002; Schmidt et al. 1998).

Functional considerations and Conclusions

These experiments demonstrate that activity in pyramidal tract neurons may modulate the activity in different reflex pathways in a very specific manner. From a functional point of view, it is probable that this convergence provides a means of modifying the magnitude of a reflex depending on circumstance. In our experiments, stimulation was applied as swing was initiated and the foot was lifted from the treadmill. This is a critical transition phase of the step cycle and a time when discharge activity in many cortical neurones is maximal (Armstrong and Drew 1984a; Drew 1993), as are the reflex responses in some muscles, and particularly the St. The large facilitation of the reflex activity evoked in the St by stimulation of the saphenous and superficial peroneal nerve would serve to increase knee flexion. This would move the limb away from any natural stimulus that would activate these nerves, especially in the case of activation of the superficial peroneal nerve inervating the dorsum. Similarly, facilitation of the responses in GL would also act to move the paw away from the stimulus by extending the ankle or counteracting any tendency towards ankle flexion (Wand et al. 1980). Suppression of the reflex responses in Srt and TA would likewise prevent the limb and paw being moved forward where they would risk contacting the stimulus again. As such, the cortical activation reinforces the most natural course of action when contacting an obstacle at swing onset. The almost reciprocal effects of pyramidal tract stimulation on the responses evoked by the Tibialis Posterior nerve serve to mostly depress the reflex responses that are evoked by this nerve. Reflex responses evoked by stimulation of the plantar surface of the paw at swing onset may not be desirable (or likely under natural circumstances) as they would tend to produce a plantarflexion of the paw.

Finally, although not tested in these experiments, it is possible that the major importance of this convergence might be to modulate reflex activity according to context. For example, PTNs increase their discharge frequency during behaviours that require modulation of gait and/or fine control over limb trajectory (Beloozerova and Sirota, 1993; Drew 1988, 1993). In addition to contributing to the modified patterns of muscle activity that these tasks require, this increased discharge would also serve to further modulate reflex excitability and might serve to enhance reflexes that serve to promote or stabilise the movement and depress those that serve to destabilise it.

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Figure Legends

Fig 1: Cortical modulation of the cutaneous reflex evoked in the left Semitendinosus (St) by stimulation of the left Saphenous nerve (contralateral to the cortical site) at the onset of swing. A: Untreated data showing the electromyographic (EMG) activity evoked in St by (from top to bottom) stimulation of the motor cortex (MC) alone, the Saphenous nerve alone and both sites simultaneously (delay = 0 ms). B: Schematic representation of the conditioning paradigm in which the cortical stimulation was applied preceding the cutaneous stimulus. C: Average EMG responses evoked in St by stimulation of, from top to bottom: the motor cortex alone, the saphenous nerve alone or by both at delays of 0, 20 and 40 ms (thick lines). Average background EMG activity in unstimulated cycles is illustrated by thin lines at the same phase; dotted lines indicate the 0.01 confidence level of the standard error of the mean for the control data. Filled areas indicate the net evoked EMG responses that we measured. Amplitude is arbitrary but constant for each trace. D,E: Controls: average EMG responses in St evoked by the motor cortex alone (D) and the cutaneous nerve alone (E) at a 20 ms delay. F: Summary of the amplitude of EMG responses evoked at different delays by the combined stimulation as a function of the algebraic sum of EMG responses evoked by the motor cortex and cutaneous nerve alone. Dotted horizontal line, 100%; shaded area, 99% confidence interval of the standard error (SE) of the mean of the algebraic sum. Black and grey, unconnected, circles (MC and Cut) are the responses evoked by the motor cortex or the Saphenous nerve alone at delays of 0 ms. Supplementary responses at 20 ms illustrate the responses illustrated in D and E, together with their algebraic sum. All illustrated muscles in this and all other figures are in

the left limb and are contralateral to the cortical site of stimulation. Abbreviations: cut, cutaneous; MC, motor cortex; N, number of stimulated step cycles; Srt, Sartorius; Stim, stimulation.

Fig 2: Modulation of left saphenous reflex effects by cortical and pyramidal tract stimulation in three selected muscles during locomotion. A-C: An example of the effects evoked by a cortical site (track 32 from the cat MC25) at different delays. Note that the unfilled peak in Fig 2C (indicated by *) is a stimulus artifact from stimulation of the Saphenous nerve. D-F: Summary of the cortical facilitation or depression of the magnitude of the evoked responses as a function of the algebraic sum of the responses evoked by the motor cortex and cutaneous nerve alone for the cortical site illustrated in Fig 2A-C (D), from the pyramid (E) from the same cat MC25, and from a cortical site in cat MC27 (F).

Fig 3: Cortical modulation of reflex effects evoked by stimulation of the left Superficial Peroneal in three selected muscles during locomotion. A-C: An example of the effects evoked by the same cortical site illustrated in Fig 2A-D (track 32 from the cat MC25). D-F: Summary of the cortical facilitation or depression of the magnitude of the evoked responses as a function of the algebraic sum of the responses evoked by the motor cortex and cutaneous nerve alone for the cortical site illustrated in A-C (D), from another cortical site from the same cat, MC25 (E), and from a cortical site in cat MC26 (F).

Fig 4: Cortical modulation of left Tibial Posterior reflex effects in three selected muscles during locomotion. A-C: An example of the effects evoked by the stimulation of a cortical

site in cat MC25 at different delays. D-E: Summary of the cortical facilitation or depression of the magnitude of the evoked responses as a function of the algebraic sum of the responses evoked by the motor cortex and cutaneous nerve alone for the cortical site illustrated in Fig 4A-C (D), and from a different cortical site in the same cat (E).

Fig 5: Cortical modulation of right (ipsilateral to the motor cortex), crossed cutaneous reflex effects in three muscles of the left hindlimb during locomotion. Stimulation was applied at the onset of swing in the left hindlimb (i.e. at the same time as in the previous figures). A-C: An example of the effects evoked by one cortical site at different delays. D: Summary of the cortical facilitation or depression of the magnitude of the evoked responses as a function of the algebraic sum of the responses evoked by the motor cortex and right cutaneous nerve alone for the cortical site illustrated in Fig 5A-C (D) in contralateral hindlimb muscles. E: effects from a different cortical site.

Fig 6: Cortical modulation of left (contralateral) cutaneous reflexes in four selected muscles during the swing phase in cat MC25. A: cortical facilitation or depression of the magnitude of the evoked responses as a function of the algebraic sum of the responses evoked by the motor cortex and left Saphenous nerve alone. B: cortical modulation of the left Superficial Peroneal reflex effects. C: cortical modulation of the left Tibial Posterior reflex effects. Each colour illustrates a different cortical site. Abbreviations: Srt: sartorius; TA: tibialis anterior; GL: gastrocnemius lateralis.

Fig 7: Proportion of cortical and pyramidal sites evoking responses that exceeded the

interval of confidence of the SE of the mean of the algebraic sum of the cortical and cutaneous nerve stimulation for left and right cutaneous reflexes in four selected muscles during swing. Gray bars illustrate the proportion of cortical sites evoking a facilitation or depression of the left (contralateral) cutaneous reflexes. Dotted lines illustrate the same information for stimulation of the pyramidal tract. The solid lines illustrate the proportion of cortical sites evoking a significant modulation on different right (ipsilateral), crossed cutaneous reflexes.

Fig. 8: Modulation of the reflex responses evoked by the 3 cutaneous nerves by conditioning stimuli to a single cortical site.

Table I

Cutaneous Nerve Stimulated

	Left cutaneous reflexes			**Right cutaneous reflexes**		
Cat:MC	Saphenous	Superficial Peroneus	Tibial Posterior	Saphenous	Superficial Peroneus	Tibial Posterior
			Stimulation of the Motor Cortex			
24	-	-	-	5	5	-
25	16	11	9	4	-	-
26	1	1	1	1	1	1
27	3	-	-	-	3	-
Total	**20**	**12**	**10**	**10**	**9**	**1**
			Stimulation of the Pyramidal Tract			
24	-	-	-	1	1	-
25	3	2	3	3	-	1
26	1	1	1	1	-	1
27	1	-	-	-	1	-
Total	**5**	**3**	**4**	**5**	**2**	**2**

Number of experiments in which cortical or pyramidal tract stimulation was combined with stimulation of different cutaneous nerves. Contralateral and ipsilateral pertain to the site of the cortical stimulation.

Table II

Relative increase in the magnitude of the reflex responses

Left cutaneous reflexes **Right cutaneous reflexes**

Saphenous

	Facilitation		Depression		Facilitation		Depression	
Muscles	mean ± SD	prop	mean ± SD	prop	mean ± SD	prop	mean ± SD	prop
St	189.5 ± 77.3	70.0	29.0 ± 20.1	60.0	190.5 ___	11.1	29.0 ± 32.3	44.4
TA	397.6 ± 576.0	30.0	46.6 ± 26.1	30.0	133.9 ± 12.5	22.2	53.0 ± 15.9	44.4
EDL	320.2 ± 389.7	30.0	39.0 ± 19.4	25.0	146.8 ± 15.5	22.2	49.5 ± 16.6	66.7
Srt	152.7 ± 38.2	15.0	53.2 ± 19.0	70.0	___ ___ ___		48.6 ± 18.7	100.0
EDB	1753.0 ___	5.0	17.8 ___	5.0	370.8 ___	11.1	36.1 ___	11.1
GL	275.5 ± 98.7	45.0	33.6 ± 21.6	15.0	183.6 ± 41.9	33.3	12.2 ± 4.0	22.2
GM	442.1 ± 252.6	20.0	___ ___ ___		212.6 ± 71.5	33.3	11.7 ___	11.1
VL	___ ___ ___		26.3 ± 32.6	10.0	140.1 ± 7.6	22.2	25.9 ± 20.9	55.6
BF	240.8 ± 3.2	10.0	31.9 ± 23.8	10.0	___ ___ ___		30.0 ± 18.4	22.2
Sol	155.4 ___	5.0	___ ___ ___		___ ___ ___		___ ___ ___	
FDL	___ ___ ___		___ ___ ___		250.2 ± 166.6	33.3	___ ___ ___	

Superficial Peroneal

	Facilitation		Depression		Facilitation		Depression	
Muscles	mean ± SD	prop	mean ± SD	prop	mean ± SD	prop	mean ± SD	prop
St	228.7 ± 56.8	80.0	29.6 ± 19.6	70.0	138.5 ___	14.3	44.6 ± 19.5	42.9
TA	228.0 ± 93.4	50.0	46.2 ± 31.8	30.0	___ ___ ___		55.8 ± 14.2	57.1
EDL	265.7 ± 154.5	60.0	45.6 ± 36.8	30.0	___ ___ ___		60.0 ± 12.1	71.4
Srt	194.6 ___	10.0	30.5 ± 26.0	100.0	132.8 ± 1.7	28.6	54.8 ± 16.5	85.7
EDB	2125 ± 2560	20.0	___ ___ ___		___ ___ ___		___ ___ ___	
GL	312.8 ± 89.6	70.0	26.9 ± 23.5	50.0	871.2 ± 1192.0	42.9	56.6 ± 7.0	28.6
GM	742.8 ± 379.6	50.0	27.7 ± 17.4	20.0	480.0 ± 184.2	28.6	51.1 ___	14.3
VL	___ ___ ___		23.7 ___	10.0	___ ___ ___		48.9 ± 10.6	28.6
BF	511.3 ± 348.8	40.0	5.3 ± 0.2	20.0	___ ___ ___		___ ___ ___	
Sol	441.1 ± 215.6	20.0	3.3 ___	10.0	___ ___ ___		___ ___ ___	
FDL	___ ___ ___		___ ___ ___		214.5 ± 25.2	28.6	___ ___ ___	

Tibial Posterior

	Facilitation		Depression		Facilitation		Depression	
Muscles	mean ±SD	prop	mean ±SD	prop	mean ±SD	prop	mean ± SD	prop
St	1055 ± 2087	60.0	26.1 ± 13.2	70.0	___ ___	___	7.5 ___	100.0
TA	198.6 ± 63.7	20.0	14.9 ± 20.2	80.0	___ ___	___	61.8 ___	100.0
EDL	246.4 ± 75.5	50.0	19.9 ± 16.7	80.0	___ ___	___	54.8 ___	100.0
Srt	137.7 ± 15.3	40.0	52.2 ± 15.2	100.0	___ ___	___	41.7 ___	100.0
EDB	___ ___	___	69.7 ± 2.9	30.0	___ ___	___	___ ___	___
GL	277.1 ± 139.3	40.0	36.2 ± 14.0	30.0	___ ___	___	___ ___	___
GM	188.9 ___	10.0	___ ___	___	___ ___	___	___ ___	___
VL	1163.0 ___	10.0	30.6 ± 20.8	40.0	___ ___	___	___ ___	___
BF	1033 ± 1527	50.0	33.5 ___	10.0	___ ___	___	___ ___	___
Sol	895.7 ___	10.0	___ ___	___	___ ___	___	___ ___	___
FDL	___ ___	___	___ ___	___	___ ___	___	___ ___	___

Cortical modulation of left and right cutaneous reflexes during locomotion in fours cats MC24-27. For each nerve, the table provides information on the mean (± standard deviation: SD) facilitation and depression in the reflex response produced by different nerves of the left and right hindlimbs (expressed as a percentage of the algebraic sum of the control values) as well as the proportion (prop)of cortical sites that produced either a facilitation or depression as a percentage of the number of sites.

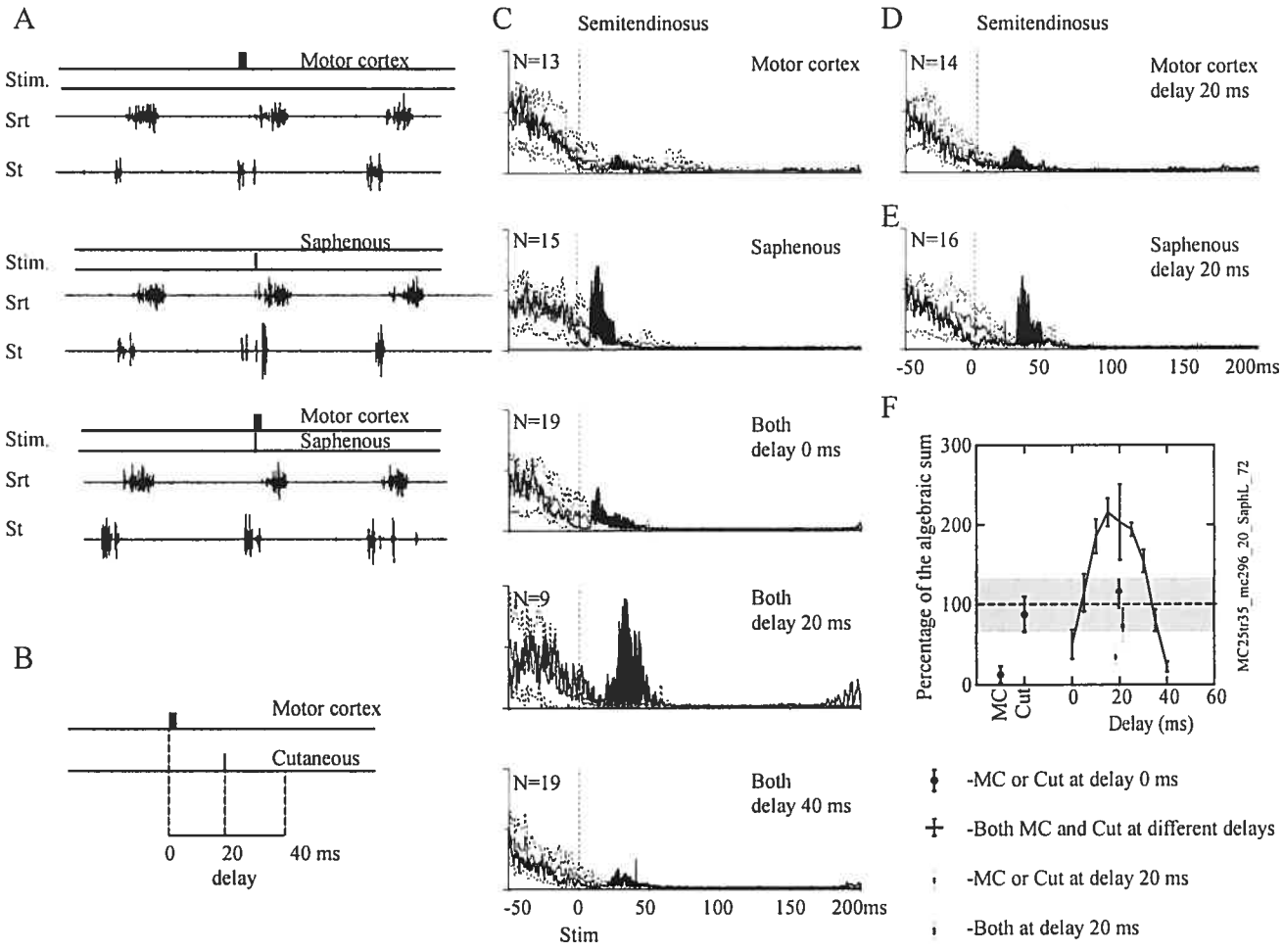
A
Motor cortex
Stim.
Srt
St
Saphenous
Stim.
Srt
St
Motor cortex
Saphenous
Stim.
Srt
St
B
Motor cortex
Cutaneous
0
20
40 ms
delay
C
Semitendinosus
N=13
Motor cortex
N=15
Saphenous
N=19
Both
delay 0 ms
N=9
Both
delay 20 ms
N=19
Both
delay 40 ms
-50
0
50
100
150
200ms
Stim
D
Semitendinosus
N=14
Motor cortex
delay 20 ms
E
N=16
Saphenous
delay 20 ms
-50
0
50
100
150
200ms
F
Percentage of the algebraic sum
300
200
100
0
MC
Cut
0
20
40
60
Delay (ms)
MC25tr35_mc296_20_SaphL_72
-MC or Cut at delay 0 ms
-Both MC and Cut at different delays
-MC or Cut at delay 20 ms
-Both at delay 20 ms

Figure 1

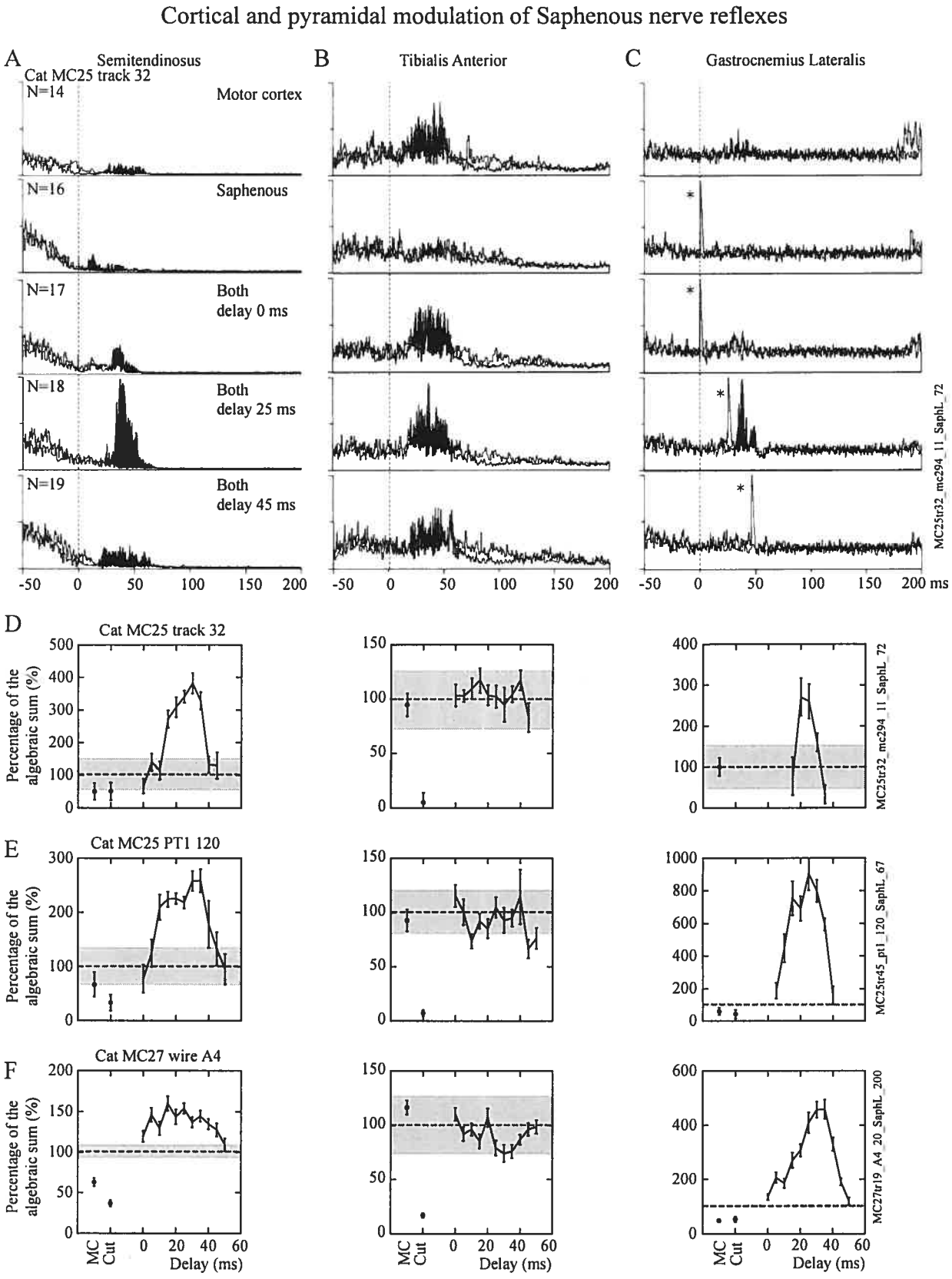
Cortical and pyramidal modulation of Saphenous nerve reflexes
A
Semitendinosus
B
Tibialis Anterior
C
Gastrocnemius Lateralis
Cat MC25 track 32
N=14
Motor cortex
N=16
Saphenous
N=17
Both
delay 0 ms
N=18
Both
delay 25 ms
N=19
Both
delay 45 ms
-50 0 50 100 150 200 ms
MC25tr32_mc294_11_SaphL_72
D
Cat MC25 track 32
E
Cat MC25 PT1 120
MC25tr45_pt1_120_SaphL_67
F
Cat MC27 wire A4
MC27tr19_A4_20_SaphL_200
Percentage of the algebraic sum (%)
MC Cut
Delay (ms)

Figure 2

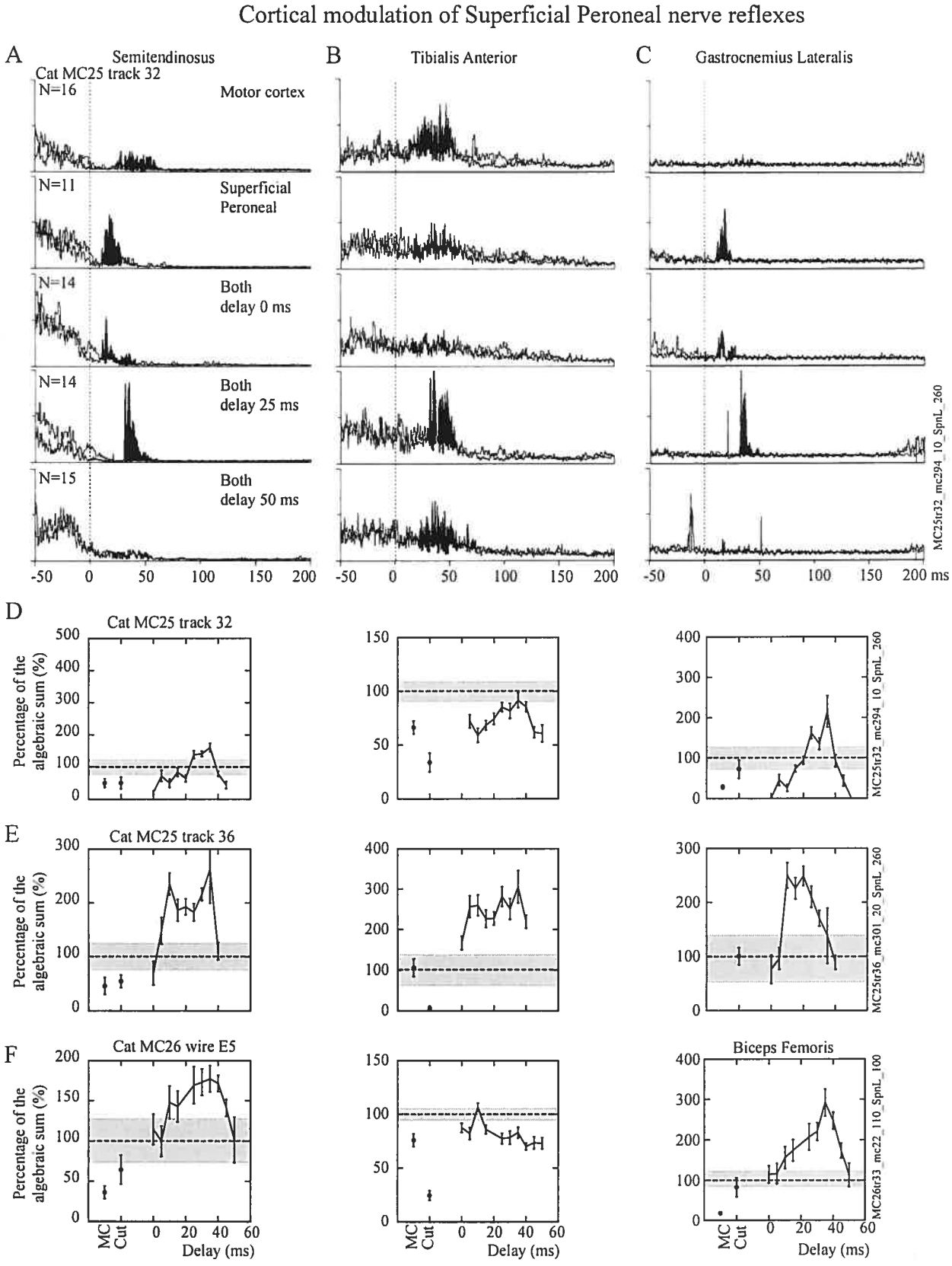
Cortical modulation of Superficial Peroneal nerve reflexes
A
Semitendinosus
Cat MC25 track 32
N=16
Motor cortex
N=11
Superficial Peroneal
N=14
Both delay 0 ms
N=14
Both delay 25 ms
N=15
Both delay 50 ms
B
Tibialis Anterior
C
Gastrocnemius Lateralis
-50 0 50 100 150 200 ms
MC25tr32_mc294_10_SpnL_260
D
Cat MC25 track 32
Percentage of the algebraic sum (%)
E
Cat MC25 track 36
MC25tr36_mc301_20_SpnL_260
F
Cat MC26 wire E5
Biceps Femoris
MC26tr33_mc22_110_SpnL_100
MC Cut
Delay (ms)

Figure 3

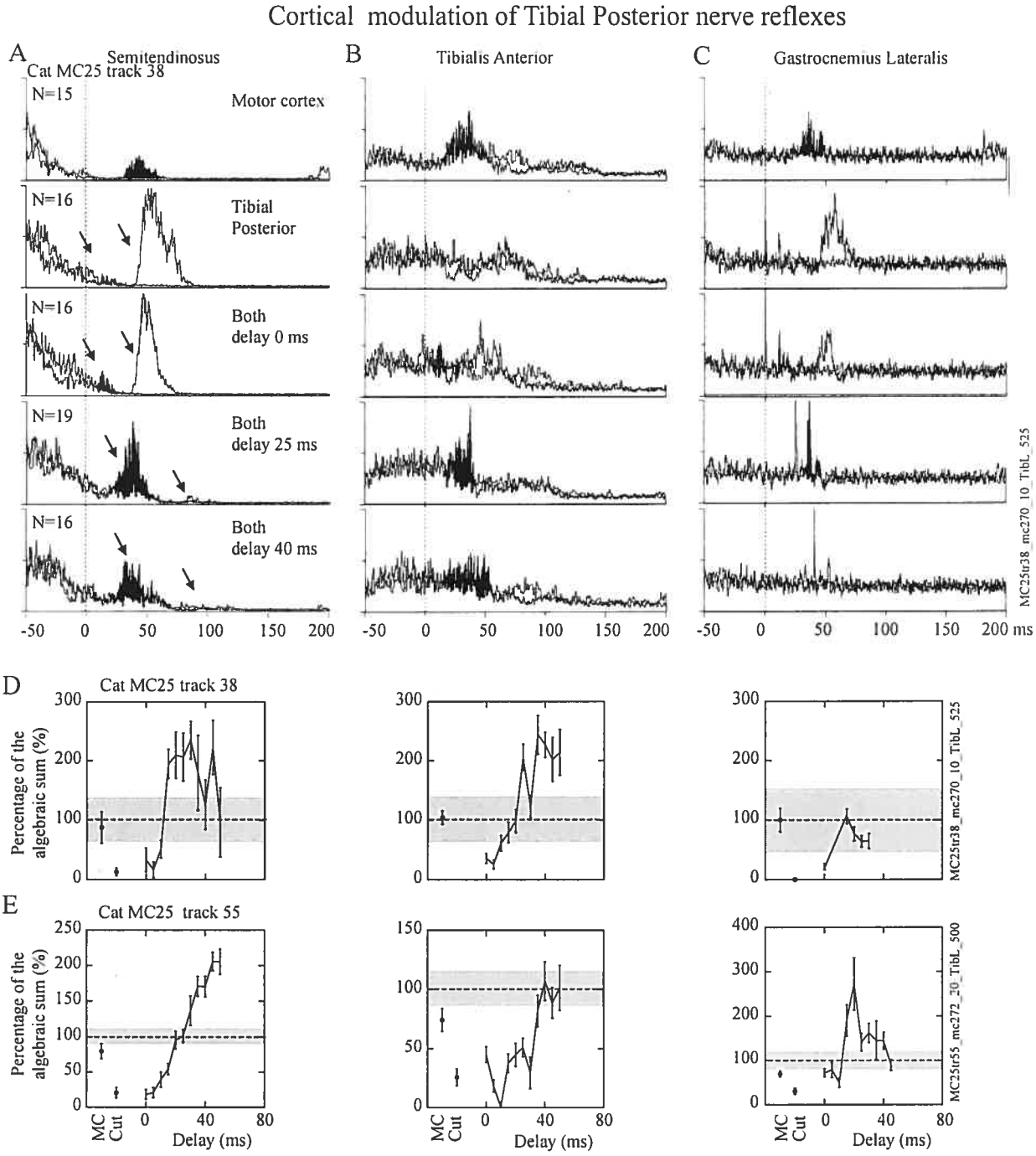
Cortical modulation of Tibial Posterior nerve reflexes
A
Semitendinosus
Cat MC25 track 38
N=15
Motor cortex
N=16
Tibial Posterior
N=16
Both delay 0 ms
N=19
Both delay 25 ms
N=16
Both delay 40 ms
B
Tibialis Anterior
C
Gastrocnemius Lateralis
-50 0 50 100 150 200 ms
MC25tr38_mc270_10_TibL_525
D
Cat MC25 track 38
Percentage of the algebraic sum (%)
E
Cat MC25 track 55
MC Cut
Delay (ms)
MC25tr38_mc270_10_TibL_525
MC25tr55_mc272_20_TibL_500

Figure 4

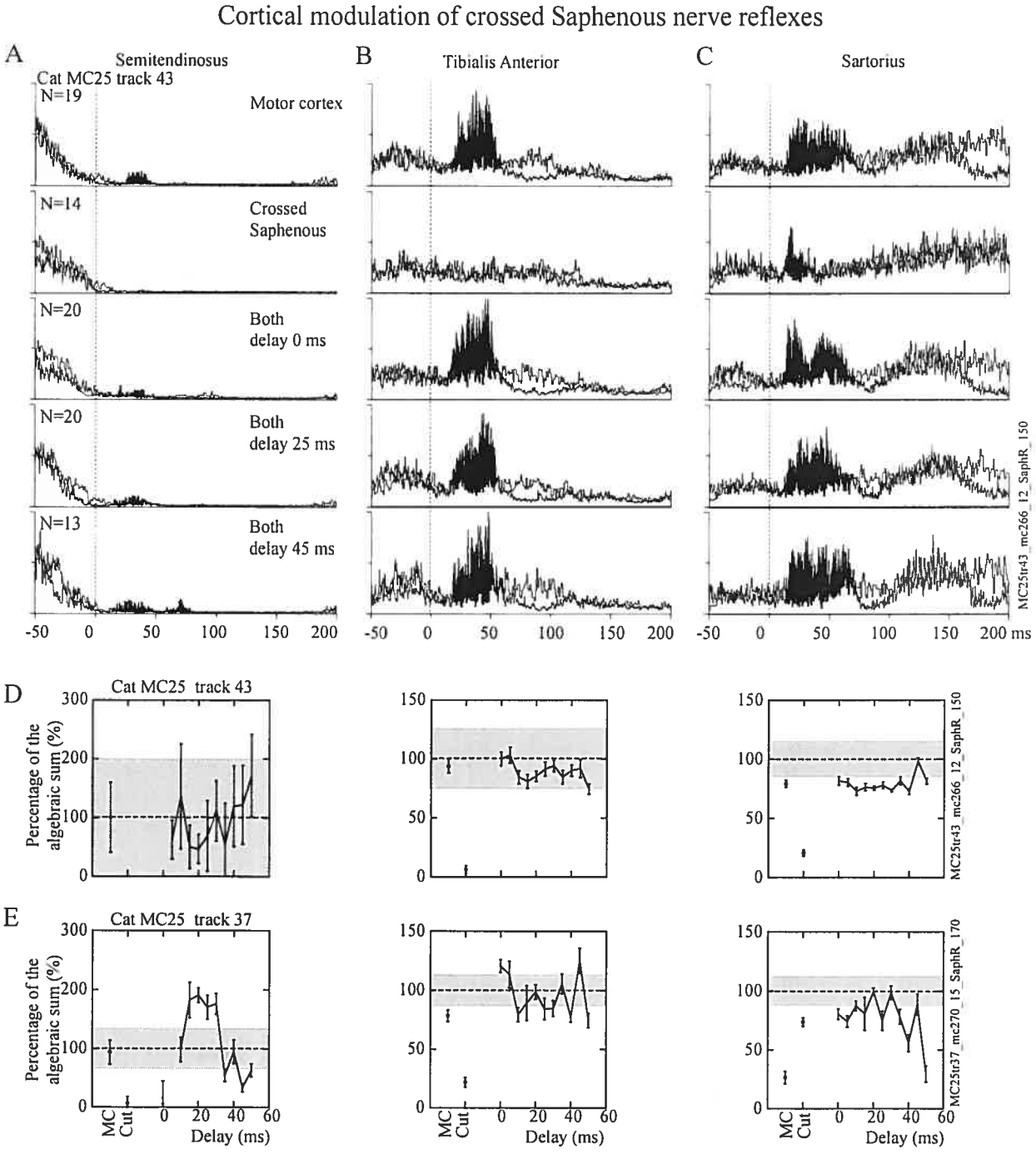
Cortical modulation of crossed Saphenous nerve reflexes
A
Semitendinosus
Cat MC25 track 43
N=19
Motor cortex
N=14
Crossed Saphenous
N=20
Both delay 0 ms
N=20
Both delay 25 ms
N=13
Both delay 45 ms
B
Tibialis Anterior
C
Sartorius
MC25tr43_mc266_12_SaphR_150
-50 0 50 100 150 200 ms
D
Cat MC25 track 43
Percentage of the algebraic sum (%)
E
Cat MC25 track 37
MC25tr37_mc270_15_SaphR_170
MC Cut
Delay (ms)

Figure 5

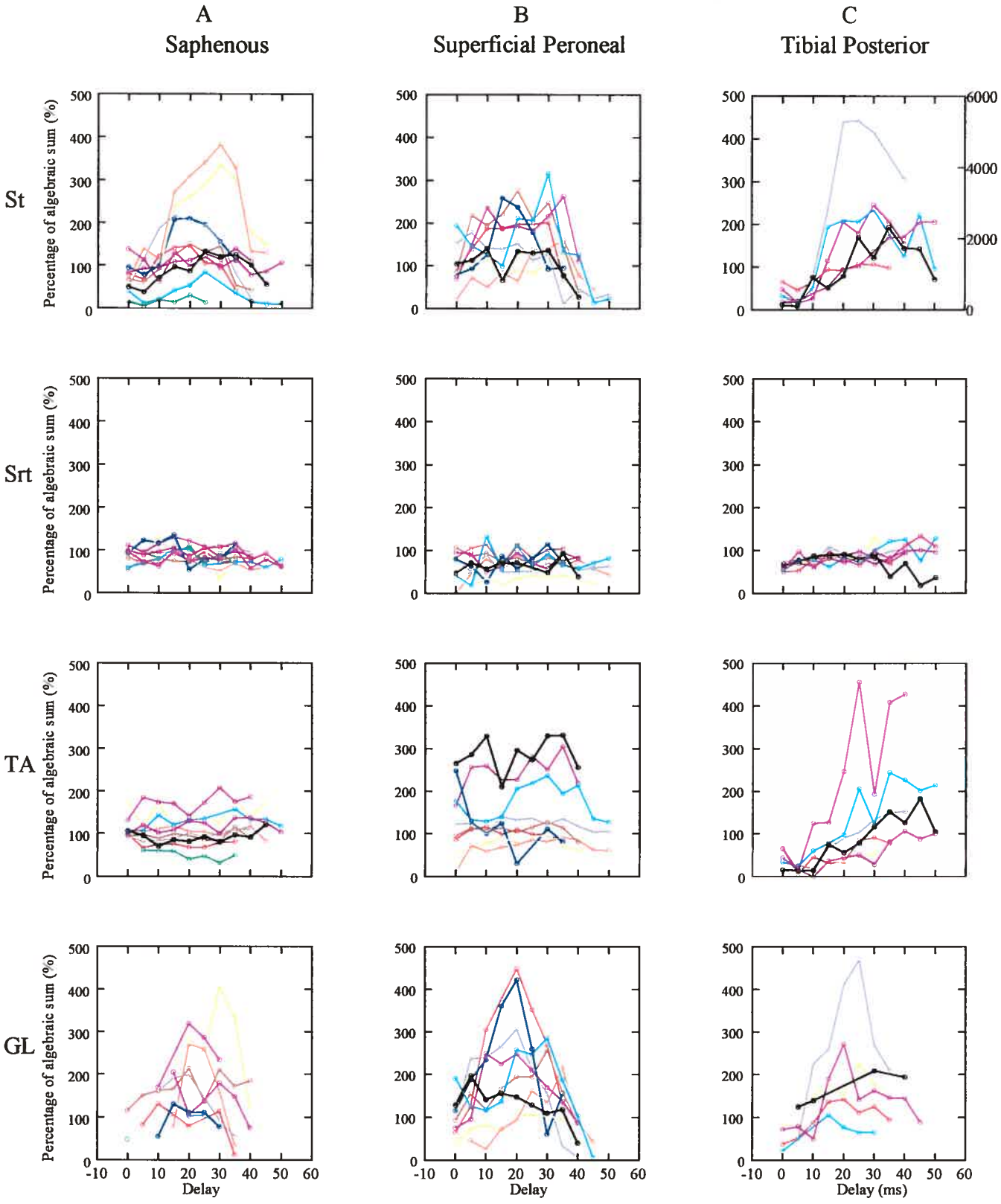
A
Saphenous
B
Superficial Peroneal
C
Tibial Posterior
St
Srt
TA
GL
Percentage of algebraic sum (%)
Delay
Delay (ms)

Figure 6

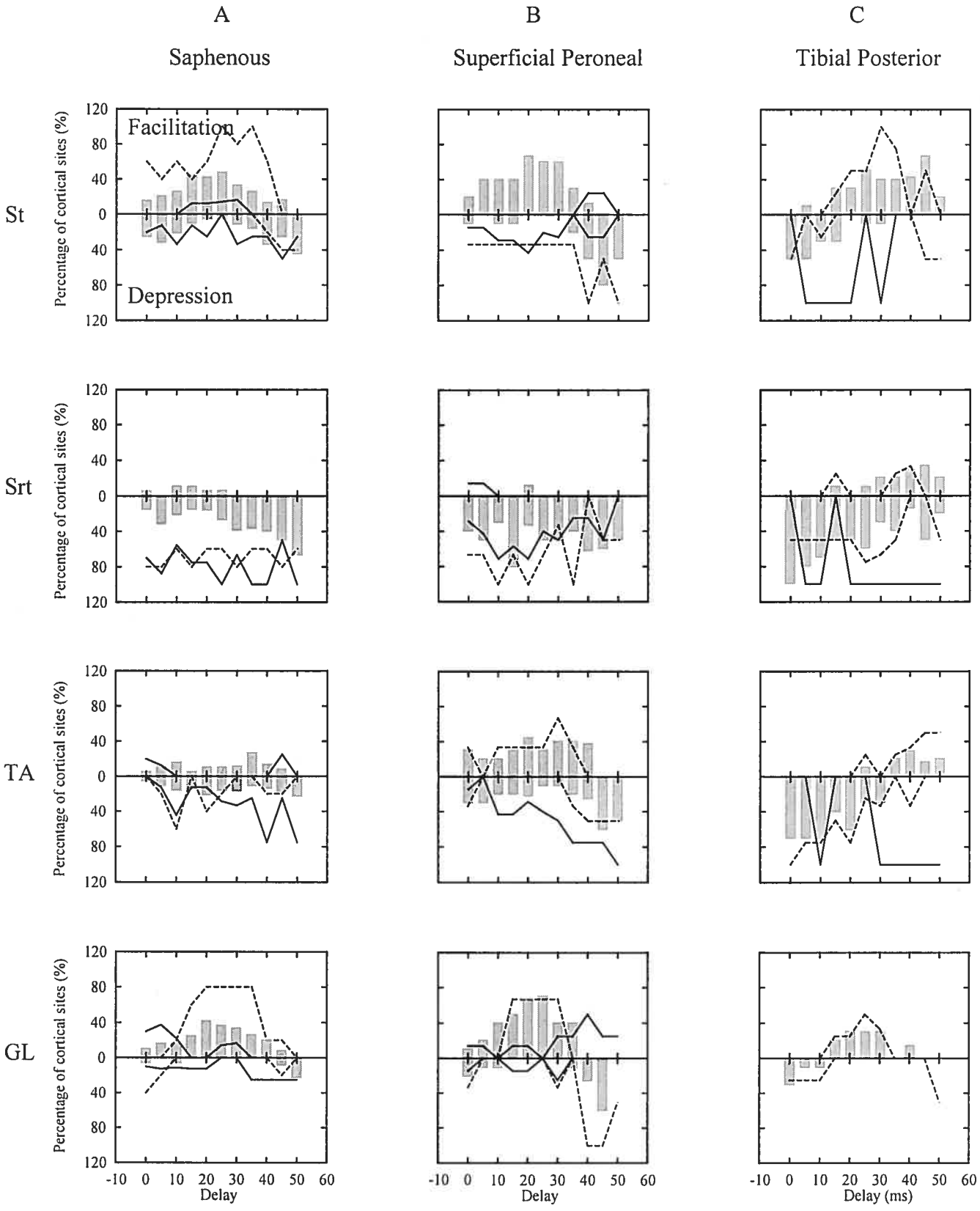
A
Saphenous
B
Superficial Peroneal
C
Tibial Posterior
St
Srt
TA
GL
Facilitation
Depression
Percentage of cortical sites (%)
120
80
40
0
40
80
120
-10 0 10 20 30 40 50 60
Delay
Delay (ms)

Figure 7

St

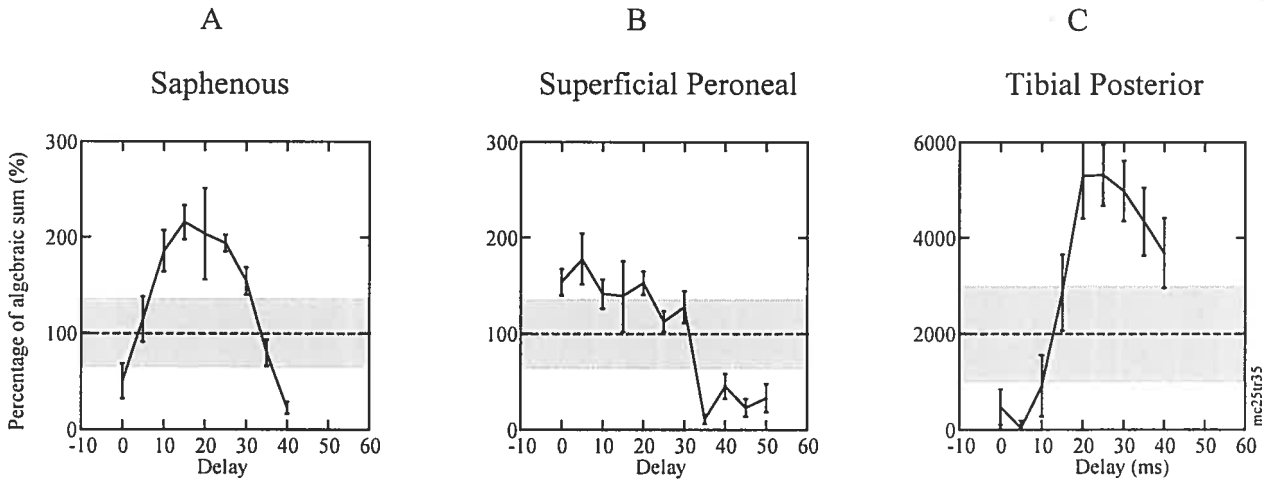
A
Saphenous
B
Superficial Peroneal
C
Tibial Posterior
Percentage of algebraic sum (%)
300
200
100
0
6000
4000
2000
-10 0 10 20 30 40 50 60
Delay
Delay (ms)
mc25tr35

Figure 8

Article # 3

Changes in corticospinal efficacy contribute to the locomotor plasticity observed following unilateral cutaneous denervation of the hindpaw

by

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Abstract

We used microwire electrodes chronically implanted into the hindlimb representation of the motor cortex as well as into the pyramidal tract to test the hypothesis that the corticospinal system contributes to the locomotor plasticity that is observed following cutaneous denervation of the cat hindpaw. A total of 23 electrodes implanted into the motor cortex in 3 cats trained to walk on a treadmill produced phase-dependent, short-latency, twitch responses in hindlimb flexor and extensor muscles during locomotion. After ≥ 1 month, we performed a unilateral cutaneous denervation of the hindpaw. Following denervation, the cats showed transient deficits in locomotion, including a dragging of the hindpaw along the treadmill belt during the swing phase of locomotion. This deficit rapidly recovered over the course of a few days. The behavioural changes were accompanied by a pronounced increase in the magnitude of the responses evoked in the different muscles by the cortical stimulation ; this increase followed a similar time-course. Changes in corticospinal efficacy were observed at all 23 cortical sites as well as at 4/5 of the pyramidal tract sites. These changes ranged up to > 1000% of control. Responses evoked by stimulation of the pyramidal tract were also increased following the denervation but to a lesser extent than those evoked by the cortical stimulation. These results suggest that the denervation produces changes in both cortical and spinal excitability that, together, produce a change in corticospinal efficacy that contributes to the observed recovery of locomotor function.

Introduction

A recent study by Bouyer and Rossignol (2003a) showed that cutaneous denervation of the cat hindpaw leads to a transient deficit in treadmill locomotion that is characterized by a dragging of the hindpaw during the swing phase of locomotion. Cats rapidly compensate for this deficit, however, by modifying the level and the pattern of activity in hindlimb flexor muscles, especially those acting around the knee and ankle.

Several lines of evidence suggest that this functional recovery of locomotion might be mediated by both a combination of spinal and supra-spinal mechanisms. For example, in contrast to the situation in non-denervated cats (Barbeau and Rossignol 1987; Belanger et al. 1996; de Leon et al. 1998, 1999; Rossignol et al. 1999, 2000), cats with a cutaneous denervation that are subsequently spinalized display marked abnormalities in the locomotor pattern, including a permanent hindpaw drag (Bouyer and Rossignol 2001; Bouyer and Rossignol 2003b). This finding, which is also observed after lesion of motor nerves (Bouyer et al. 2001; Carrier et al., 1997) and dorsal rhizotomy (Goldberger 1977, 1988) suggests that the denervation produced permanent changes in the spinal circuitry. At the same time, the deficits emphasize that the spinal plasticity is insufficient to completely compensate for the effects of the denervation, emphasizing the necessity of supraspinal descending influences for adequate recovery of function.

It is probable that at least a part of the supraspinal descending signal originates from the motor cortex. There is abundant evidence from lesion (Chambers and Liu 1957; Eidelberg and Yu 1981; Jiang and Drew 1996; Laursen and Wiesendanger 1966), single unit recording (Widajewicz et al. 1994; Kably and Drew 1998; Drew et al. 2002) and intracortical microstimulation (ICMS) (Bretzner and Drew 2005a) studies, that the motor cortex plays an important role in the control of the hindlimb during locomotion in cats,

particularly in situations that require a fine control over the paw placement or limb trajectory. In addition, electrolytic lesion of the hindlimb representation of the cat's motor cortex diminishes and delays the functional recovery following chronic cutaneous denervation of the hindpaw (Bouyer et al. 2000). Moreover, conditioning experiments have demonstrated that information from corticospinal pathway and cutaneous afferent pathways is integrated on spinal interneuronal pathways that influence the level of motoneurone activity both in the anaesthetized preparation (Fleshman et al. 1988; Lundberg et al. 1962; Lundberg and Voorhoeve 1962; Pinter et al. 1982) and in the intact cat, during locomotion (Bretzner and Drew, 2005b).

A contribution from the motor cortex in the functional recovery of locomotion following denervation is also supported by several lines of evidence in other species and in other tasks. For example, expansion of the motor cortical representation of different muscles has been reported following facial nerve section (Sanes et al. 1988, 1990; Donoghue et al. 1990) or forelimb amputation in adult (Sanes et al. 1990) and developing (Donoghue and Sanes 1987, 1988) rats. A similar expansion has been demonstrated in the cortical representation of the amputated arm compared to the unaffected side in humans (Cohen et al. 1991; Ridding and Rothwell 1995) and non-human primates (Schieber and Deuel 1997).

There is also some evidence that changes in cortical organization may also be complemented by changes in corticospinal efficacy. Enhanced responses to cortical stimulation, with respect to the unaffected side, have been reported in muscles proximal to an ischaemic nerve block of the arm (Brasil-Neto et al. 1993; McNulty et al. 2002; Ridding and Rothwell 1995, 1997; Rossi et al. 1998; Rossini et al. 1996; Ziemann et al. 1998a,b, 2001, 2002), as well as in muscles proximal to the stump in amputated humans (Chen et al. 1998).

The present experiments were designed to determine whether similar changes in corticospinal efficacy occur in our cutaneous denervation model and whether they could contribute to the recovery of locomotion observed during locomotion in the cat. To this end, we studied the responses evoked in selected hindlimb muscles by cortical stimulation applied during different phases of the locomotor cycle. The use of chronically implanted electrodes allowed each cat to serve as its own control so that the magnitude of the responses evoked following denervation could be directly related to the control responses obtained in the intact animal. In addition, to try to determine whether any changes in corticospinal efficacy were because of increased cortical excitability or changes in spinal excitability, we also examined the effects of stimulation of the pyramidal tract and the cutaneous nerves on the side contralateral to the denervation.

The results show a clear increase in corticospinal efficacy following denervation that we suggest is mediated by changes in both cortical and spinal excitability.

Methods

Care and training

Experiments were carried out on five male cats (weights 4.2-5.5 kg) trained to walk at a comfortable and constant speed (*circa* 0.35-0.45 m/s) on a treadmill. Cats were carefully selected on the basis of their willingness to walk for uninterrupted periods ≥ 20 minutes. These were the same five animals that were used in our previous experiments investigating the contribution of the motor cortex to the structure and the timing of hindlimb locomotion (Bretzner and Drew 2005a) and the corticospinal facilitation of cutaneous reflexes (Bretzner and Drew 2005b) during locomotion in the free walking cat.

Surgical Procedures

Surgical Implantation

Most of the surgical procedures used in these experiments, including details of general anesthesia, are reported in Bretzner and Drew (2005a,b). In brief, in three cats (MC23, 24 27), microwire electrodes (Tri-ML insulated stainless steel: 25 μm diameter) attached to a miniature connector (Neuralynx: EIB27) were manually inserted into the posterior bank of the cruciate sulcus that contains the hindlimb representation of the motor cortex (Bretzner and Drew 2005a; Widajewicz et al. 1994; Armstrong and Drew 1984; Nieoullon and Rispal-Padel 1976). Appropriate positioning of the microwires was facilitated by recording neuronal activity and applying ICMS as the wires were inserted. The cortex was covered with a homeostatic material (Sterispon) and the microwire connector was attached to the cat's cranium with dental acrylic. Microwires were also implanted in the pyramidal tract at P7 (Drew 1993) in cats MC24-27 to allow comparison with the

responses evoked by ICMS.

One to two weeks after recovery from the initial surgery, multiple pairs of Teflon-insulated, braided stainless steel wires were implanted into selected muscles of the fore- and hindlimbs to record EMG activity during locomotion (see Bretzner and Drew 2005a for a list of these muscles and their major functions).

In addition, in cats MC24-27, cuff electrodes (Julien and Rossignol 1982) were also implanted around the cutaneous nerves Saphenous, Superficial Peroneus and Tibial Posterior, of the right hindlimb, ipsilateral to the motor cortex and contralateral to the denervated hindlimb (Bretzner and Drew 2005b) (see below).

Cutaneous Denervation

After a period of two to four months during which we obtained our control records, a unilateral cutaneous denervation of the hindpaw was performed according to the surgical procedures detailed by Bouyer and Rossignol (2003a). In brief, the 5 cutaneous nerves inervating the left hindpaw: the saphenous, sural, tibial, superficial peroneal nerves and the cutaneous branch of the deep peroneal nerve were surgically transected. To prevent regeneration of the nerves, the proximal end was covered with a homeostatic material (gelfoam) and a flexible vinyl polysiloxane cap (Reprosil, Dentsply International, Milford, DE). Receptive fields were tested daily with a sharp probe for the rest of the experiment to ensure the absence of regeneration. In cat MC25, the sural nerve was accidentally left intact, whereas all other nerves were cut. This nerve was transected 38 days later. Cat MC27 developed allodynia and some dystonia and was treated with Baclofen, a GABAb receptor agonist acting mainly at the spinal level. Stimulation sessions in this cat were always performed 24 hrs after the last application of Baclofen. Detailed data from this cat are not

presented but, as the general trend of the results were the same in this cat as in the other two, summary results are presented in Fig. 6.

Pyramidotomy

To test the contribution of the corticospinal tract to the recovery of locomotion following the cutaneous denervation of the hindpaw, a pyramidotomy just rostral to the level of the decussation (Armstrong and Drew 1984) was performed in two cats, MC23-24. In brief, the pyramid was exposed at the level of the foramen magnun by a parapharyngeal approach and was divided with a pair of fine forceps. Recordings were pursued for ~20 days following this lesion.

Penicillin (Novopharm) (40000 UI/kg iv) and analgesics: buprenorphine hypochloride (5 μg/kg) were provided at the beginning and at the end of each surgery, and for at least 48 hours following each surgery. Antibiotics (cephadroxil: 100-200 mg/day) were administrated daily for the duration of the experiment. All surgical and experimental procedures followed the recommendations of the Canadian Council for the Protection of Animals and were approved by the local ethics committee.

Protocol

In initial experiments, we determined the threshold of all cortical and pyramidal microwires, as well as the cutaneous cuff electrodes both with the cat at rest and during the onset of the swing period during locomotion. Cortical and pyramidal microwires were tested by delivering trains of stimuli (cathodal current, 11 pulses at 330 Hz, pulse duration 0.2 ms), while cutaneous cuff electrodes were tested with a single pulse of the same duration.

In addition, we also determined the strength at which each wire would be stimulated for the remainder of the experimental protocol. This intensity was normally set so as to ensure that the stimulation evoked robust responses during swing in most flexor muscles while ensuring that these responses were well below any saturation level. This ensured that both increases and decreases in the magnitude of the responses evoked following denervation would be detectable.

Once the stimulus intensities had been determined, one of more electrodes was stimulated on each day over a period of 2 - 4 months. For each wire, we initially verified the threshold during the swing phase of locomotion and then applied stimulation, at the predetermined and fixed level, throughout the step cycle. Ten to fifteen repetitions were made at each delay in the following pseudorandom order: 50-150-300-500-700-900-0-100-200-400-600-800 and 1,000 ms after the onset of the activity in the sartorius (Srt). For cortical wires, the delay was then set to 50 ms and 15-20 stimuli were applied at intensities of 20, 25, 35, 50, 75, 100 and 150 μA. A similar procedure was also followed with stimulation of the pyramidal and cutaneous electrodes except that in these cases, the range of stimuli was determined separately for each electrode. During this control period, we tried to ensure that each electrode was tested a minimum of 3 times, evenly spaced over the control period. In addition, the impedance of the microwires was tested ~ 1/week and we also tested each cortical wire for the presence of unit activity. When units were isolated, they were tested to determine if they could be antidromically activated from one of the wires in the pyramidal tract. On selected days, we also recorded sessions of locomotion without stimulation to allow us to assess any changes in the background level of EMG activity either during the control period or following the denervation.

A similar protocol was used after the denervation with the exception that we placed more emphasis on stimulating each wire at different phases of the step cycle than on stimulating with different intensities at a single phase. This allowed us to check most wires at least once, and sometimes twice, in the week following the denervation.

In all experiments, evoked EMG responses were digitized on-line at a frequency of 5 kHz for ≥ 25 ms before and ≥ 150 ms after the onset of the stimulus train. EMGs were band-pass filtered between 100 Hz and 3 kHz. In addition, a continuous record of the EMG activity during locomotion was also digitized at 1 kHz. All of the unstimulated sessions of locomotion were video-taped and a digital time code allowed these videos to be synchronized to the EMG recordings.

Data analysis

Data were analysed as previously described (Bretzner and Drew 2005a,b; Rho et al. 1999). The responses evoked by all of the stimuli were computed-rectified and averaged and plotted on a display monitor. The average activity from a similar time period taken from unstimulated cycles was superimposed on this display (see Fig. 3C) (Drew and Rossignol, 1984). The onset and offset of the response were determined manually using the interval of confidence ($P<0.01$) of the standard error (SE) of the mean of the control activity as a guideline. Evoked responses were included in the analysis if their latency was ≤ 50ms and their duration exceeded 5ms.

For the statistical analysis of phase dependant responses, the net amplitude of the evoked EMG responses from each individual trial was computed by subtracting the mean area of the control response relative to the phase of the step cycle from that of the evoked

response within a given latency range determined on the basis of the averages. Each individual value was then allocated to one of the 10 groups and the mean and SE of the responses was calculated (see Fig. 4).

To compare background EMG patterns between sessions before and after the denervation, data segments consisting of ~40 consecutive steps at a constant speed were chosen using the videotapes of the experiments. The onset and offset of each burst of activity in a given muscle was selected by an interactive custom software. These measures were used to calculate the burst duration and the integrated amplitude of the burst, as well as the phase of activity of the muscle relative to the onset of activity in the left sartorius. For display purposes, the activity was computer rectified and averaged with respect to the onset of the period of activity in the left sartorius.

To obtain a baseline value for our statistical analysis, we made a weighted average of all of the series of data obtained prior to the denervation and calculated the interval of confidence ($p < 0.01$) of the SE of the mean. Averaged evoked responses measured after denervation were considered to be significantly increased or decreased if they fell outside of this confidence level.

Histology

At the end of all experimental manipulations, small electrolytic lesions (20-50 μA DC cathodal current) were made through selected wires. The cat was deeply anaesthetized and perfused *per aortum* with formaldehyde. The brain was removed, sectioned and stained with cresyl violet. In cats with a pyramidal lesion, sections of the brainstem and the cervical and lumbar enlargements were stained using the Swank and Davenport method (Carleton, 1967) to reveal degenerating myelin.

Results

Behaviour: functional recovery following a unilateral cutaneous denervation

The behavioural effects of a bilateral cutaneous denervation of the hindpaw have been described in detail by Bouyer and Rossignol (2003a). As there were only minor differences in the effects observed after a unilateral denervation, the behavioural effects of our intervention will not be described in detail.

In brief, the unilateral denervation always induced a transient deficit that was characterized by the cat dragging the dorsum of the hindpaw along the treadmill during the swing phase of locomotion. This period generally lasted ≤ 1 week. Inspection of the video recordings suggests that during this initial stage the limb was quite stiff and there was less excursion in all joints of the hindlimb as described by Bouyer and Rossignol (2003a). Later, all of the cats exhibited a prolonged stance phase whereby the limb was extended further behind the body than during the pre-lesion control. This was normally associated with an exaggerated knee flexion at swing onset. In addition, these cats with unilateral denervation normally exhibited a distinct limp, indicative of asymmetric locomotion.

Changes in EMG activity during the recovery period were, for the most part, compatible with these behavioural changes as illustrated for one cat in Fig 1. At six days following the denervation, the major change in activity was observed in the tibialis anterior (TA) with smaller changes in the Srt, the extensor digitorum brevis (EDB) and the left vastus lateralis (lVL). At this stage the cat lightly dragged the dorsum along the treadmill belt. Subsequently, at day 10, there was a major increase in the magnitude of the semitendinosus (St) and the EDB and a diminution in the activity of the Srt and the TA. The level of activity of the ipsilateral, left, VL was slightly increased. At this stage, the cat no

longer dragged its hindpaw. Over the following three months the St remained elevated and there was a further progressive increase in the level of activity of the iVL: the level of activity of the Srt returned to slightly above control levels. At this stage, the stance phase of the denervated limb was increased and there was an exaggerated knee flexion. There was also a significant increase of activity in the right VL, which was associated with increased elevation of the body during the swing phase of the denervated limb.

Similar compensatory changes in behavior and EMG activity were seen in the other four cats used in this study (Fig 2), although there were some variations in the exact nature of the changes. In cat MC26, for example, St and TA both showed maintained increases in activity while Srt showed a transitory increase. Activity in both VLs also increased for the first month but subsequently decreased on the denervated side. In EDB, there was a major change in the overall activity of the muscle, which discharged throughout the period of stance in the period immediately following the denervation instead of only at foot contact as in the control. In cat MC25, changes in Srt, TA, EDB and VL were similar to those in MC26, while St showed a relatively smaller increase, that was only evident following the transection of the sural nerve at day 38 post-denervation (see Methods). Completion of the denervation also caused further increases in activity in the St, TA and VL. In cat MC24, the initial changes in Srt, St, TA and both VLs were similar to those observed in MC26, although the levels of EMG activity quickly returned to control levels. The EDB showed a significant decrease in the level of activity in contrast to former cats.

Stimulation of the motor cortex

Changes in corticospinal efficacy elicited after denervation were investigated in three cats (MC23, 24 and 27) by recording and comparing the EMG responses evoked by

intracortical microstimulation during locomotion before and after denervation. Changes in the corticospinal efficacy were essentially similar in all cats. However, because of the possible complications engendered by the dystonia observed in cat MC27, emphasis will be placed on the results from cats MC23 and MC24.

During control locomotion (pre-denervation), stimulation of the motor cortex evoked phase dependent responses in all recorded hindlimb muscles (see Bretzner and Drew, 2005a; Fig 3A) Both the absolute magnitude and the phase dependency of the responses was relatively constant over the period of the control recordings. As such, calculation of the weighted mean and the interval of confidence of the SE of the mean for these control recordings resulted in a narrow band encompassing the individual responses (Fig. 3B). This interval of confidence was used as a basis for determining changes in response magnitude or phase dependency following the denervation (see Methods)

Following the denervation, there were frequently large, statistical increases in the magnitude of the evoked responses as well as of the phase dependency. Figure 4 illustrates a typical example of the responses evoked by cortical stimulation before and after cutaneous denervation during locomotion in cat MC24. Prior to the denervation, stimulation during swing evoked a weak, short latency decrease in activity in the hip flexor Srt, together with transient increased responses in the knee flexor St and the ankle flexor TA (Fig. 4A *left*). Two days after the denervation, the decrease in Srt activity produced by the cortical stimulation was lost and there was a small increase in the amplitude of the St and the TA. In addition, the increase in activity in the TA was substantially prolonged leading to a major increase in the overall magnitude of the response. At 40 days, the short latency responses in both the St and the TA were clearly more pronounced, as was the longer latency response

in the TA. In addition, the stimulus now also evoked a substantial longer latency response in the St. Short latency increased responses were now also evoked in the Srt.

More pronounced responses following denervation were also observed during stance (4B). Before the denervation, stimulation evoked small responses in TA and EDB, and a small transient decrease in the level of activity of the VL. Two days following the denervation, the responses in TA and the EDB were larger and there was also a clear evoked response in the St. The transient decrease in activity of the VL was also much more pronounced. At 40 days, responses in St, TA, EDB and in the extensor VL were still more pronounced and there was also a clear response in the Srt. Note that all of these responses in the flexor muscles occurred at a time in the step cycle when these muscles were inactive.

Figure 5 (left column) summarises the net amplitude of changes in the phase dependent responses evoked by the cortical site illustrated in Fig. 4 before (shaded area) and 2 (dotted line) and 40 (solid line) days following the denervation. Before denervation, responses in the hip flexor Srt were weakly decreased during early swing (Fig 4A) and weakly increased during late swing; there were no responses during stance (Fig 4B). Two days after denervation, (dotted line) the responses during late swing and early stance, were more pronounced. At forty days post-denervation, (solid line) increased responses were observed throughout the step cycle, including stance, but were maximal in late swing. As illustrated in Fig 4A, the denervation markedly increased the duration of the cortically evoked responses in the St and TA in this cat. In this example, the response was divided into a short-latency response, equivalent to the response evoked before denervation, and a longer-latency response that was absent before denervation. Fig. 5A illustrates that the denervation increased both the short latency response and the total magnitude of the

response (inset) throughout the step cycle. Moreover, the short latency response evoked during stance, when the muscle was inactive, was only slightly smaller than that evoked during the period of activity of the muscle, at the end of stance and the onset of swing. Responses in EDB were increased during stance at day 2 post-denervation and were still more pronounced later. In the knee extensor VL, the decreased responses produced during stance before denervation, were more and more pronounced following the denervation. Very similar changes in the phase dependent responses were evoked by stimulation at 35μA from another cortical site, D6, in the same cat MC24 (5B), although the responses evoked in the EDB were smaller and more variable than those illustrated in Fig 5A.

The nature of these responses was very similar in the other two cats used in this part of the study. Fig 5C, for example, shows the changes in the responses evoked by cortical microstimulation at 25 μA at a site in cat MC23. As in cat MC24, denervation resulted in an increase in the magnitude of the responses evoked in the flexor muscles, Srt, St and TA during swing. However, in this cat, no excitatory responses were observed in stance even at 2-3 months following denervation. In addition in this site there were never any long-latency responses evoked in St and TA. This was the case for all 11 sites stimulated in cat MC23. Responses in EDB were small and variable (as in Fig 5B), and the transient decrease in activity in the VL was much increased following denervation, as in the examples in Figs 5A and B.

Although not illustrated, there were also significant changes observed in other hindlimb muscles. Increased responses in the ankle flexor, Extensor Digitorum Longus, followed the same tendency as that in the TA. Changes in responses of the type observed as the knee extensor, VL, were also observed in the other recorded extensors, including the lateral and medial heads of the Gastrocnemius, the Flexor Digitorum Longus and the

Soleus. Increased responses were also observed in the hip extensor, the Biceps Femoris.

Figure 6 summarizes the time course of the percentage change of the net amplitude of responses evoked during locomotion for all stimulated cortical sites in cats MC23, 24 and MC27. As illustrated for the examples in Figs 4 and 5, the denervation resulted in a rapid increase in the magnitude of the evoked responses in all muscles, and from all cortical sites, in all cats. In the illustrated flexor muscles (Srt, St and TA), increases to > 250 % of control magnitude were observed in 10/23 wires in the three cats within 2-5 days of the denervation and in some cases, changes of > 500 % were seen. In the VL, responses were also more pronounced, in this case representing a more pronounced decrease in activity (plotted as a negative value in Fig. 6). At most cortical sites, there was a rapid change in the magnitude of the response within a few days of the denervation. This was normally followed by a progressive increase in the amplitude of the evoked responses throughout the period of study (2-3 months).

Stimulation through most cortical electrodes evoked increased responses in most of the muscles that we recorded. Inspection of Figs. 4 - 5, for example show that stimulation at site D4 in cat MC24 (black line in Fig. 6A) evoked increased responses in all of the flexor muscles, as well as a more pronounced decrease of activity in the VL. However, inspection of Fig. 6 also suggests that the relative change in activity evoked in the different muscles varied from one electrode to another. For example, the wire D4 was one of the most effective electrodes in producing increased activity in the EDB and the TA but evoked weak changes in Srt, when compared to those evoked from other electrodes. In contrast wire D6 in the same cat (green line in Fig. 6A) produced relatively large increases in the level of activity in the Srt but relatively weak responses in the EDB. Similar differential effects

can also be observed for many of the electrodes in cats MC23 and MC27. These differential effects are quantified in Fig. 7A for wires D4 and D6 from cat MC24 as well as for two others, wires A2 (orange line in Fig 6A) and A6 (brown line in Fig. 6A) in the same cat. Inspection of this figure clearly shows the differential nature of the responses evoked in Srt and TA by wires D4 and D6 and shows a similar property for wire A2 which also produced the largest responses in the Srt. In contrast wire A6 produced similar increases in the Srt, St and EDB but only small changes in the activity of the TA. Inspection of Fig. 7B shows a similar characteristic for the changes in corticospinal efficacy observed in cat MC23. For example, wire B1 (blue line in Fig. 6) produced very large increases in the magnitude of the response evoked in the St but relatively small changes in the other flexor muscles. In contrast, wire B6 (yellow line in Fig. 6) produced strong changes only in the activity of the EDB.

In addition to increasing the relative magnitude of responses evoked by the motor cortex at any one stimulus intensity, the denervation also increased the gain of the response. Figure 8A illustrates the relationship between the magnitude of the response evoked in the St at the onset of swing and the intensity of the stimulation for two electrodes in cat MC23. Before the denervation (dotted lines), increasing stimulus intensity produced a relatively linear increase in the response magnitude up to a value of 75 μA. Intensities greater than this value produced no further increase in magnitude, leading to the plateau observed in the graphs. Following the denervation, the nature of this relationship was modified such that not only were larger responses evoked for any given intensity but also the responses became progressively increased at higher intensities. In other words, the gain of the relationship was modified as can be appreciated from the increased slopes of the relationships; this is

particularly evident for wire B6 (Fig. 8A, *right*). In addition, in most wires, it was also clear that the amplitude of the responses evoked at the lowest intensities used were also increased (Fig. 8A, *left*). This suggests that there was also a decrease in the threshold of the responses, although as we used a fixed series of intensities for these experiments, this was not tested directly.

Cortical versus spinal plasticity

To try to determine the extent to which the increased corticospinal efficacy might be due to changes in the efficacy of the motor cortex or spinal interneuronal networks, we investigated changes in the magnitude of the responses evoked by the pyramidal tract and crossed cutaneous reflexes in four cats (MC24-27). Because EMG responses evoked by stimulation of the pyramidal tract are independent of the level of cortical activity (Schmied and Fetz, 1987), they provide an indication of the effect of changes in excitability of spinal interneuronal networks on corticospinal efficacy. Similarly, stimulation of the cutaneous nerves also provides an indication of changes in spinal excitability.

Stimulation of the pyramidal tract

Figure 9 illustrates a typical example of the responses evoked during the swing and stance phases of the step cycle by stimulation of the pyramidal tract before and after cutaneous denervation in cat MC26. Prior to the denervation, stimulation during swing evoked an increase in activity in the hip flexor Srt, together with increased responses in the knee flexor St, the ankle flexor TA and the knee extensor, VL. Nine days after the denervation, there was a slight decrease in the magnitude of the Srt and the VL, while responses were slightly more pronounced in the St and TA. At 52 days post-denervation,

the responses in the Srt and the St were increased, while that in TA was relatively unchanged. Before denervation, the only response evoked in stance was a small transient decrease in VL. Following the denervation, the decrease in the activity in the VL was similar to that observed in the control recording while there were now small responses evoked in Srt and TA.

Figure 10 (left column) summarises the net amplitude of the changes in the phase dependent responses evoked by the stimulation of the pyramidal tract illustrated in Fig. 9 before (shaded area) and following the denervation (dotted and solid lines). In general, the magnitude of the responses evoked following the denervation were slightly increased above control levels in all 3 flexor muscles during the swing phase of locomotion although these changes were less than those observed from stimulation of the individual cortical electrodes (see Figs. 5,6 and below). There were also slight increases in amplitude during the stance phase in the TA. Responses in EDB and in VL were relatively little changed. Similar changes were observed in cat MC25 although the relative magnitude of the increase in responses of St and TA was greater than in cat MC 26. In cat MC24, no changes in response amplitude were observed after denervation.

Stimulation of cutaneous crossed reflexes

Figure 11 illustrates a typical example of the responses evoked in four left hindlimb muscles and one right hindlimb muscle by stimulation of the right superficial peroneal nerve during locomotion before and after the cutaneous denervation in cat MC24. Stimulation during the swing phase of the denervated hindlimb (Fig. 11A) before denervation, evoked responses in the hip flexor, Srt, and the ankle flexor, TA during the normal period of activity. Following the denervation, the magnitude of the evoked responses were relatively

stable at day 5 as well as at day 35. During the stance phase of the left hindlimb (Fig. 11B) before denervation, weak responses were evoked in the EDB and the VL. Denervation had little effect on the magnitude of any of these responses. Because the stimulation during the stance phase of the denervated limb corresponded to the swing phase of the stimulated limb, large responses were also evoked in the St of the stimulated limb (rSt). The magnitude of these responses was also unaffected by the stimulation.

Figure 12 summarises these results and emphasizes the absence of large changes in the net amplitude of phase dependent responses evoked by the stimulation of the right superficial peroneal nerve following the denervation in cat MC24 (Fig. 11 and 12A), as well as in other cats MC25 (Fig. 12B) and MC26 (Fig. 12C).

Summary

The relative changes in the magnitude of the responses evoked in the St, Srt and the TA by stimulation of the pyramidal tract and the superficial peroneal nerve are summarized in Fig. 13. In cats MC25 and MC26, stimulation of the pyramidal tract (Fig. 13A) evoked changes of $\geq 200\%$ in the magnitude of the TA and Srt and $> 500\%$ in the magnitude of the St; there were no changes following stimulation in cat MC24. There were no significant changes in the magnitude of the EDB or the VL. Stimulation of the superficial peroneal nerve in all 3 cats (Fig. 13B) produced only small increases in activity following the denervation. In a similar manner, the responses evoked by stimulation of the saphenous (Fig. 13C) and tibial posterior nerves (Fig. 13D) were also relatively unchanged after denervation.

Pyramidotomy

To determine whether the integrity of the corticospinal tract is necessary for the changes in the magnitude of the responses following the denervation we attempted to lesion the pyramidal tract at the level of the medulla oblongata in 2 cats. In one of these cats (MC23) the lesion almost completely transected the pyramidal tract as illustrated in Fig. 14A. Inspection of this figure shows the physical extent of the damage in the right pyramid (top) and the course of the degenerating fibres in, from top to bottom, the pyramidal decussation, and the cervical and lumbar spinal cord. Degenerating myelin is clear in both the left dorsolateral and right ventromedial funiculus at the cervical level of the spinal cord and is also visible in a similar location in the lumbar spinal cord, demonstrating that corticospinal fibres inervating lumbar regions were transected by the lesion. Following this lesion, the cat dragged both the dorsum of both the forepaw and the hindpaw along the treadmill during the swing phase of locomotion. In the forelimb, this deficit recovered over a period of a few days while the hindlimb continued to drag lightly for the remainder of the experiment . Microstimulation through the cortical wires at the same intensities used prior to the lesion was ineffective in producing muscles responses following the pyramidotomy, either at rest or during locomotion. Fig. 14 B shows one example in which stimulation following the denervation but prior to the pyramidotomy produced clear short latency responses in the St and TA at a strength of 25 μA. Following the pyramidotomy, these responses are lost even at current intensities of 75 μA.

Discussion

Cutaneous denervation of the hindpaw produced locomotor deficits that were rapidly compensated by modifying the level of the background EMG activity in both flexor and extensor muscles. This recovery of function was accompanied by rapid increases in the magnitude of the responses that were evoked by cortical stimulation. We suggest that this increase in corticospinal efficacy contributes to the recovery of locomotor function. Changes in the magnitude of the responses evoked by stimulation of the pyramidal tract or by contralateral cutaneous nerves were relatively small or non-existent suggesting that substantial changes in corticospinal excitability contributed to the observed increase in corticospinal efficacy.

Functional recovery following the denervation

The overall changes that we observed following the denervation were very similar to those detailed by Bouyer and Rossignol (2003a) following a bilateral cutaneous denervation. As in their study, the major deficit that we observed was a transient inability to flex the leg sufficiently to prevent the paw dragging on the treadmill surface at the onset of swing.The cats rapidly compensated for this deficit by modulating the level of activity in different flexor and extensor muscles throughout the limb. Similar rapid changes in the level of EMG activity have been described after other interventions designed to examine locomotor plasticity, notably after lumbosacral rhizotomy (Goldberger, 1977, 1988; Goldberger and Murray, 1974), and motor neurectomy of either ankle flexors (Carrier et al., 1997) or ankle extensors (Whelan and Pearson 1997; Pearson et al., 1999; Pearson and Misiaszek, 2000; Misiaszek and Pearson, 2002; Pearson et al., 2003).

In our study, as in that of Bouyer and Rossignol (2003a), a major contribution to the recovery of function was made by increased activity in the St. This increased activity would serve to increase the flexion of the knee and raise the paw away from the treadmill belt. Changes in most other muscles, however, were more variable and there was no one clear pattern that repeated itself in the different cats. This suggests that each cat devised its own unique strategy to compensate for the deficit by differentially modifying the activity in hip, knee and flexor muscles. In this respect, it is interesting to note that there were frequently reciprocal changes in the level of activity in the hip and ankle flexors over the recovery period suggesting that the cats continued to modify their strategy during the recovery period. Lastly, it should also be noted that because the lesion that we used was unilateral there were also compensatory changes in the other hindlimb, consisting of an increase in the level of the extensor muscles, such as the VL. We suggest that this increase in extensor muscle activity would serve to elevate the level of the pelvis during the swing phase of the denervated limb and thus also contribute to the compensation.

Unilateral denervation increased corticospinal efficacy

The major finding from these experiments was that the unilateral denervation led to a rapid increase in the magnitude of the responses evoked by stimulation of the motor cortex. We consider these increased responses as being indicative of an overall change in the efficacy of the corticospinal projection. Several factors support this suggestion and argue against the results being produced by other, unrelated, changes, such as movement of the cortical wires.

First, we recorded control activity over a period of several months before the denervation and the magnitude of the evoked responses changed very little over this period

(see e.g. Fig. 3). Second, changes in corticospinal efficacy were observed from stimulation of nearly all of the implanted electrodes and in all of these cases the change in corticospinal efficacy began shortly after the denervation. (Fig. 6). Third, we successfully antidromically activated several neurones recorded from these electrodes by stimulating the pyramidal tract both before and after the denervation (although never the same neurone). Fourth, there were no changes in the impedance of the electrodes over time. The contention that changes in corticospinal efficacy are independent of any changes in the properties or location of the microwire electrodes are fully in agreement with previous studies that have also argued that such electrodes produce constant effects over extended periods of time (Armstrong and Drew 1985; Palmer 1990; Palmer et al. 1985).

We believe that the changes in corticospinal efficacy that we observed underlie the accompanying changes in the functional recovery of locomotion. This is supported by several lines of evidence. For example, changes in the level of the EMG activity occurred in the first few days following the denervation and progressively increased over the next few weeks before reaching a plateau. The changes in corticospinal efficacy followed a similar time-course. Although it is possible that the changes in the magnitude of the evoked responses are simply a function of the increased level of background EMG activity, we feel that this is unlikely. Figure 4B, for example, shows that, following the denervation, there were substantial responses evoked in some flexor muscles during the stance phase of locomotion at a time when these muscles are inactive and presumably hyper-polarized (Jordan 1984). The increased responses are, therefore, more likely due to changes in cortical or spinal, interneuronal, excitability (see next section).

Further support for the view that these changes in corticospinal efficacy contribute

to the recovery of function comes from the two cats in which we performed a pyramidotomy following several months of recovery. Both of these animals showed immediate deficits following the transection, characterized by dragging of the fore-and hindpaws. This is characteristic of the results of damage to the corticospinal system at any level (see Armstrong 1986; Drew et al. 1996). However, in otherwise intact cats, these deficits are transitory and there is complete recovery over a period of several days. In the cats with a unilateral denervation, the deficits in the hindpaw remained over a period of several weeks while those in the forepaw quickly disappeared. This suggests that after damage of the corticospinal system, the animals were no longer able to fully compensate for the denervation. Similarly, our preliminary results (Bouyer et al. 2000) show that a lesion to the hindlimb representation of the motor cortex prior to hindpaw denervation delays or prevents recovery. Together, these results support a view that the cortex contributes to this recovery of function.

There are few other studies that have specifically examined changes in corticospinal efficacy following insult to either the peripheral or central nervous system. Moreover, even those that have examined this issue have mostly studied only the immediate changes that follow a temporary intervention. For example, evidence for changes in corticospinal efficacy have been observed both following anaesthesia (Murphy et al., 2003; Rossi et al., 1998) and ischaemic nerve block (Brasil-Neto et al., 1992, 1993; Ridding and Rothwell, 1995, 1997; Ziemman et al., 1998a,b, 2001, 2002; McNulty et al., 2002) of a limb. Such changes, however, immediately disappear after removal of the anaesthesia or nerve block and are likely to involve different mechanisms from those underlying the long-term changes observed in this study.

While changes in corticospinal efficacy were observed from most of the sites that

we studied in these experiments, the nature of the changes evoked from any one wire could be quite different. As demonstrated by Fig. 7, the magnitude of the relative changes evoked from different wires in different muscles could vary quite widely. For example, cortical site D4 in cat MC24 produced large changes in the activity of the St, TA and EDB, but not the Srt and TA while site B1 in cat MC23 produced large changes in St but not in the other flexor muscles. This variability argues against the changes being the result of some homogeneous, global change in activity and argues for some specificity in the action exerted from different regions of the motor cortex. Although the present experiments did not allow us to relate these specific changes to changes in the pattern of EMG activity, one may speculate that the relative changes in corticospinal efficacy are related to the relative changes in the level of the EMG activity required in the different muscles that are influenced from any one cortical site. However, because stimulation at most cortical sites influenced the activity of multiple muscles, to differing extent, it is difficult to relate changes at any one site to specific changes in muscle activation patterns. Moreover, it should be emphasized that the initial denervation resulted in a decrease in the level of activity of most muscles, resulting in the paw drag, and that the compensation in the level of activity in some muscles may result only in a return to pre-denervation levels, as in cat MC24 (Fig. 2C). As such, the increase in corticospinal efficacy at some cortical sites might result only in a restoration of the level of EMG activity and not an increase in the level of activity. This further complicates the possibility of correlating changes in corticospinal efficacy directly with changes in the level of EMG activity.

Mechanisms

There are several possible explanations for the changes in corticospinal efficacy that

we observed in these experiments. These can be broadly divided into changes in excitability of the spinal interneuronal networks onto which the motor cortex impinges or changes in excitability of the cortical sites stimulated. We tried to differentiate between these possibilities in these experiments by also studying the effects of the denervation on the effects evoked by stimulation of the pyramidal tract and by stimulation of cutaneous nerves in the contralateral, intact, hindlimb.

The responses evoked by stimulation of the pyramidal tract would be expected to be independent of any changes in corticospinal efficacy. This was supported by direct measurement of the effects of pyramidal tract stimulation on excitability (Schmied and Fetz 1987) as well as by inference from the differences in the effects evoked by transcranial magnetic stimulation (TMS) which activates cortical neurones directly and transcranial electrical stimulation, which activates corticospinal axons directly (Edgley et al. 1990). In our experiments, changes in corticospinal efficacy were clearly observed following pyramidal tract stimulation (although not universally) but were relatively weaker than those observed from the stimulation of the motor cortex. Nevertheless, the existence of the changes in excitability does suggest that there is some change in the excitability of the spinal interneuronal pathways onto which these cortical sites impinge. This would be compatible with the results from the experiments of Bouyer and Rossignol (2003b) showing changes in the locomotor capacity of cats spinalized following a denervation. As they have argued, this strongly suggests the existence of long-term, plastic, changes in spinal circuits.

We also found that there was no change in the magnitude of the crossed reflex responses evoked by stimulation of the contralateral cutaneous nerves (Figs. 11 and 12). This supports our suggestion made in a preceding section that the changes in efficacy are not the result of any global changes in excitability. This result also suggests that the

interneuronal populations that mediate the signals conveyed by the corticospinal tracts are separate from those that are activated by the afferents from the contralateral limb. This is compatible with the results from our previous work (Bretzner and Drew 2005b) showing that conditioning stimuli to the motor cortex generally facilitate the reflex responses from the limb contralateral to the stimulation site (corresponding to the denervated limb in this study) but depresses the crossed reflex responses.

While we argue for some changes in spinal excitability, the relative difference in the magnitude of the changes in corticospinal efficacy evoked from the cortical and pyramidal tract stimulation also suggests that changes in cortical excitability contribute to the changes that we observed. This is also supported by our finding that lesion of the pyramidal tract or of the hindlimb representation of the motor cortex (Bouyer et al. 2000) subsequent to functional recovery leads to a restoration of many of the initial deficits, suggesting that changes in spinal excitability are insufficient for a full recovery (see also Bouyer and Rossignol 2003b). Given that the changes in functional recovery and corticospinal efficacy occur rapidly following the denervation, it is possible that at least the initial changes in corticospinal efficacy are caused by increases in the discharge frequency of pyramidal tract neurones implicated in regulating hindlimb muscle timing and activity during locomotion (Widajewicz et al. 1994; Drew et al. 2002). Indeed, such increased activity in the corticospinal (and probably the rubrospinal) pathway will be needed to compensate for the decreased spinal excitability that might be expected following the loss of major portion of the cutaneous input from the hindpaw.

It is also probable that there are concomitant changes in cortical motor representation of muscles as has been described following facial nerve section or

amputation (see Introduction). Although we could not address this issue directly because our electrodes were chronically implanted, a change in cortical representation is implicit in the finding that stimulation in a given site, at a given strength, frequently recruited muscles that were not activated by stimulation preceding the denervation.

Conclusions

This study clearly shows that there is a cortical contribution to the mechanisms that underlie functional recovery of locomotion following the loss of cutaneous input from the paw. This serves to emphasize the importance of the corticospinal tract in the regulation of locomotion, demonstrating that the motor cortex not only contributes to voluntary modifications of the gait pattern but also has the capacity to modulate the base locomotor pattern in response to injury. Indeed, it is probable that the motor cortex will contribute to step by step modifications of gait under a wide variety of circumstances. The study also serves to emphasize the close interaction and integration of supraspinal and peripheral inputs in the regulation of locomotion. Taken together with the results presented in a previous publication (Bretzner and Drew 2005b), it seems clear that corticospinal and cutaneous pathways act through common interneuronal populations to modulate the activity of the hindlimb musculature during locomotion. Lastly, the results serve to emphasize that damage to the peripheral nervous system can produce plastic changes in the corticospinal system that have the capacity to produce major changes in the efficacy of this pathway in modulating motor activity.

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Figure Legends

Fig 1: An example of the changes in EMG activity following a unilateral cutaneous denervation of the hindpaw in cat MC23. A: EMG activity in selected muscles 24 days before denervation (gray) and at days 6, 10 and 82 post denervation (black) (N=35-45 step cycles). B: The mean and standard error (SE) of the mean of the integrated amplitude of the burst following denervation is plotted as a percentage of the overall weighted mean of the control responses obtained from 4 experiments (each of 35 - 45 step cycles) prior to the denervation. The shaded area illustrates the 99% confidence interval of the SE of the weighted mean. Abbreviations: EDB: Extensor Digitorum Brevis; l: left; r, right; Srt: Sartorius; St: Semitendinosus; TA: Tibialis Anterior; VL: Vastus Lateralis. Note that left is on the same side as the denervation but contralateral to the implanted motor cortex.

Fig. 2: Summary of the changes in the integrated amplitude of the EMG activity following the cutaneous denervation in three other cats MC24-6. Note that the vertical dotted line at Day 38 in the plots for cat MC25 identifies the time of the delayed denervation of the Sural nerve (see Methods). As in Fig 1, the shaded area illustrates the 99% confidence interval of the control responses before denervation.

Fig. 3: Phase dependency of the responses evoked by stimulation of the motor cortex. A: Computer-rectified and averaged responses evoked in St evoked by stimulation of the motor cortex at 10 different phases of the step cycle. B: The integrated responses are plotted as a function of the step cycle for 3 different days before denervation. The shaded area illustrates the 99 % confidence interval of the SE of the control responses as calculated from the

weighted average. Units on the ordinate are arbitrary.

Fig. 4: Increased corticospinal efficacy following the cutaneous denervation. A: Average of EMG responses, evoked during the swing phase (Group 2) at a current strength of 35 μA before and after denervation.. For the St and the TA, the figure identifies both the short (black) and the longer (gray) latency responses. B: Average EMG responses, evoked by the same microwire at the same current strength during the stance phase (Group 6). Note that the gain of display for a given EMG is constant in all traces. Values in parentheses indicate the number of stimulated cycles and the average phase.

Fig. 5: Summary of the changes in magnitude of the responses evoked, before and after denervation, in five muscles from three different cortical sites (A, B and C), plotted as a function of the step cycle. A: same experiment as in Fig. 4.B: data from another cortical site, D6, from the same cat MC24 at a current strength of 35 μA. C: data from a site in another cat MC23 at a current strength of 25 μA. For St and TA in A and B, the graphs plot both the short latency reponses and the overall magnitude of the response (including both the black and gray filled regions of Fig. 4): the latter is included as an inset. The shaded area illustrates the 99 % confidence interval of the control responses before denervation (based on 6 sessions for both cortical sites in MC24 and 3 sessions in MC23.

Fig. 6: Percentage change and time course of the changes in the relative magnitude of the responses evoked by motor cortex stimulation in three cats MC24 (A), MC23 (B) and MC27 (C) following the denervation. The relative magnitude of the responses was calculated as a percentage of the weighted mean response evoked before denervation. Data

values are taken from the swing phase for Srt (group 3), St and TA (group 2) and during the stance phase for the toe flexor, EDB (gp 7) and the knee extensor, VL (gp 8). The site, D4, illustrated in Figs. 4 and 5A is represented by the black line in Fig 6A and the site, D6, illustrated in Fig. 5B, by the green line. The site illustrated in Fig 5C is represented by the brown line in Fig 6B. Seven sites are illustrated for cat MC24 (6A), 11 sites for cat MC23 (6B) and 5 for cat MC27 (6C). Note that in B, the ordinate has been divided because of the large responses evoked in some wires.

Fig. 7: The relative magnitude of the responses evoked in selected muscles from four different cortical sites in cat MC24 (A) and MC23 (B) following the denervation.

Fig. 8: A,B: Change in the magnitude of the responses as a function of the intensity of the stimulation before (dotted lines) and after (solid lines) denervation for two electrodes in cat MC23.

Fig. 9: Responses evoked by stimulation of the pyramidal tract at a strength of 100 μA following cutaneous denervation in cat MC26 before and after denervation during swing (9A: group 1) and stance (9B: group 6) phase of the step cycle.

Fig. 10: Summary of changes in the spinal efficacy evoked by the stimulation of the pyramidal tract for three different cats, A, B and C. The figure is organised as in Fig. 5.

Fig. 11: Responses evoked by stimulation of the superficial peroneal nerve at a strength of 200 μA (2XT) before and after denervation. Note that the first four muscles belong to the denervated hindlimb, while the last one, the right Srt, belongs to the contralateral hindlimb,

in which the cutaneous stimulation was applied. Organisation as in Figs. 4 and 9.

Fig. 12: Summary of responses evoked by stimulation of the superficial peroneal nerve in 3 different cats. Figure organized as for Figs. 5 and 10.

Fig. 13: Percentage change and time course of the changes in the relative magnitude of the responses in the Srt, St and TA evoked by the stimulation of the pyramidal tract (A), the superficial peroneal nerve (B), the saphenous nerve ©, and the tibial nerve (D).

Fig. 14: A: histological sections from cat MC23 showing the site of the lesion of the pyramidal tract on the right side (top), and the degenerating axons (black dots) at (from top to bottom) the level of the decussation, the cervical spinal cord and the lumbar spinal cord. B: Responses evoked by cortical stimulation at 25 μA at one site before pyramidotomy (top) and at 75 μA following pyramidotomy (bottom).

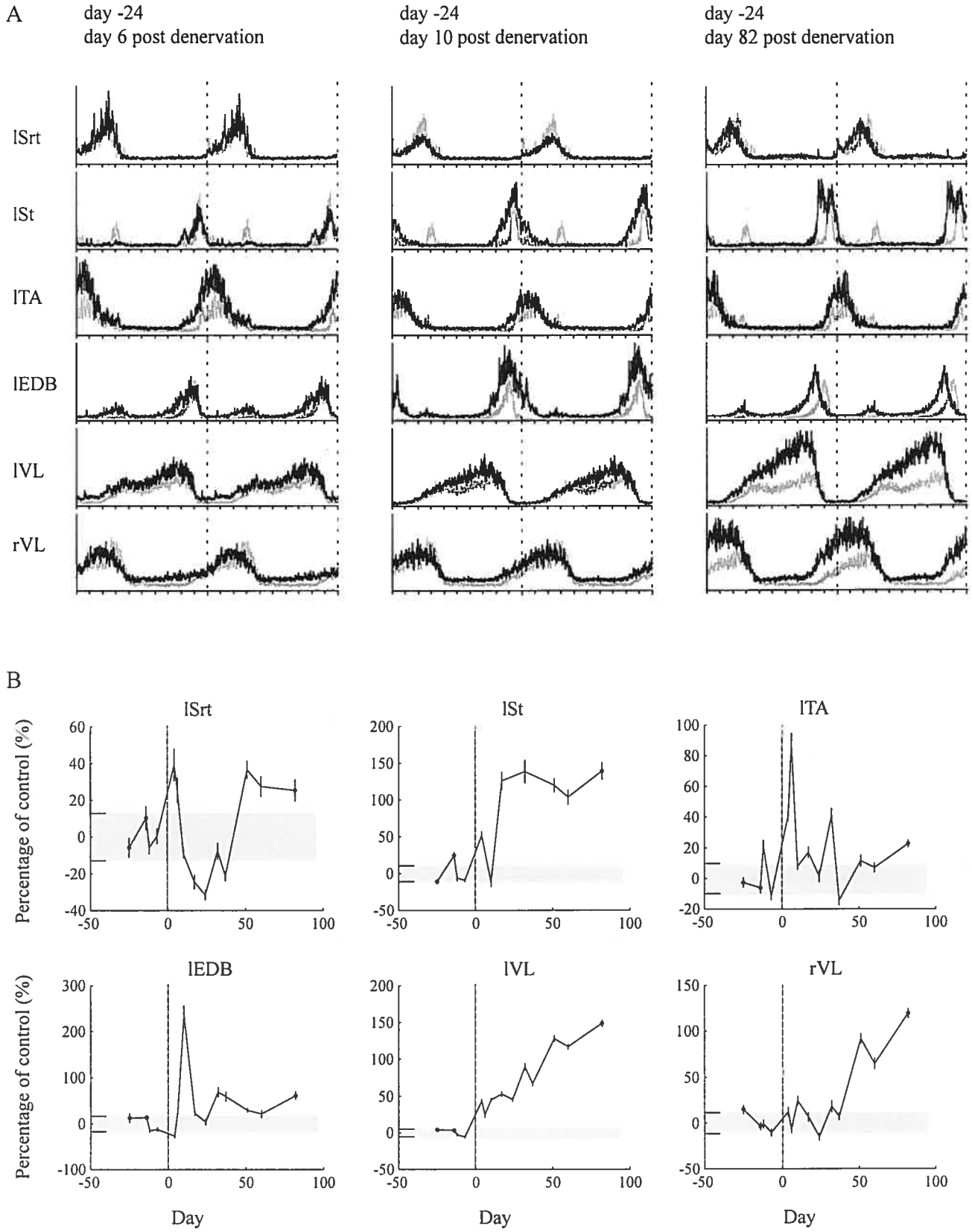

A
day -24
day 6 post denervation
day -24
day 10 post denervation
day -24
day 82 post denervation
lSrt
lSt
lTA
lEDB
lVL
rVL
B
lSrt
lSt
lTA
lEDB
lVL
rVL
Percentage of control (%)
Percentage of control (%)
Day
Day
Day

Figure 1

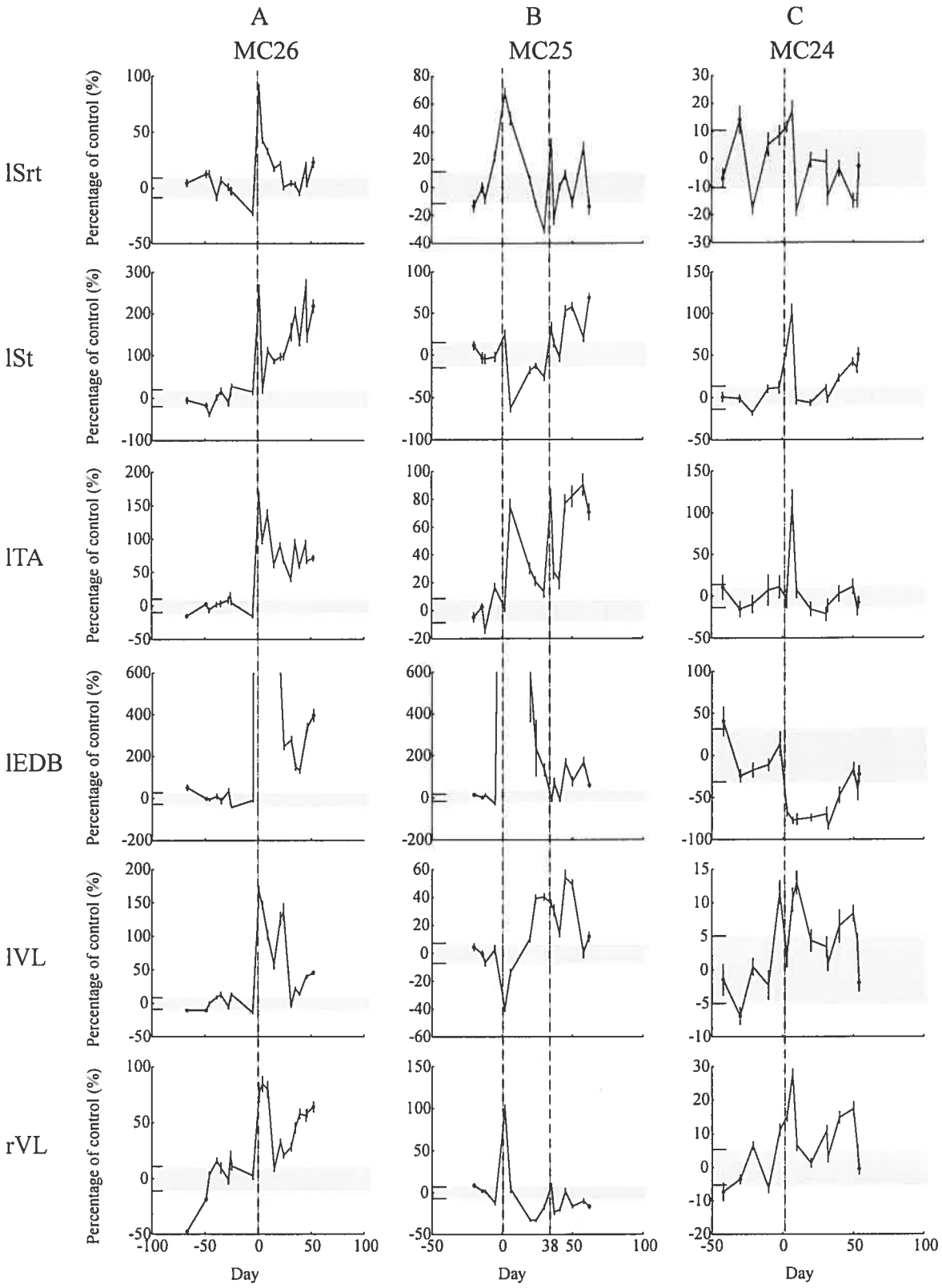
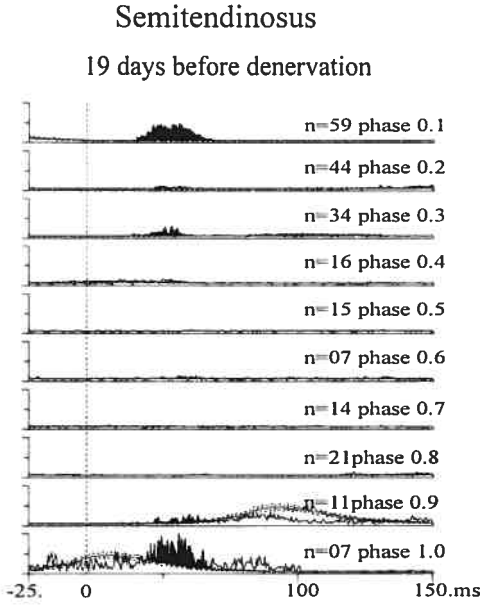

A
MC26
B
MC25
C
MC24
lSrt
lSt
lTA
lEDB
lVL
rVL
Percentage of control (%)
Day

Figure 2

A


Semitendinosus
19 days before denervation
n=59 phase 0.1
n=44 phase 0.2
n=34 phase 0.3
n=16 phase 0.4
n=15 phase 0.5
n=07 phase 0.6
n=14 phase 0.7
n=21phase 0.8
n=11phase 0.9
n=07 phase 1.0
-25.
0
100
150.ms

B

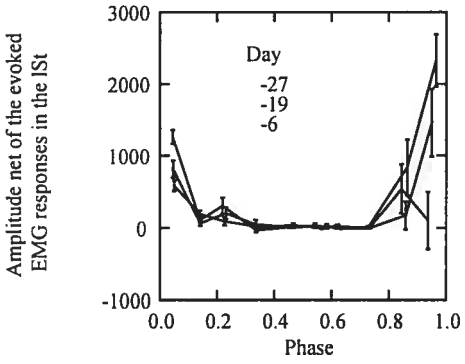

Amplitude net of the evoked EMG responses in the ISt
3000
2000
1000
0
-1000
Day
-27
-19
-6
0.0
0.2
0.4
0.6
0.8
1.0
Phase

Figure 3

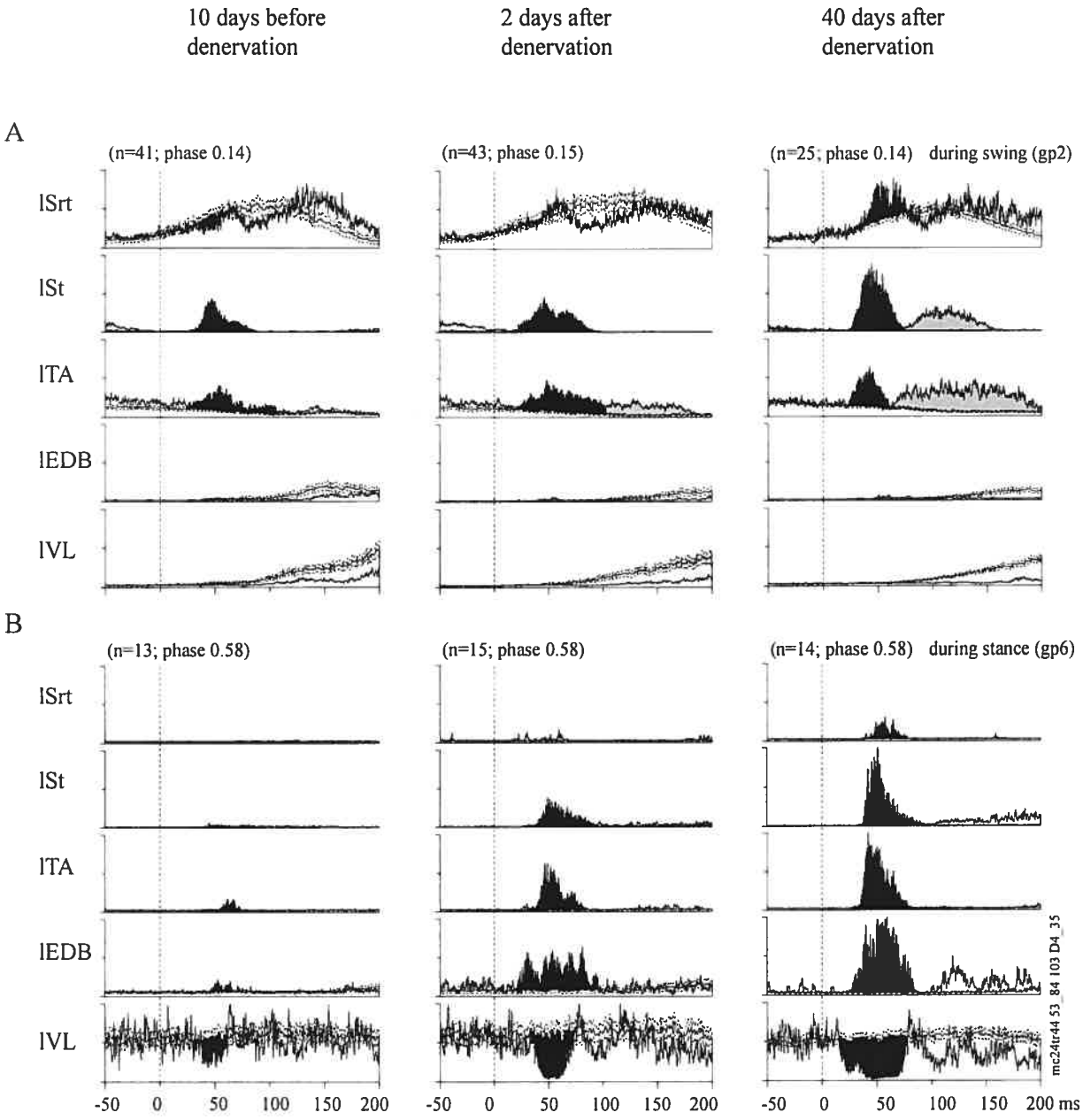

10 days before denervation
2 days after denervation
40 days after denervation
A
(n=41; phase 0.14)
(n=43; phase 0.15)
(n=25; phase 0.14) during swing (gp2)
lSrt
lSt
lTA
lEDB
lVL
B
(n=13; phase 0.58)
(n=15; phase 0.58)
(n=14; phase 0.58) during stance (gp6)
lSrt
lSt
lTA
lEDB
lVL
-50 0 50 100 150 200
-50 0 50 100 150 200
-50 0 50 100 150 200 ms
mc24tr44 53_84 103 D4_35

Figure 4

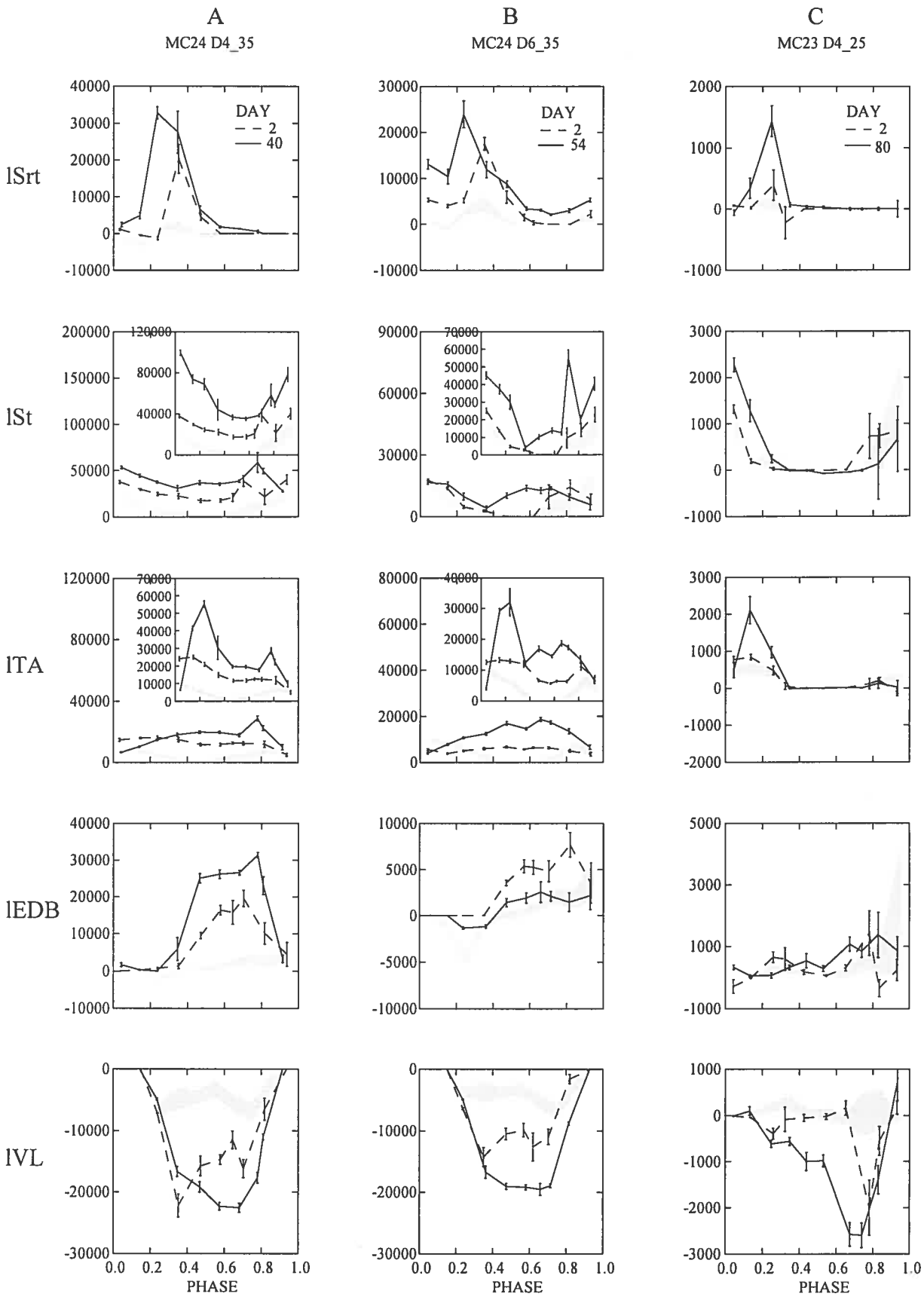

A
MC24 D4_35
B
MC24 D6_35
C
MC23 D4_25
lSrt
lSt
lTA
lEDB
lVL
DAY
2
40
54
80
PHASE

Figure 5

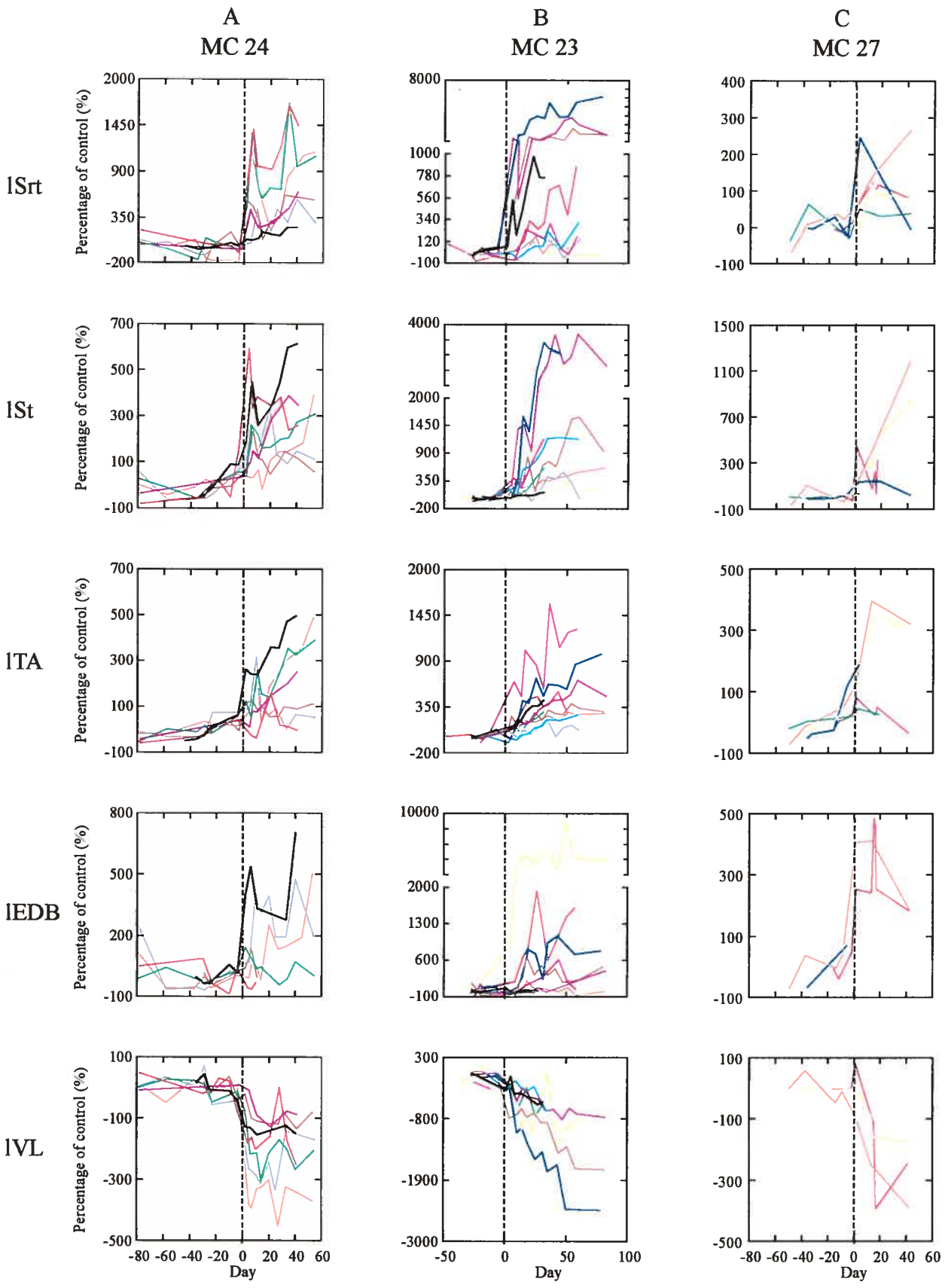

A
MC 24
B
MC 23
C
MC 27
lSrt
lSt
lTA
lEDB
lVL
Percentage of control (%)
Day

Figure 6

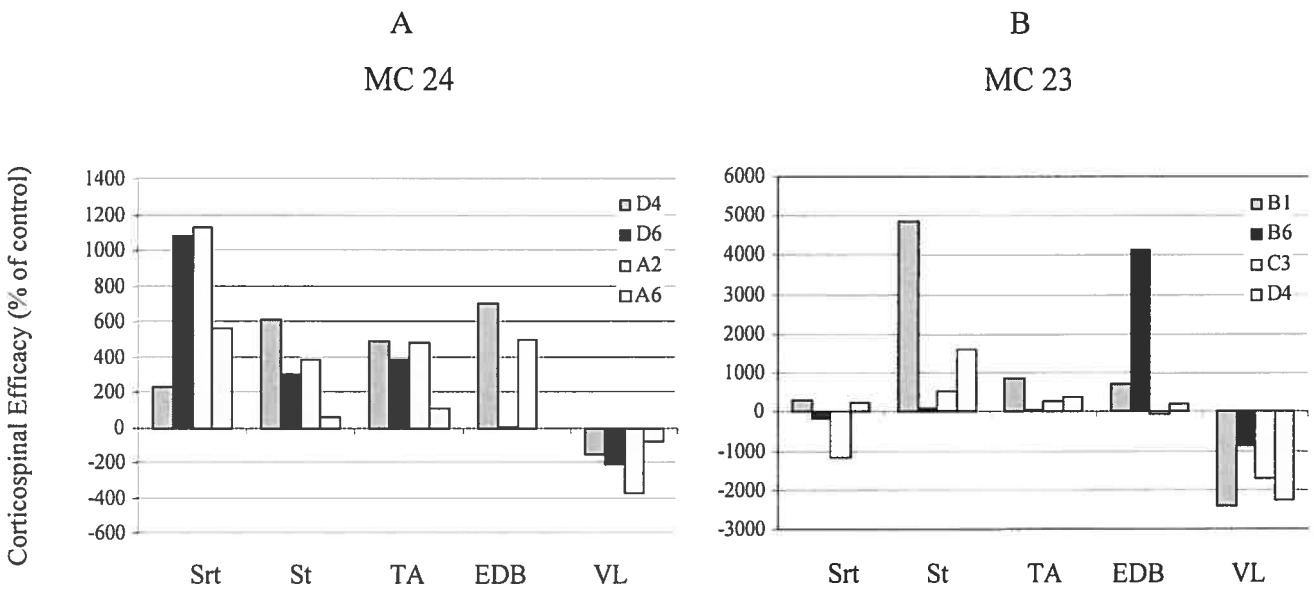

A
MC 24
B
MC 23
Corticospinal Efficacy (% of control)
1400
1200
1000
800
600
400
200
0
-200
-400
-600
D4
D6
A2
A6
Srt
St
TA
EDB
VL
6000
5000
4000
3000
2000
1000
0
-1000
-2000
-3000
B1
B6
C3
D4
Srt
St
TA
EDB
VL

Figure 7

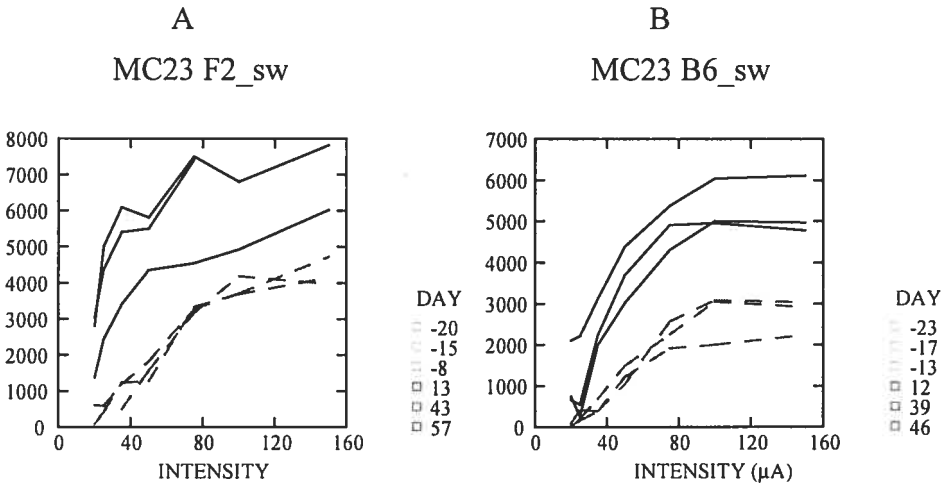

A
MC23 F2_sw
B
MC23 B6_sw
8000
7000
6000
5000
4000
3000
2000
1000
0
0
40
80
120
160
INTENSITY
DAY
-20
-15
-8
13
43
57
7000
6000
5000
4000
3000
2000
1000
0
0
40
80
120
160
INTENSITY (µA)
DAY
-23
-17
-13
12
39
46

Figure 8

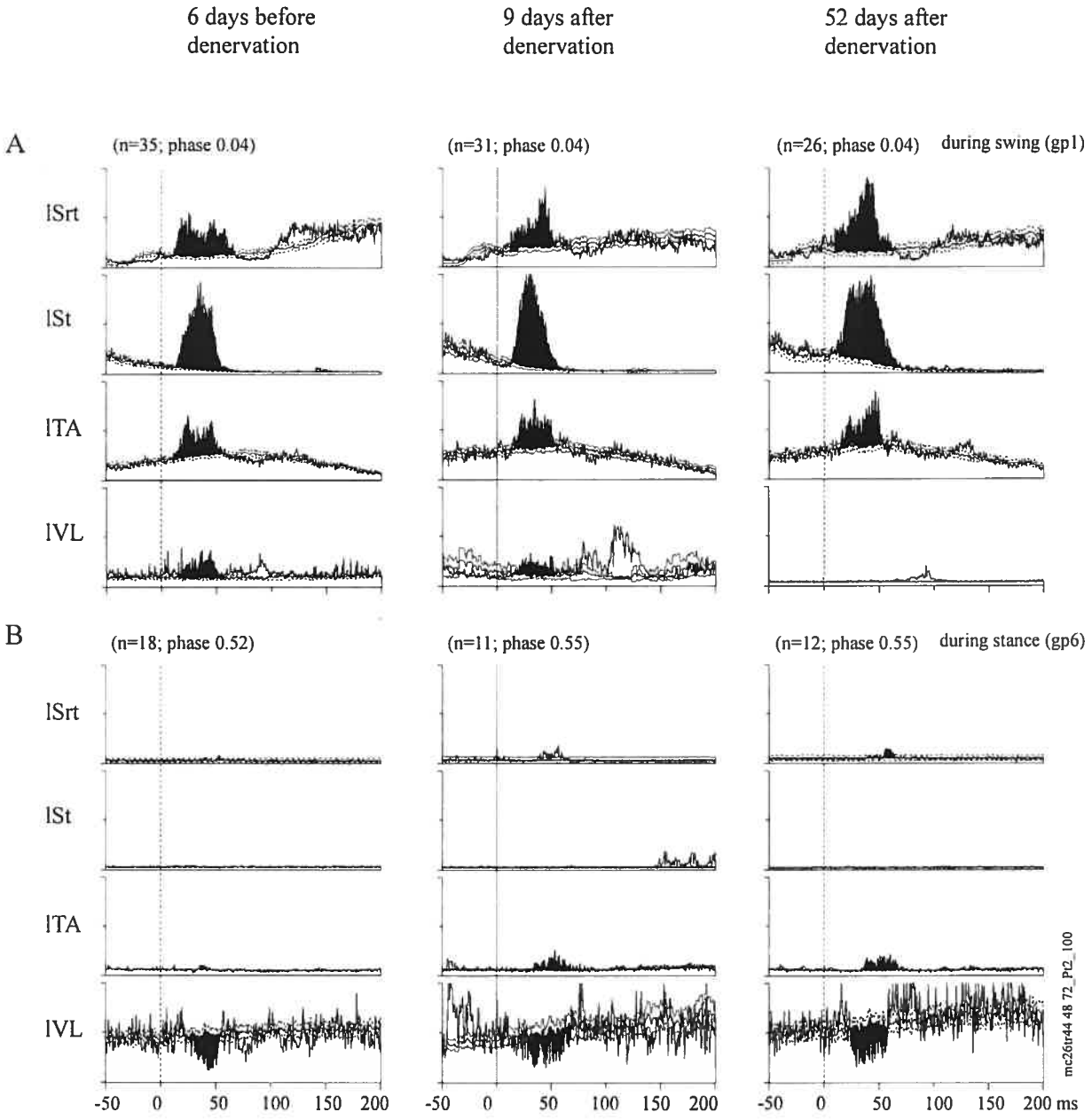

6 days before denervation
9 days after denervation
52 days after denervation
A
(n=35; phase 0.04)
(n=31; phase 0.04)
(n=26; phase 0.04)
during swing (gp1)
lSrt
lSt
lTA
lVL
B
(n=18; phase 0.52)
(n=11; phase 0.55)
(n=12; phase 0.55)
during stance (gp6)
lSrt
lSt
lTA
lVL
-50 0 50 100 150 200
-50 0 50 100 150 200
-50 0 50 100 150 200 ms
mc26r44 48 72_Pt2_100

Figure 9

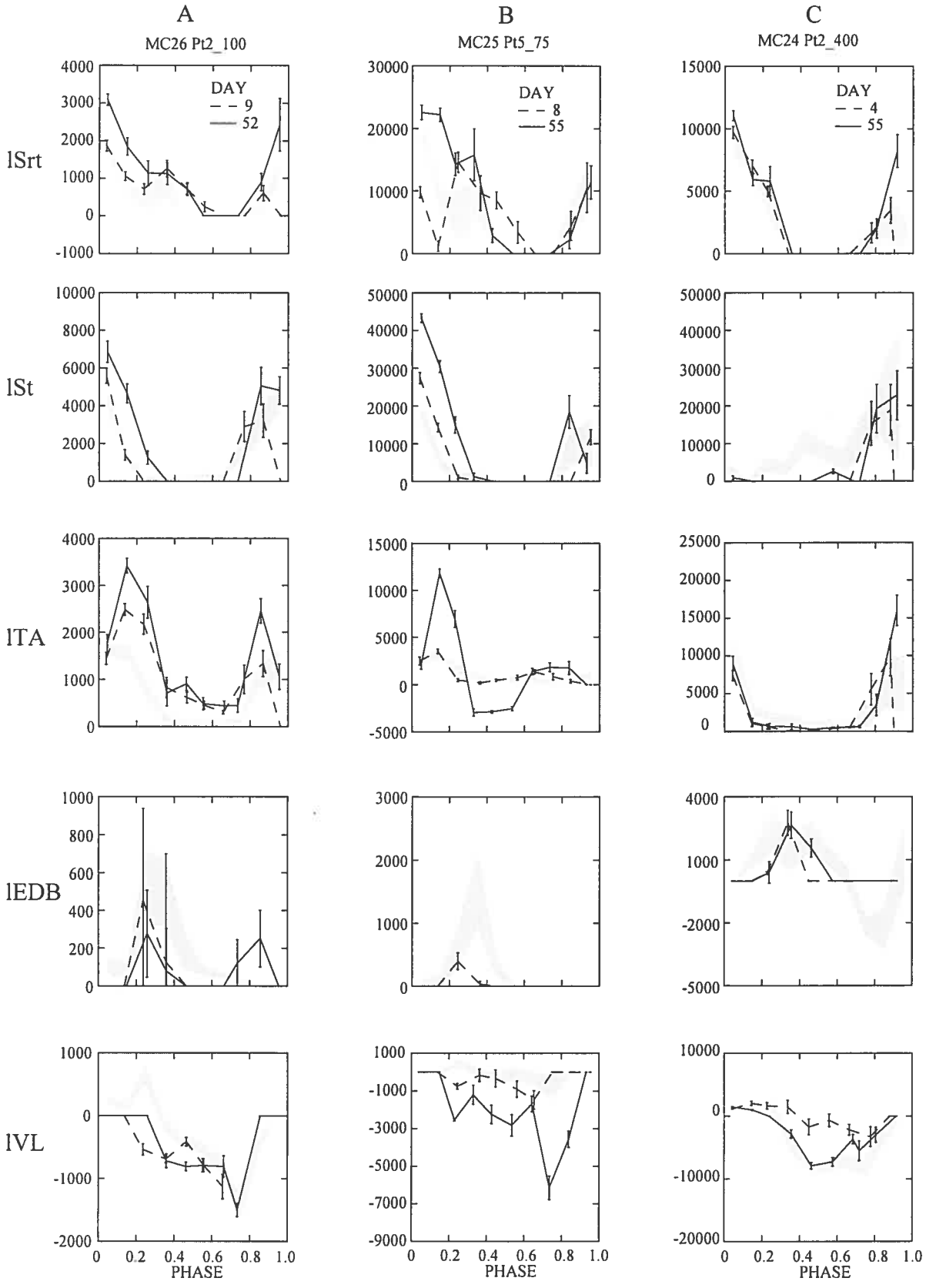

A
MC26 Pt2_100
B
MC25 Pt5_75
C
MC24 Pt2_400
DAY
9
52
DAY
8
55
DAY
4
55
lSrt
lSt
lTA
lEDB
lVL
PHASE
PHASE
PHASE

Figure 10

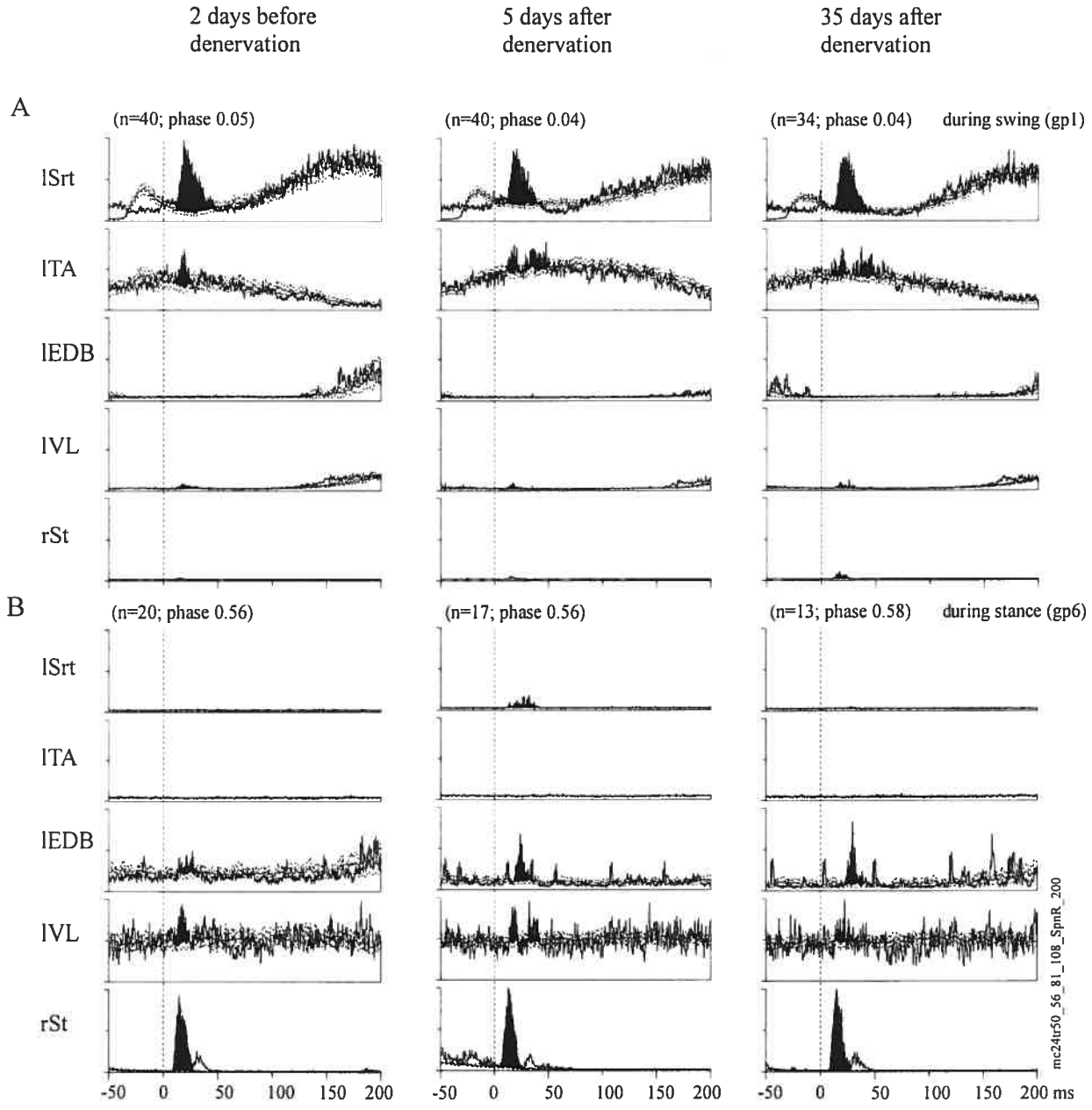

2 days before denervation
5 days after denervation
35 days after denervation
A
(n=40; phase 0.05)
(n=40; phase 0.04)
(n=34; phase 0.04)
during swing (gp1)
lSrt
lTA
lEDB
lVL
rSt
B
(n=20; phase 0.56)
(n=17; phase 0.56)
(n=13; phase 0.58)
during stance (gp6)
lSrt
lTA
lEDB
lVL
rSt
-50 0 50 100 150 200
-50 0 50 100 150 200
-50 0 50 100 150 200 ms
mc24tr50_56_81_108_SpnR_200

Figure 11

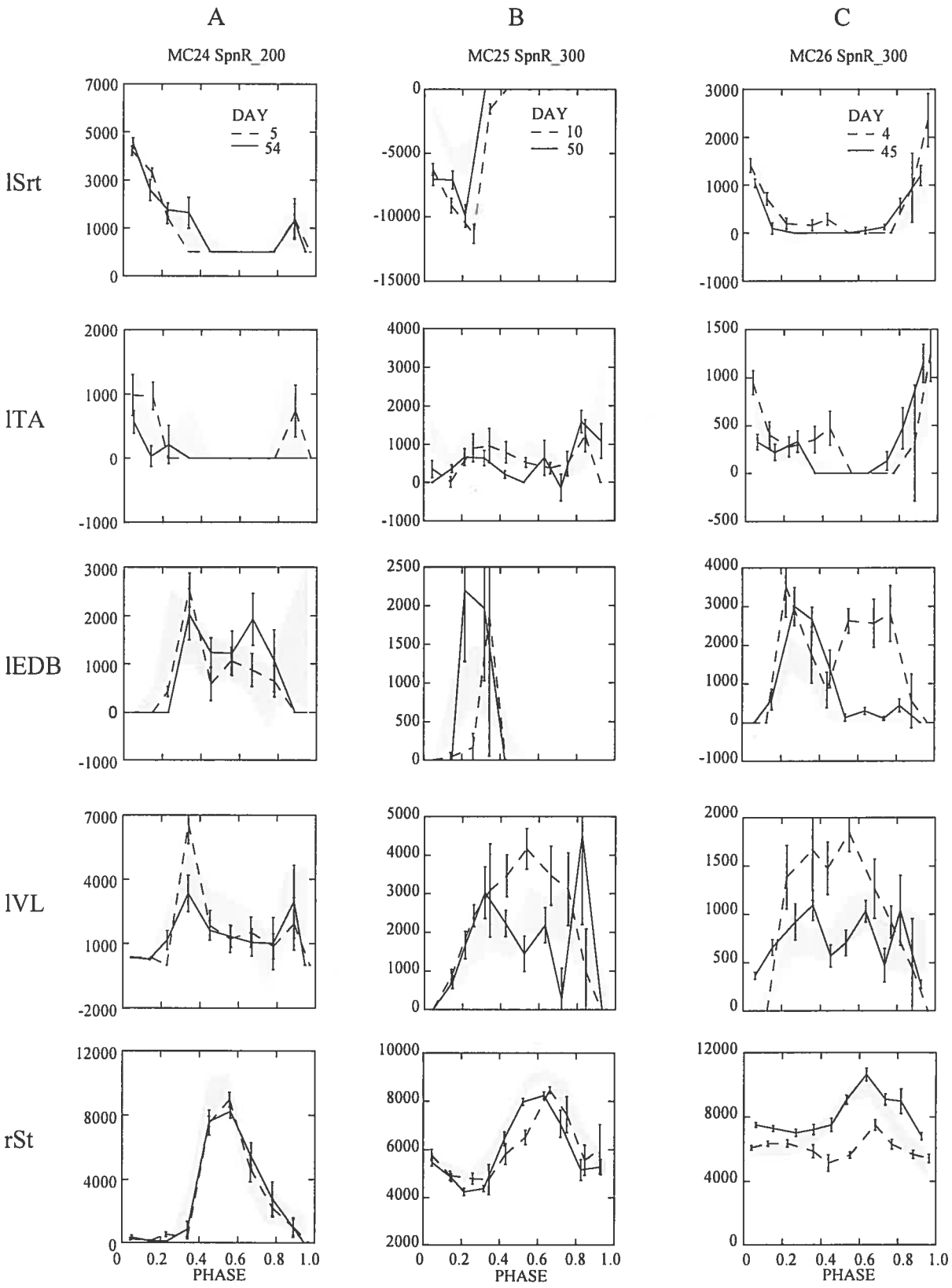

A
B
C
MC24 SpnR_200
MC25 SpnR_300
MC26 SpnR_300
DAY
5
54
DAY
10
50
DAY
4
45
lSrt
lTA
lEDB
lVL
rSt
PHASE
PHASE
PHASE

Figure 12

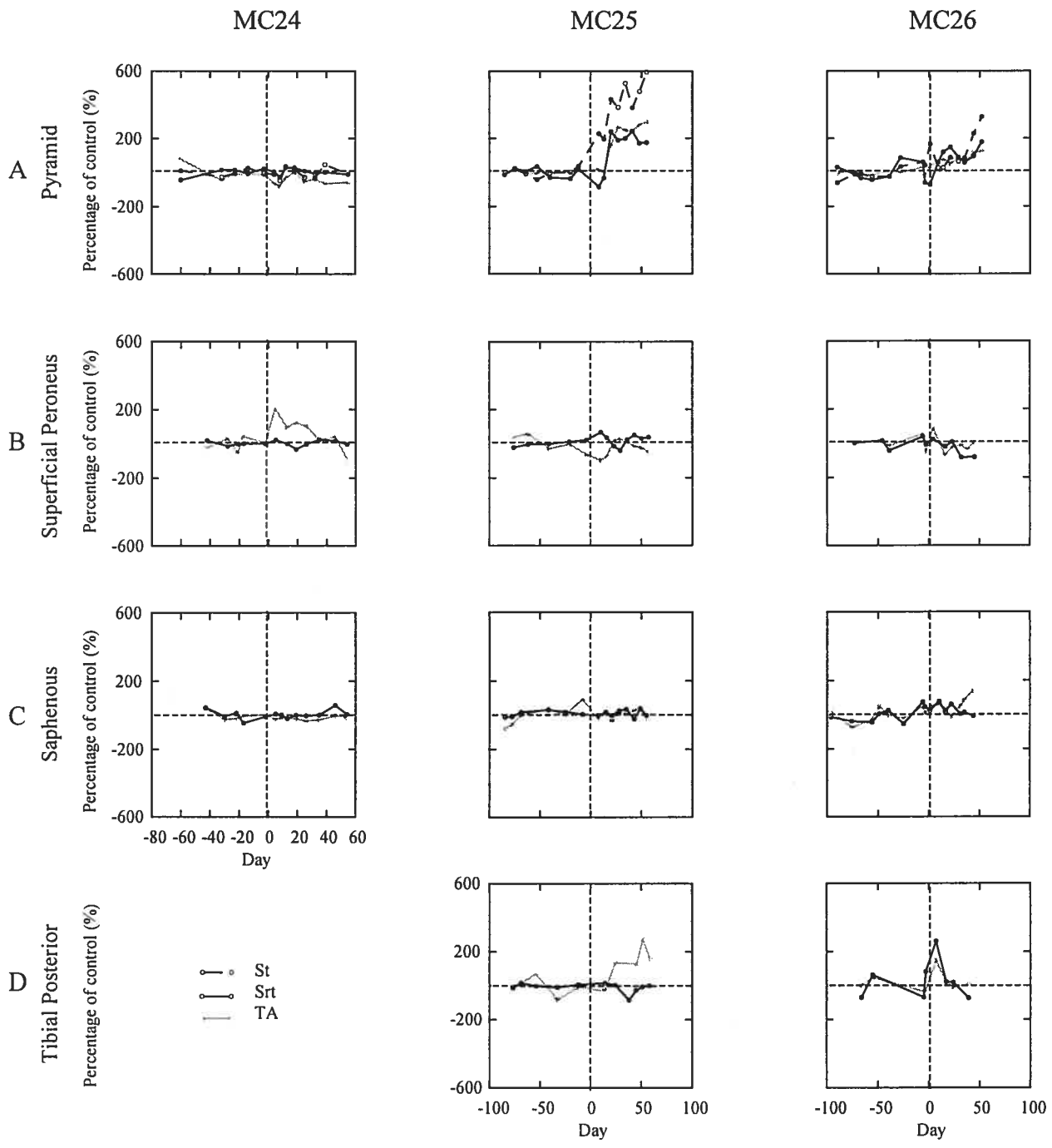

MC24
MC25
MC26
A
Pyramid
B
Superficial Peroneus
C
Saphenous
D
Tibial Posterior
Percentage of control (%)
600
200
-200
-600
-80 -60 -40 -20 0 20 40 60
-100 -50 0 50 100
Day
St
Srt
TA

Figure 13

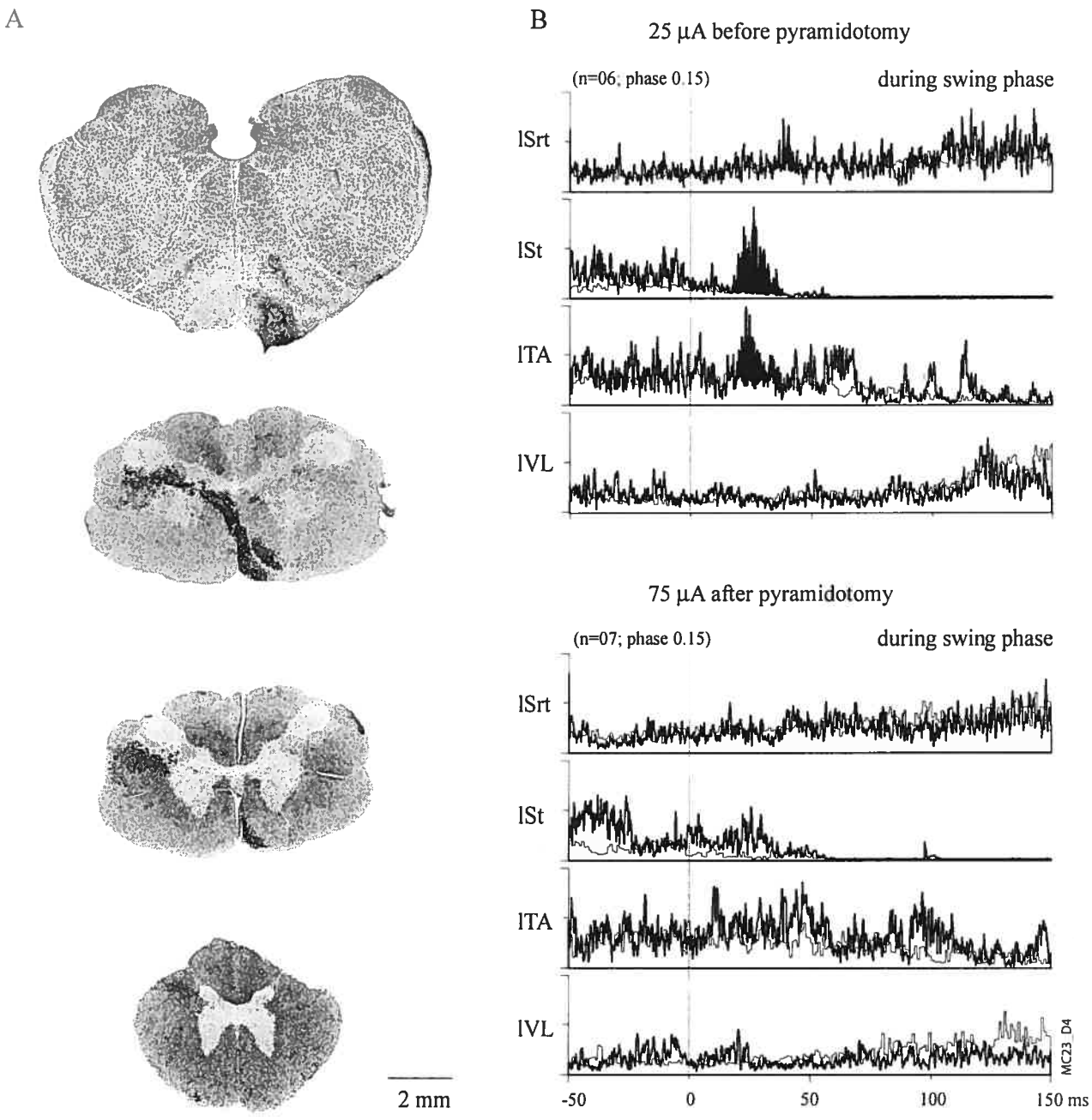

A
B
25 µA before pyramidotomy
(n=06; phase 0.15)
during swing phase
lSrt
lSt
lTA
lVL
75 µA after pyramidotomy
(n=07; phase 0.15)
during swing phase
lSrt
lSt
lTA
lVL
MC23_D4
-50
0
50
100
150 ms
2 mm

Figure 14

Discussion Générale et Conclusion

Les trois chapitres qui viennent d'être présentés démontrent que le cortex moteur contribue au contrôle de la locomotion du membre postérieur, qu'il module de manière spécifique les réflexes cutanés lors de la marche et enfin qu'il contribue à la récupération fonctionnelle de la marche à la suite d'une dénervation cutanée de la patte postérieure. Dans les prochaines sections, nous discuterons plus en détails de nos résultats et du rôle du cortex moteur et des afférences cutanées dans le contrôle et la plasticité du système locomoteur.

Rôle du cortex moteur dans le contrôle de la marche

Il ne fait aucun doute que le cortex moteur est important dans le contrôle de la marche, tout au moins chez l'homme. Une lésion bilatérale de la voie pyramidale ou des accidents cérébraux vasculaires du cortex moteur (Holmes 1915 ; Nathan 1994 ; Knutsson et Richards 1979) conduisent à de sévères incapacités et même des paralysies de la jambe chez l'homme, alors qu'ils affectent peu la marche sur surface plane chez le chat. Seules les conditions plus complexes tels que l'enjambement d'un obstacle ou la marche sur les barreaux d'une échelle semblent exiger l'intégrité du cortex moteur chez le chat. Néanmoins de récentes études ont également suggéré l'importance du cortex moteur dans la récupération fonctionnelle de la marche sur une surface plane à la suite d'une altération du système nerveux périphérique (Bouyer et Rossignol 2003a,b ; Bouyer et al. 2000). De plus des études d'enregistrements cellulaires dans notre laboratoire ont suggéré une certaine contribution du cortex moteur dans la régulation de l'amplitude et la durée des activités musculaires du membre postérieur chez le chat intact lors de la marche (Drew et al. 2002 ; Kably et Drew 1998b ; Widajewicz et al. 1994). Notre étude a donc consisté à déterminer et à clarifier la nature de la contribution du cortex moteur dans le contrôle du membre postérieur chez le chat marchant librement.

Dans les prochaines sections, nous discuterons de nos résultats suggérant que le cortex moteur agit sur l'amplitude et la durée des activités musculaires et le rythme locomoteur. Nous discuterons également des évidences suggérant une certaine spécificité dans le recrutement cortical des muscles de la patte postérieure au repos et

lors de la marche, et l'intérêt d'étudier cette spécificité du contrôle locomoteur lors de différents paradigmes comportementaux. Enfin nous traiterons des évidences suggérant un contrôle cortical sur le membre postérieur indépendant de celui du membre antérieur chez le chat.

Action corticale sur la structure des activités musculaires

Nous avons démontré que le cortex moteur est capable de modifier de manière importante l'amplitude et la durée des bouffées des muscles du membre postérieur lors de la marche chez le chat, suggérant un contrôle cortical sur la structure du cycle de marche. En général, les réponses dans les fléchisseurs étaient augmentées lors de la phase de balancement, tandis qu'elles étaient diminuées dans les extenseurs lors de la phase d'appui. Cette modulation des réponses en fonction de la phase du cycle de marche était également évoquée de façon semblable par la stimulation de la pyramide chez le chat. Parce que les réponses évoquées par la stimulation de la pyramide sont indépendantes du niveau d'activité corticale (Schmied et Fetz 1987), elles fournissent une indication du niveau d'excitabilité des réseaux interneuronaux spinaux par rapport à l'efficacité corticospinal. Ceci suggère donc que cette dépendance à la phase serait probablement due, en grande partie, aux changements d'excitabilité des réseaux interneuronaux spinaux chez le chat. Chez l'homme, les réponses évoquées dans les muscles de la jambe par une stimulation trans-crânienne magnétique (TMS) sont modulées de manière similaire au cours du cycle de marche (Capaday et al. 1999 ; Petersen et al. 1998, 2001 ; Schubert et al. 1997), mais il n'existe pas d'évidence suggérant que cette modulation est corticale ou spinale.

Action corticale sur le rythme locomoteur

Nous avons aussi montré que le cortex moteur est capable d'agir sur le rythme locomoteur du membre postérieur chez le chat, alors qu'aucune évidence de raccourcissement du cycle de marche n'a été rapporté à ce jour chez l'homme (Capaday et al 1999). Ceci peut sembler surprenant, compte tenu des évidences indirectes chez le primate non humain suggérant l'existence de fortes projections mono-synaptique (Porter et Lemon 1993) et de la forte contribution corticale au contrôle de la marche chez l'homme (Nathan 1994 ; Nielsen et al. 1997 ; Knutsson et Richards 1979). Néanmoins, l'absence de réinitialisation du rythme locomoteur chez l'homme pourrait s'expliquer du fait de l'utilisation de techniques très indirectes (la stimulation trans-crânienne magnétique) dans des zones du cortex difficilement accessibles (la représentation de la jambe) (Petersen et al. 2003 ; Rothwell 2003). En effet, on ne connaît pas encore avec certitude le mode d'activation de la stimulation magnétique dans la représentation de la jambe : indirectement via des cellules corticales qui activerait des cellules de la voie corticospinale ou directement au soma ou au premier nœud de Ranvier des axones des cellules corticospinales. De plus, le large territoire cortical recruté par la stimulation magnétique pourrait activer autant de cellules excitatrices qu'inhibitrices, annulant l'existence d'une certaine spécificité à travers la représentation corticale de la jambe.

Action spécifique du cortex moteur dans le contrôle de la patte postérieure

Nous avons rapporté une certaine spécificité dans la représentation corticale de la patte postérieure chez le chat semblable à celle observée dans celle du membre antérieur

(Armstrong et Drew 1985 ; Nieoullon et Rispal Padel 1976). La microstimulation au seuil dans la représentation du membre postérieur évoquait fréquemment des mouvements autour d'une ou de deux articulations au repos. Cependant un certain nombre de ces sites perdait de leur spécificité lors de la marche par rapport au repos, à cause d'un recrutement de nouveaux muscles, probablement dû à une augmentation dans l'excitabilité spinale ou corticale lors de la marche.

En effet, un site cortical pourrait influencer l'activité d'un muscle directement à travers des connexions corticospinales ou indirectement via des connexions cortico-corticospinales (Jankowska et al. 1975). Ceci pourrait être résolu en partie par l'utilisation d'une facilitation post-stimulus à l'aide d'un stimulus unique et d'un moyennage sur un grand nombre d'essais des réponses électromyographiques (EMGs) (Cheney et Fetz 1985 ; Lemon et al. 1987). Chez le primate, il a été montré qu'une telle stimulation à 10 μA évoque une facilitation post-stimulus dans les réponses EMGs qui ressemblent beaucoup, en terme d'organisation et d'amplitude, aux réponses observées lors d'un ``spike trigger averaging`` (STA). Une telle méthode permettrait ainsi d'étudier plus spécifiquement la connectivité fonctionnelle de neurones pyramidaux dans un rayon de 90 μm, ce qui serait beaucoup plus précis que ce que nous avons obtenu dans notre étude lors de l'utilisation d'un court train de stimuli (Stoney et al. 1968).

Cependant une telle technique est difficilement transposable chez le chat, car ce dernier ne semble pas posséder de connections corticospinales mono-synaptiques contrairement au primate et à l'homme, ce qui explique l'absence d'effets lors d'un STA. Néanmoins, quelques résultats préliminaires ont été reportés lors de l'utilisation

d'un stimulus unique appliqué à une fréquence de 25 Hz durant la marche (Drew 1988). L'étude montrait qu'un site de la représentation corticale du membre antérieur évoquait une facilitation évidente dans trois muscles fléchisseurs de la patte antérieure durant la phase de balancement, et plus faiblement dans un muscle fléchisseur durant la phase d'appui. L'existence d'une cellule ou d'un groupe circonscrit de neurones pyramidaux facilitant trois muscles synergistes est d'ailleurs en accord avec la nature divergente et distribuée des projections corticospinales dans le segment cervical (Shinoda et al. 1976, 1986; Futami et al. 1979).

Cette technique pourrait donc être utilisée pour étudier de manière plus subtile et plus circonscrite l'efficacité corticospinale depuis différents sites de la représentation corticale du membre postérieur lors de différentes phases du cycle de marche.

Spécificité lors de la marche et lors de différents paradigmes comportementaux

Bien que nous ayons rapporté une diminution dans la spécificité d'un certain nombre de sites corticaux lors de la marche à cause d'un recrutement de nouveaux muscles, des différences dans l'amplitude relative des réponses évoquées étaient tout de même encore mesurables. Par exemple, alors que certains sites corticaux évoquaient des réponses plus grandes dans le Tibialis Anterior et le Sartorius comparativement au Semitendinosus, d'autres sites évoquaient des réponses inverses durant la marche. Cette spécificité, bien que diminuée lors de la marche, est en accord avec les études d'enregistrements cellulaires dans le laboratoire qui ont montré que certains groupes de neurones corticaux augmentent leur taux de décharge en phase avec certains groupes de muscles requis lors

de l'enjambement d'un obstacle (Widajewicz et al. 1994 ; Kably et Drew 1998 ; Drew et al. 2002).

Les résultats de ces dernières études nous amènent également à penser qu'il serait intéressant d'investiguer la spécificité du cortex moteur non pas seulement dans le contexte de la marche mais lors d'autres paradigmes comportementaux, tel que l'enjambement d'un obstacle, afin de moduler les niveaux d'excitabilité corticale et spinale. En effet, une récente étude chez l'homme a montré que l'amplitude des réponses EMGs évoquées par TMS augmente lorsque le sujet doit utiliser des indices visuels pour placer son pied correctement, comparativement à une marche normale sur un tapis roulant (Schubert et al. 1997). Ces résultats suggèrent donc une augmentation dans le niveau d'excitabilité corticale et/ou spinale de certains muscles et par conséquent une spécificité fonctionnelle du cortex moteur qui dépendrait du contexte. Les changements dans l'amplitude relative des réponses évoquées par un court train de stimuli lors de la phase de levée de la patte au niveau de l'obstacle, ou au-delà de l'obstacle, pourraient alors être comparés à ceux obtenus lors de la marche, qui nous servirait de référentiel, ainsi que ceux obtenus lors de la marche sur une échelle horizontale, qui sollicite un placement fin du bout de la patte sur les barreaux. La comparaison des corrélations décharge neuronale - activité musculaire et des changements d'efficacité corticospinale dans l'activité de différents muscles depuis différents sites distribués à travers le cortex moteur nous permettrait alors de déterminer l'existence d'une spécificité fonctionnelle ainsi que peut être une topographie du cortex moteur dans le contrôle du membre postérieur lors de ces divers paradigmes comportementaux.

Indépendance du contrôle cortical entre les pattes antérieures et postérieures

Widajewicz et al. (1994) ont précédemment suggéré un contrôle cortical de la patte postérieure indépendant de celui de la patte antérieure, que notre étude supporte. En effet, un court train de stimuli dans la représentation corticale de la patte postérieure évoquait des changements dans l'amplitude et la durée des activités musculaires de la patte postérieure durant la marche, avec de très rare effet dans la patte antérieure. Un long train de stimuli appliqué dans cette même représentation en plus d'évoquer des changements dans l'amplitude et la durée des muscles de la patte postérieure, raccourcissait la durée du cycle de marche de cette patte, en affectant à un degré moindre et dans un second temps les autres pattes. Cette indépendance dans le contrôle cortical de l'amplitude et la durée des activités musculaires, ainsi que du rythme locomoteur entre les pattes postérieures et antérieures est en accord avec les études anatomiques ayant démontré que les neurones corticospinaux de la représentation de la patte antérieure ou postérieure projettent de manière spécifique dans le segment cervical ou lombaire (Armand 1984 ; Armand et Aurenty 1977). Ces évidences suggèrent donc un contrôle cortical de la patte postérieure précis et indépendant du reste du corps nécessaire lors de l'enjambement d'un obstacle.

D'autre part, nous avons aussi observé l'existence de quelques sites corticaux de la représentation de la patte postérieure dans la portion la plus latérale du cortex crucié qui étaient capables d'évoquer des effets dans les pattes postérieures et antérieures. Ceci corrobore les études cellulaires de Widajewicz et al. (1994) qui ont montré également des cellules dans cette région qui étaient modulées avec l'activité musculaire des pattes

antérieures et postérieures. Ces observations sont en accord avec les quelques évidences anatomiques ayant rapporté l'existence de quelques neurones corticospinaux entre les représentations des pattes antérieures et postérieures qui projettent à la fois dans le segment cervical et lombaire (Armand, 1978 ; Hayes et Rustioni, 1981). L'existence de telles projections corticospinales dans les deux segments spinaux permettrait la flexibilité nécessaire au système nerveux central pour coordonner les interactions entre les pattes antérieures et postérieures comme par exemple lors du gallop chez les quadrupèdes.

En résumé, notre étude a démontré que le cortex moteur contribuait activement et spécifiquement au contrôle de la locomotion du membre postérieur sur un tapis roulant. Elle suggère que le cortex moteur agit sur le contrôle du membre postérieur de manière aussi spécifique que sur le membre antérieur. Enfin, ce contrôle cortical du membre postérieur semble également indépendant du contrôle cortical du membre antérieur, suggérant l'apparition d'un contrôle cortical indépendant sur les membres au cours de l'évolution phylogénétique. En effet, le contrôle cortical sur la patte postérieure chez les marsupiaux est probablement retransmis par les voies descendantes du tronc cérébral ou par les neurones propriospinaux car il n'existe pas de projections corticospinales dans le segment lombaire. Par contre, le chat possède quelques projections corticospinales dans le segment cervical et lombaire, ainsi que des projections spécifiques dans les segments cervicaux et lombaires, qui lui permettraient à la fois de coordonner les synergies motrices entre les pattes antérieures et postérieures ainsi que d'ajuster un contrôle plus

fin sur la patte postérieure. Enfin, l'importance du cortex dans le contrôle de la marche chez l'homme et la présence de fortes projections mono- et poly-synaptiques dans le segment lombaire chez le primate non-humain suggèrent l'émergence et la spécialisation au cours de l'évolution d'une voie corticospinale dans le segment lombaire, qui aurait permis l'apparition de la bipédie parallèlement à la dextérité de la main chez l'homme.

Modulation corticale des réflexes cutanés lors de la marche du membre postérieur

La locomotion est modulée par différentes structures supra-spinales et divers signaux provenant de la périphérie qui modulent et informent de la réalisation de ces mouvements. Comme l'a démontré notre première étude, le cortex moteur semble contribuer à la régulation de la marche en modifiant la structure et le rythme du cycle de marche sur un tapis roulant (Bretzner et Drew 2005a). Parmi les signaux provenant de la périphérie, le feed-back cutané fournit également une importante information produisant des réponses réflexes intégrées dans le cycle de marche permettant à l'animal de maintenir un rythme locomoteur normal (Rossignol et al. 1988). Enfin, de récentes études (Bouyer et Rossignol 2003a,b) ont montré que les informations cutanées contribuent également à la régulation du rythme locomoteur de base car des déficits importants surviennent à la suite d'une dénervation cutanée chronique du bout de la patte. Cependant, alors que de nombreuses évidences supportent l'idée que le feed-back cutané pourrait moduler l'activité corticale, peu d'information suggère que l'activité corticale pourrait moduler les réflexes cutanés durant la marche. Notre seconde étude a donc consisté à déterminer si le cortex moteur est capable de moduler les effets de plusieurs réflexes cutanés chez le chat marchant librement.

Dans les prochaines sections, nous discuterons du lieu d'intégration de la modulation corticale des réflexes cutanés qui pourrait avoir lieu aussi bien au niveau cortical qu'au niveau spinal. Nous aborderons la spécificité de cette modulation selon le site cortical, le réflexe cutané, ainsi que le muscle enregistré. Nous discuterons ensuite de la modulation

corticale des réflexes proprioceptifs, ainsi que des interactions entre les réflexes cutanés et le cortex moteur lors de différents paradigmes comportementaux.

Intégration corticale versus spinale

Un certain nombre d'évidences chez l'animal suggèrent que l'information cutanée, en modulant l'activité des neurones corticaux durant la marche (Chapin et Woodward 1986 ; Marple-Horvat et Armstrong 1999 ; Palmer et al. 1985), pourrait moduler le niveau d'excitabilité corticale et par extension altérer le volet descendant corticospinal. De récentes évidences ont suggéré qu'un tel mécanisme pourrait exister. En effet, il existe chez l'homme des réflexes cutanés à longues latences qui pourraient être trans-corticaux (Duysens et al. 1990 ; Yang et Stein 1990), car ils sont absents dans le côté lésé chez les patients hémiplégiques (Zehr et al. 1998) et ils sont modulés par une stimulation trans-crânienne magnétique au cours de la marche (Christensen et al. 1999, 2000 ; Pijnappels et al, 1998).

Bien que les inputs corticaux et cutanés pourraient être intégrés au niveau cortical, nous suggérons plutôt que l'intégration ait lieu au niveau spinal. En effet, nous avons observé que la stimulation de la pyramide semble moduler les effets des réflexes cutanés de manière aussi forte que la stimulation corticale au cours de la marche. De plus, la courbe de modulation des effets des réflexes cutanés présente en générale des changements optimums autour de 15 à 25 ms, suggérant que les interactions aient lieu à courts délais. Étant donné que la latence moyenne d'activation des muscles suivants une microstimulation intracorticale de la représentation de la patte postérieure est autour de 20 à 25 ms (Bretzner et Drew 2005a) et que la latence moyenne des réponses corticales

évoquées par la stimulation des nerfs cutanés de la patte antérieure est de 12 à 16 ms (Marple-Horvat et Armstrong 1999), il semble que la latence minimale d'une réponse musculaire évoquait par un réflexe trans-cortical devrait donc être autour de 40 ms, ce qui est beaucoup trop long pour expliquer nos résultats. De plus, un certain nombre d'études a rapporté une facilitation corticale des dépolarisations des afférences primaires cutanées (Rudomin et al. 2004 ; Rudomin et Schmidt 1999), ainsi qu'une convergence des inputs corticaux et cutanés dans des interneurones inter-segmentaires lombaires (Lundberg 1964 ; Lundberg et al. 1962).

Ces évidences suggèrent donc que les interactions entre le cortex moteur et les réflexes cutanés à courtes latences auraient lieu au niveau spinal lors de la marche chez le chat.

Spécificité

Nous avons également reporté des différences dans les modulations corticales des effets des réflexes cutanés lors de la marche, selon le site cortical, le réflexe cutané et le muscle enregistré. Les différences dans les réponses résultant des interactions corticales et cutanées selon le site cortical corroborent notre première étude (Bretzner et Drew 2005a) suggérant que différentes régions du cortex moteur possèderaient des terminaisons différentes sur des populations interneuronales régulant l'activité de muscles agissant autour de différentes articulations de la patte. Les différences dans les réponses résultant des interactions corticales et cutanées selon le réflexe cutané corroborent également les nombreuses études en locomotion fictive suggérant l'existence de réseaux spinaux différents selon le site de stimulation cutanée sur le dos

ou la surface plantaire de l'extrémité de la patte postérieure et la phase du cycle de marche stimulée (Burke 1999 ; Degtyarenko et al. 1996, 1998 ; Moschovakis et al. 1991). Enfin, les différences dans les réponses résultant des interactions corticales et cutanées selon le muscle enregistré suggèrent aussi une convergence des inputs corticaux et cutanés dans des réseaux interneuronaux spinaux spécifiques régulant l'activité de différents groupes de muscles synergistes.

Les différences reportées dans les patrons de modulations corticales des réflexes cutanés suggèrent non pas une interaction globale et homogène mais spécifique. Ces évidences supportent également la possibilité d'une organisation modulaire des réseaux interneuronaux spinaux comme cela a été avancé dans les études du réflexe de retrait (Shouenborg 2002). En effet, Schouenborg et al. (1990) en étudiant le réflexe de retrait chez le rat ont proposé le concept d'une organisation modulaire, dans chacun desquels (module) chaque muscle ou groupe de muscles aurait un champ récepteur cutané séparé correspondant à une aire de peau qui est étiré par le même groupe de muscles lors d'une contraction (Schouenborg et Weng 1994). Une telle organisation modulaire permettrait d'expliquer les différences observées dans notre étude selon le réflexe cutané et le muscle enregistré chez le chat marchant librement. Elle permettrait également d'expliquer les différences reportées selon le site de stimulation cutanée sur le dos ou la surface plantaire de la patte postérieure et la phase du cycle de marche durant un épisode de locomotion fictive chez le chat paralysé (Burke 1999 ; Degtyarenko et al. 1996, 1998 ; Moschovakis et al. 1991).

Modulation corticale des réflexes proprioceptifs

Il existe une importante littérature portant sur la convergence anatomique des inputs corticaux et proprioceptifs dans le segment lombaire, sur la capacité des afférences proprioceptives à moduler et entretenir le rythme locomoteur en locomotion fictive (Pearson et Rossignol 1991 ; Saltiel et Rossignol 2004a,b), ainsi que sur la capacité du cortex moteur à réinitialiser le rythme locomoteur chez le chat intact (Bretzner et Drew 2005a). D'après ces évidences, il serait très intéressant d'étudier la modulation corticale des réflexes proprioceptifs au cours du cycle de marche. Cette étude permettrait sans doute de déterminer les afférences proprioceptives les plus efficaces pour réinitialiser le rythme locomoteur.

Interactions entre les réflexes cutanés et le cortex moteur lors de différents paradigmes comportementaux

L'existence de différents réflexes cutanés selon le site de stimulation cutanée et la phase du cycle de marche en locomotion fictive (Burke 1999 ; Degtyarenko et al. 1996, 1998 ; Moschovakis et al. 1991), ainsi que de différents réflexes de retrait selon le site cutané (Schouenborg 2002) suggère qu'il existe probablement une spécificité contextuelle des réflexes cutanés. En variant le contexte à l'aide de différents paradigmes comportementaux, nous pourrions alors changer le niveau d'excitabilité du système nerveux et ainsi déterminer et approfondir le degré d'interaction entre les informations périphériques et le contrôle descendant supraspinal. Un paradigme intéressant serait d'intégrer des perturbations reproductibles lors d'un mouvement

périodique et lui aussi reproductible tel que l'enjambement d'un obstacle lors de la marche sur un tapis roulant.

En effet, lors de l'enjambement d'un obstacle, le chat doit lever la patte suffisamment haut pour passer au dessus de l'obstacle. Les réseaux neuronaux convoyant le signal cutané provenant du dos de la patte pourrait donc être beaucoup plus sollicité pour éviter de frapper l'obstacle. Inversement, lors de la phase de transfert au-dessus et au-delà de l'obstacle, les réseaux neuronaux convoyant le signal cutané provenant de la surface plantaire pourraient être beaucoup plus sollicités afin d'éviter l'obstacle et ajuster un placement correct de la patte au sol.

La contribution du cortex moteur dans la phase d'élévation et de transfert de la patte lors de l'enjambement d'un obstacle (Drew et al. 2002 ; Kably et Drew 1998 ; Widajewicz et al. 1994), pourrait alors moduler ces différents réflexes cutanés. Le cortex moteur pourrait ainsi faciliter les effets du réflexe cutané du dos de la patte par un retrait lors de la phase de transfert. Inversement il pourrait faciliter les effets du réflexe cutané de la surface plantaire de la patte par une phase de transfert prolongée lors du passage au-dessus de l'obstacle. Enfin, le cortex moteur pourrait également faciliter les effets du réflexe cutané de la surface plantaire par un ajustement de la patte lors du contact au sol au-delà de l'obstacle. La modulation corticale des effets des réflexes cutanés lors de l'enjambement d'un obstacle ainsi que lors du placement fin de la patte sur les barreaux d'une échelle horizontale pourrait être comparée à ceux durant la marche, qui servirait de référentiel.

Ces paradigmes comportementaux en impliquant une intégration visuelle, il est probable que les interactions entre les inputs corticaux et cutanés aient lieu au niveau

cortical ainsi qu'au niveau spinal. L'intégration corticale des réflexes à longues latences permettrait d'ajuster et de préparer à l'avance la commande motrice pour enjamber l'obstacle. L'intégration spinale des réflexes à courtes latences permettrait de corriger les perturbations de dernières minutes dans le cas où la patte toucherait l'obstacle.

Plasticité du cortex moteur à la suite d'une dénervation cutanée de la patte postérieure

Nous avons rapporté dans notre troisième étude que la dénervation cutanée de la patte postérieure est rapidement compensée par différentes stratégies motrices consistant à modifier le niveau d'activité de base dans certains muscles en particulier les fléchisseurs. Cette récupération s'accompagne d'une augmentation rapide dans l'amplitude des réponses évoquées par le cortex moteur. Nous suggérons que cette augmentation d'efficacité corticospinale contribue à la récupération fonctionnelle. Les changements dans l'amplitude des réponses évoquées par la stimulation de la pyramide suggèrent que l'augmentation d'efficacité corticospinale serait due à une augmentation d'excitabilité spinale. Cependant les faibles changements d'efficacité spinale par rapport aux changements d'efficacité corticospinale nous laissent penser que ces changements sont probablement à la fois corticaux et spinaux. Enfin les faibles changements dans l'efficacité spinale évoquée par les nerfs cutanés contralatéraux suggèrent, que les changements survenant au niveau spinal ne sont pas globaux mais spécifiques.

Dans les prochaines sections, nous discuterons des différents aspects de cette récupération fonctionnelle, de cette plasticité et des mécanismes sous-jacents à cette plasticité. Enfin, nous aborderons les autres structures supraspinales et spinales qui pourraient contribuer à la récupération fonctionnelle de la marche après une dénervation.

Récupération fonctionnelle

Nous avons rapporté dans cette étude, similairement à celle de Bouyer et Rossignol (2003a), que la majeure contribution à la récupération de la marche était une augmentation dans le niveau d'activité de base du fléchisseur du genou, le Semitendinosus. Cette augmentation consistait à augmenter la flexion du genou afin d'élever la patte pour prévenir le traînement de la patte sur le tapis roulant. Les changements dans les autres muscles étaient par contre beaucoup plus variables au cours de la période de récupération, ce qui ne nous a pas permis de déterminer un patron particulier entre les chats. Ceci suggère que chaque chat aurait utilisé une stratégie compensatrice qui lui était propre. Cette variabilité est en accord avec diverses études ayant étudié la récupération fonctionnelle de la marche après une section des racines dorsales (Goldberger 1977 ; Goldberger et Murray 1974), une section de nerf moteur (Carrier et al. 1997) ou encore une dénervation cutanée bilatérale (Bouyer et Rossignol 2003a). De plus, des changements réciproques dans le niveau d'activité de certains muscles fléchisseurs n'étaient pas rares dans un même chat au cours de la période de récupération, suggérant également une adaptation dans les stratégies motrices individuelles au cours du temps. Cette variabilité individuelle dans le patron locomoteur, également observée à la suite d'une dénervation cutanée bilatérale (Bouyer et Rossignol 2003a), suggère des interactions entre les mécanismes de plasticité corticale et spinale au jour le jour résultant probablement de l'entraînement journalier.

Plasticité corticale

Nous avons rapporté une augmentation significative dans l'amplitude des réponses évoquées par la stimulation du cortex moteur. Cette augmentation d'efficacité corticospinale semble contribuer aux changements accompagnant la récupération fonctionnelle, car les modifications dans le niveau d'activité de base des muscles semblent suivre les changements d'efficacité corticospinale à la suite de la dénervation. De plus, nous avons également rapporté des changements dans l'efficacité corticospinale dans certains muscles fléchisseurs durant la phase d'appui, lorsque ces muscles sont inactifs et probablement hyper-polarisés (Jordan 1984). Ces évidences suggèrent que cette augmentation d'efficacité corticospinale pourrait résulter de changements dans l'excitabilité corticale ou des interneurones spinaux.

D'autres évidences supportent également que les changements dans l'efficacité corticospinale contribuent à la récupération fonctionnelle. Une section de la voie corticospinale abolit immédiatement la récupération de la marche après dénervation, entraînant un traînement des pattes antérieure et postérieure. Ce déficit est caractéristique d'un dommage de la voie corticospinale. Cependant alors que chez le chat intact, ces déficits sont transitoires et complètement compensés en quelques jours (Armstrong 1986 ; Drew et al. 1996). Chez les chats avec une dénervation, les déficits de la patte postérieure perdurent sur une période de plusieurs semaines, tandis que ceux de la patte antérieure disparaissent rapidement. Ces observations suggèrent qu'après une section de la voie corticospinale, les animaux mettent plus longtemps pour compenser la dénervation. De manière similaire, des études préliminaires (Bouyer et al 2000) ont montré que la lésion de la représentation corticale de la patte postérieure avant une

dénervation de la patte postérieure retarde ou empêche la récupération. Tous ces résultats suggèrent donc que le cortex contribue à la récupération fonctionnelle de la marche

Quelques études ultérieures ont montré également des changements dans l'efficacité corticospinale à la suite d'altérations périphériques transitoires, comme des anesthésies (Murphy et al. 2003 ; Rossi et al. 1998) ou un blocage ischémique (Brasil-Neto et al. 1992, 1993 ; Ridding et Rothwell, 1995, 1997 ; Ziemman et al. 1998a,b, 2001, 2002 ; McNulty et al. 2002) d'un bras ou d'une jambe chez l'homme. Cependant, ces changements disparaissaient immédiatement après retrait de l'anesthésie ou du blocage, ce qui suggère probablement des mécanismes différents de ceux que nous avons observés.

Bien que nous ayons observé des changements dans l'efficacité corticospinale depuis l'ensemble des sites, la nature de ces changements étaient différents d'un site cortical à l'autre. Cette variabilité suggère que les changements ne sont probablement pas dus à des changements globaux et homogènes, mais qu'ils résultent d'une certaine spécificité dans les actions exercées depuis différentes régions du cortex moteur. Cette variabilité corrobore d'ailleurs nos deux premières études (Bretzner et Drew 2005a,b) suggérant que différentes régions du cortex moteur possèderaient des terminaisons différentes sur des populations interneuronales spinales régulant l'activité de muscles agissant autour de différentes articulations de la patte.

Mécanismes corticaux ou spinaux

L'augmentation d'efficacité corticospinale pourrait résulter de changements d'excitabilité au niveau du cortex moteur, ainsi que des réseaux interneuronaux spinaux. Nous avons essayé de distinguer ces mécanismes en étudiant les effets de la dénervation sur les réponses évoquées par la stimulation de la voie pyramidale et par la stimulation des nerfs cutanés de la patte intacte, contralatérale à la patte dénervée.

Nous avons rapporté dans certains cas des changements dans l'amplitude des réponses évoquées par la stimulation de la voie pyramidale mais ceux ci étaient relativement faibles par rapport à ceux observés lors de la stimulation du cortex moteur. Néanmoins, l'existence de changements dans l'excitabilité des réseaux interneuronaux spinaux contactés par le cortex moteur est compatible avec les expériences de Bouyer et Rossignol (2003b) qui ont reporté une plasticité spinale compensatrice chez les chats spinaux après une dénervation.

Nous avons également observé une absence de changements dans l'amplitude des réponses évoquées par la stimulation des nerfs cutanés contralatéraux. Ceci supporte notre suggestion que les changements ne résultent pas de changements globaux d'excitabilité. Ce résultat suggère également que les populations d'interneurones spinaux qui sont contactées par la voie corticospinale sont séparées des populations interneuronales activées par les afférences de la patte contralatérale. Ces suggestions corroborent les résultats de notre seconde étude (Bretzner et Drew 2005b) montrant que la stimulation conditionnée du cortex moteur facilite généralement les réponses évoquées par les réflexes cutanés de la patte contralatérale au site cortical de stimulation

(correspondant à la patte dénervée) mais déprime les réponses évoquées par les réflexes cutanées croisées.

Alors que nos résultats suggèrent des modifications dans l'excitabilité spinale, les différences relatives entre les changements d'efficacité évoqués par la stimulation du cortex moteur et la stimulation de la voie pyramidale suggèrent que l'augmentation dans l'excitabilité corticale contribue de manière importante aux modifications observées dans le patron locomoteur. Ceci est d'ailleurs supporté par l'abolition d'une récupération complète dans le cas de lésion de la voie pyramidale, de la lésion de la représentation corticale de la patte postérieure (Bouyer et al. 2000), ainsi que dans le cas de section complète de la moelle épinière (Bouyer et Rossignol 2003b). De plus quelques études ont précédemment démontré une augmentation dans l'amplitude des potentiels locaux corticaux évoqués par la stimulation du noyau interposé ainsi que du noyau thalamique ventro-latéral lors d'un conditionnement cutané de flexion du membre antérieur (Meftah et Rispal-Padel 1994, 1995 ; Meftah et Rispal-Padel 1997), suggérant une augmentation d'excitabilité corticale.

Mécanismes cellulaires de cette plasticité corticale

Ces changements d'excitabilité corticale pourrait résulter d'une augmentation du taux de décharge des neurones de la voie pyramidale qui réguleraient le patron et le rythme locomoteur à la suite de la dénervation. En effet, l'activité cellulaire de la représentation corticale de la patte postérieure du chat est modulée au cours de la marche (Drew et al. 2002 ; Kably et Drew 1998b ; Widajewicz et al. 1994). Certains

groupes de neurones corticaux augmentent leur taux de décharge en phase avec certains groupes de muscles requis lors de l'enjambement ou lors du placement de la patte au-delà de l'obstacle. Cette activité augmentée dans les neurones de la voie corticospinale compenserait alors la diminution d'excitabilité spinale résultant de la perte des informations cutanées du bout de la patte lors de la récupération de la marche.

Bien que difficile, il serait possible de tester cela en enregistrant et en comparant les patrons d'activité cellulaire de neurones pyramidaux avant et après dénervation, à l'aide de microfils implantés chroniquement dans la représentation corticale de la patte postérieure.

Il est aussi possible que ces changements d'excitabilité corticospinale résultent de changements concomitants dans la taille des représentations corticales, comme cela a été montré lors de section du nerf facial ou d'amputation (Donoghue et al. 1990 ; Sanes et al. 1988, 1990). Bien que nos électrodes implantées chroniquement ne nous permettent pas de répondre à cette question, nous avons observé un certain nombre de sites qui recrutaient des nouveaux muscles à la suite de la dénervation, suggérant indirectement des changements dans les représentations corticales.

Un moyen simple de tester cela serait de cartographier la représentation corticale de la patte postérieure et d'évaluer le niveau d'inhibition ou de facilitation intra-cortical entre deux sites corticaux à l'intérieur de cette représentation avant et après dénervation.

En résumé, notre étude suggère que le cortex moteur ne contribue pas seulement au contrôle des mouvements volontaires lors de la marche mais également aux mécanismes sous-jacents à la récupération fonctionnelle de la marche à la suite d'une perte d'information cutanée. Cette étude suggère aussi que la voie corticospinale et les réseaux des afférences cutanés interagissent dans la régulation et dans la récupération fonctionnelle de la marche. Nous avons précédemment montré dans notre seconde étude que les inputs corticaux et cutanées sont intégrés dans des populations interneuronales spinales spécifique lors de la marche (Bretzner et Drew 2005b). La présente étude démontre que la voie corticospinale agit probablement via ces mêmes populations interneuronales spinales lors de la récupération de la marche.

D'autres sources supra-spinales de compensation fonctionnelle

La dénervation cutanée bilatérale, ainsi qu'unilatérale, est rapidement compensée par une augmentation dans le niveau d'activité des muscles fléchisseurs, qui semblent survenir d'une augmentation d'efficacité du cortex moteur et dans une moindre mesure du réseau inter-neuronal spinal de la voie corticospinale. Cependant d'autres structures supra-spinales, comme le noyau rouge, le noyau vestibulaire ainsi que la formation réticulée pourraient également contribuer à la récupération fonctionnelle de la marche après dénervation.

Il est connu que le niveau d'activité des muscles fléchisseurs est sous le contrôle du cortex moteur ainsi que du noyau rouge. Le noyau rouge pourrait donc également participer à la récupération fonctionnelle de la marche après dénervation cutanée pour

compenser le traînement de la patte en augmentant le niveau d'activité des muscles fléchisseurs. En plus des changements dans le niveau d'activité des muscles fléchisseurs de la patte dénervée, nous avons observé un allongement dans la phase d'appui et dans le niveau d'activité des extenseurs de la patte dénervée lors de la marche. Puisque le niveau d'activité des muscles extenseurs semble être sous le contrôle du noyau vestibulaire (Orlovsky 1972a), il pourrait également participer à la récupération fonctionnelle de la marche après dénervation cutanée, en augmentant le niveau d'activité des muscles extenseurs. À ces changements dans le niveau d'activité des fléchisseurs et extenseurs de la patte dénervée, nous avons également observé un changement dans la coordination entre la patte dénervée et celle non dénervée. Puis qu'il existe un certain nombre d'évidences suggérant que la formation réticulée serait impliquée dans le contrôle de la posture et de la coordination inter-membre chez le chat, nous supposons que cette structure pourrait être impliquée dans les ajustement posturaux des membres postérieurs à la suite de la dénervation unilatérale, augmentant le niveau d'activité dans les muscles fléchisseurs de la patte dénervée et dans les muscles extenseurs de la patte contralatérale non-dénervée.

Pour tester cette contribution, il suffirait donc de mesurer les changements d'efficacité synaptique évoquée par la stimulation des structures supraspinales et par la stimulation d'électrodes placées caudalement dans leur voie supraspinale respective, de manière à distinguer les changements supraspinaux des changements spinaux.

De plus, le cortex moteur semble également envoyer une copie éfférente de la commande motrice dans la formation réticulée pontique et médullaire (Kably et Drew 1998a,b) via de fortes projections cortico-réticulaires (Matsuyama et Drew 1997 ; Rho

et al. 1997) chez le chat intact, ce qui pourrait réguler les ajustements posturaux de la formation réticulée et les ajustements plus subtils corticaux lors de la récupération fonctionnelle de la marche après dénervation. Pour tester la contribution du cortex moteur à la formation réticulée, les potentiels de champs locaux de la formation réticulée évoqués par la stimulation du cortex moteur pourraient être enregistrés avant et après dénervation. Une augmentation dans l'amplitude des potentiels de champs évoqués par le cortex moteur suggèreraient alors une augmentation de la contribution corticale à la formation réticulée.

Il existe donc différentes structures supra-spinales qui pourraient contribuer à la récupération fonctionnelle de la marche via un changement d'efficacité depuis les structures elle-mêmes ou encore via les réseaux inter-neuronaux spinaux recrutés par ces différentes voies supra-spinales.

Compensation fonctionnelle provenant du système nerveux périphérique

Bien que nous n'ayons pas rapporté de changements significatifs dans le niveau d'excitabilité des réseaux spinaux inter-neuronaux recrutés par les réflexes cutanés croisés, des changements pourraient provenir des afférences proprioceptives des muscles de la patte dénervée ou encore par l'étirement de la surface de la peau au-dessus du site de dénervation de la patte.

Des études ultérieures qui ont investigué la récupération fonctionnelle de la marche à la suite d'une neurectomie des extenseurs de la cheville ont suggéré une compensation fonctionnelle d'origine proprioceptive (voir *Introduction générale*). Après une neurectomie de la plupart des extenseurs de la cheville, le niveau d'excitabilité du réseau

spinal interneuronal recruté par les afférences proprioceptives de l'extenseur restant de la cheville augmente, suggérant une compensation d'origine proprioceptive. Dans notre étude sur la récupération fonctionnelle de la marche à la suite d'une dénervation cutanée, les afférences proprioceptives pourraient donc compenser la perte d'information sensorielle cutanée et contribuer à la récupération fonctionnelle de la marche. Nous pourrions éventuellement nous attendre à des changements provenant préférentiellement des afférences proprioceptives des muscles impliqués dans la récupération, soient les fléchisseurs du genou et de la cheville. Pour tester cela, il nous faudrait simplement mesurer les changements d'efficacité synaptique évoqués par la stimulation des diverses afférences proprioceptives des muscles de la patte dénervée durant la marche avant et après dénervation cutanée.

Une autre source du système nerveux périphérique qui pourrait contribuer à la récupération de la marche après dénervation cutanée du bout de la patte serait l'étirement de la surface corporelle proximale au site de dénervation. Pour tester cela, nous pourrions mesurer l'efficacité synaptique évoquée par la stimulation de la surface de la peau ou de nerfs cutanés proximaux aux nerfs cutanés sectionnés durant la marche avant et après dénervation.

Si ces hypothèses étaient confirmées, cela suggérerait que les afférences proprioceptives ainsi que la surface de la peau proximale au site de dénervation contribueraient activement à la récupération fonctionnelle de la marche après une dénervation cutanée.

Conclusion

Ces différentes études montrent que le cortex moteur présente une certaine spécificité dans le contrôle du membre postérieure lors de la locomotion. Notre première étude suggère que différentes régions du cortex moteur possèdent des terminaisons différentes sur des populations interneuronales spinales, régulant l'activité de muscles agissant autour de différentes articulations de la patte lors de la marche. Notre seconde étude suggère également que ces différentes régions du cortex moteur modulent différemment les effets de réflexes cutanés lors de la marche, probablement via différentes sous populations interneuronales spinales. Enfin notre troisième étude est une première à ma connaissance à démontrer une contribution corticale et spinale lors de la récupération fonctionnelle de la marche, probablement via ces mêmes populations interneuronales spinales dans lesquelles convergent les signaux corticaux et cutanés chez l'animal intact.

Ces études montrent à quel point le cortex moteur interagit avec des réseaux interneuronaux spinaux dans le contrôle de la locomotion, dans l'intégration des informations cutanées ainsi que lors de la récupération de la marche après une dénervation cutanée. Ces évidences qui suggèrent une forte interaction entre le cortex moteur et les réseaux interneuronaux spinaux chez le chat contrastent avec les études chez l'homme, qui suggèrent de fortes connexions monosynaptiques. Ces différences ouvrent la question à savoir jusqu'à quel point il est opportun d'étudier les mécanismes

anatomiques, physiologiques et comportementaux régulant le contrôle locomoteur chez un animal si différent de l'homme?

Alors que le cortex moteur est essentiel au contrôle de la marche chez l'homme, il semble être facultatif chez le chat. En effet les chats sont capables d'une marche spontanée sur un tapis roulant après une section complète de la moelle épinière. Sur la base de ces évidences, nous aurions pu nous attendre principalement à des changements au niveau spinal lors de la récupération fonctionnelle de la marche après une dénervation cutanée. Ce qui s'est avéré faux. En effet, nous avons observé une augmentation d'excitabilité corticospinale, qui semble contribuer à la récupération fonctionnelle, car elle disparaissait après une section de la voie pyramidale. Ces évidences suggèrent donc que même chez un animal capable d'une locomotion spontanée après une section complète de la moelle épinière, le cortex moteur semble essentiel à la récupération de la marche après une dénervation ou lors de l'enjambement d'un obstacle (Jiang et Drew 1996). Ce qui rapproche le chat du genre humain.

Concernant la contribution du cortex moteur dans le contrôle locomoteur chez le sujet intact, il existe des similitudes et des différences entre ces deux espèces. Le cortex moteur semble être capable de modifier l'amplitude et la durée des activités musculaires en fonction de la phase du cycle de marche aussi bien chez le chat que chez l'homme. Mais alors qu'il semble être capable de réinitialiser le rythme locomoteur chez le chat parce qu'il est capable de raccourcir la durée du cycle locomoteur, il n'existe aucune évidence chez l'homme (Capaday et al. 1999). Ceci est surprenant et paradoxal compte tenu des fortes projections monosynaptiques et de la forte contribution corticale au contrôle de la marche chez l'homme. Par ailleurs, le cortex moteur est la seule structure

supraspinale à être capable d'agir sur le rythme locomoteur chez le chat marchant librement. Toutes ces évidences suggèrent donc une contribution importante dans le contrôle locomoteur autant chez le chat que chez l'homme. Alors pourquoi tant de différences?

Bien qu'il ne fait aucun doute qu'il existe des différences sur le plan phylogénétique entre ces deux espèces, il apparaît tout de même que les différences reportées dans le contrôle cortical de la locomotion pourraient résulter principalement de l'utilisation de techniques indirectes et inadéquates chez l'homme. En effet nous avons abordé au cours de cette discussion le fait que la stimulation trans-crânienne magnétique est une méthode controversée pour investiguer le contrôle de la marche. Le mode d'activation de la stimulation magnétique dans la représentation de la jambe pourrait être indirecte via des cellules corticales qui activerait des cellules de la voie corticospinale ou directe au soma ou au premier nœud de Ranvier des axones des cellules corticospinales. Ceci laisse penser que cette incapacité à modifier le rythme locomoteur pourrait donc résulter non pas d'une différence entre espèce mais d'un artéfact méthodologique.

En terminant, il nous semble que les informations obtenues dans ces expériences chez le chat améliorent notre compréhension des mécanismes corticaux et spinaux qui sous-tendent le contrôle de la locomotion. Il nous semble également que ces informations originales ont des implications importantes dans notre appréciation des mécanismes qui sous-tendent la récupération à la suite d'une altération du système nerveux périphérique, qui devrait avoir de futures applications dans les protocoles de réhabilitation chez l'homme.

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