NMDA RECEPTORS PLAY A ROLE IN SPINALLY-ORGANIZED LOCOMOTION IN THE DECEREBRATE CAT

A Thesis
Presented to the
University of Manitoba

In Partial Fulfilment of the Requirements
for the Degree

Master of Science
in
Physiology

by

Jennifer R. Douglas

ISBN 0-315-76847-9

NMDA RECEPTORS PLAY A ROLE IN SPINALLY-ORGANIZED LOCOMOTION IN THE DECEREBRATE CAT

BY

JENNIFER R. DOUGLAS

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The White Rabbit put on his spectacles. "Where shall I begin,
please your Majesty?" he said.
"Begin at the beginning," the King said, very gravely, "and go on
till you come to the end: then stop."

- Lewis Carroll
Alice's Adventures in Wonderland

Now that I've come to the end (of this project at least), I'd like to thank the people who have helped me to get here.

Thanks to the members of my committee (Drs. Larry Jordan, Dave McCrea and Susan Shefchyk) for their encouragement and enthusiasm right from the start.

A special thanks to Larry, my supervisor, for all the spirited discussions over the last two years that have shown me how exciting science can be. I've learned from you how even the smallest observation can lead to a whole new set of possibilities. (And someday I might even be able to listen to a country & western tune without wincing.)

Thanks to Xiao, for being able to keep smiling through even the worst flops, for cheering during the successes, and for being such a good friend. If anyone can make a decerebrate cat walk through sheer will-power, I think it must be us.

Thanks to Kim, for caring so much about the welfare of the animals. I've learned a lot about how experimental animals should be treated by watching you at work.

Thanks to Gilles and Gilbert for software that allows even this computer-illiterate to wade through data painlessly.

Thanks to Brian, who got our experiments rolling with his arrival a year ago and who has been keeping me on my toes ever since.

Thanks to old friends from home and new friends in Winnipeg, for helping me to take off the spectacles and keep everything in perspective ("Tell me again what 'physiology' is?").

Finally, thanks to my parents, for giving me such a wonderful beginning. This thesis is dedicated to you.

ABSTRACT

The involvement of excitatory amino acids in spinally-organized rhythmic activity has been suggested by work with *in vitro* preparations of the lamprey, frog embryo and newborn rat. The present study was designed to determine whether spinal N-Methyl-D-aspartate (NMDA) receptors play a role in locomotion in an *in vivo*, adult mammalian preparation.

Experiments were performed on precollicular, postmammillary decerebrate cats. Cannulae were positioned underneath the dorsal roots in the lumbar region of the spinal cord for intrathecal drug infusions. Rostral diffusion of the drugs was prevented by tying a suture around the spinal cord at the thirteenth thoracic vertebra securely enough to occlude the subarachnoid space. Locomotion was monitored by electromyograms in treadmill locomotion experiments or electroneurograms in fictive locomotion experiments.

Hindlimb treadmill and fictive locomotion induced by electrical stimulation of the mesencephalic locomotor region in the midbrain was blocked by intrathecal infusion of the NMDA receptor antagonist 2-Amino-5-phosphonovalerate (APV) to the lumbar region of the spinal cord. Hindlimb fictive locomotion was elicited in resting animals by intrathecal administration of NMDA along with the excitatory amino acid uptake blocker dihydrokainate. These results are consistent with previous findings that NMDA receptors play a role in locomotion. However, they do not indicate the mechanism of their involvement. Possible sites of action of NMDA are discussed, including the interneurons involved in the pattern generation circuitry, descending and sensory inputs to the spinal cord, and motoneurons.

LIST OF ABBREVIATIONS

AB	anterior biceps
BI	biceps femoris
LG	lateral head of gastrocnemius
MG	medial head of gastrocnemius
QUAD	quadriceps femoris
SART	sartorius
ST	semitendinosus
TA	tibialis anterior
TRI	triceps femoris
CNS	central nervous system
CSF	cerebrospinal fluid
EAA	excitatory amino acid
EMG	electromyogram
ENG	electroneurogram
EPSP	excitatory post synaptic potential
L	lumbar
MLR	mesencephalic locomotor region
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
APB (AP4)	2-Amino-4-phosphonobutanoate
APH (AP7)	2-Amino-7-phosphonoheptanoate
APV (AP5)	2-Amino-5-phosphonovalerate
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione
CPP	3-((-)-2-Carboxypiperzin-4-yl)propyl-1-phosphonate
δDGG	δ-D-Glutamylglycine
DHK	dihydrokainate
DNQX	6,7-Dinitroquinoxaline-2,3-dione
NMDA	N-Methyl-D-aspartate
PDA	*cis*-2,3-Piperidine dicarboxylate

INTRODUCTION

Neuroscientists have known for 30 years that L-glutamate has an excitatory action on vertebrate central nervous system (CNS) neurons; however, it has only been in the last decade that significant progress has been made in our understanding of excitatory amino acid (EAA) neurotransmission. The development of selective agonists and antagonists for various EAA receptor subtypes in the early 1980's, coupled with the availability of new *in vitro* electrophysiological recording techniques, have led to rapid advances in recent years. The following survey of the EAA literature will focus primarily on the spinal cord, and is intended to establish the foundation upon which the experiments presented in this thesis are based. The classification and distribution of EAA receptors will be described first, followed by a discussion of EAA involvement in monosynaptic and polysynaptic pathways in the spinal cord. This will lead to an examination of what is known about the roles played by EAAs in spinally-organized rhythmic activity.

Excitatory amino acids appear to mediate most of the synaptic transmission along excitatory pathways (Monaghan et al 1989). The powerful depolarizing effects of the dicarboxylic amino acids L-glutamate and on cat interneurons and motoneurons were first reported in the late 1950's (Curtis et al 1959, 1960), and these two compounds are still widely believed to be the most likely EAA transmitter candidates (Stone and Burton 1988, Collingridge and Lester 1989).

The first indication that there might be different populations of EAA receptors came with the finding that L-glutamate was a more potent agonist upon dorsal horn interneurons, whereas L-aspartate was more potent upon ventral horn Renshaw cells when applied electrophoretically in the spinal cord of anesthetized cats (Duggan et al 1974, Johnston et al

1974). The mixed agonist actions of L-glutamate and L-aspartate on several receptor subtypes were initially not fully grasped, and attempts to develop antagonists for the "glutamate preferring" and "aspartate preferring" receptors did not meet with much success (reviewed in Mayer and Westbrook 1987). The breakthrough came with the development of a set of compounds which selectively blocked the responses induced by the glutamate analogue N-Methyl-D-aspartate (NMDA) (McLennan et al 1981, Watkins 1981). The introduction of a second generation of more potent and selective NMDA antagonists, led by 2-Amino-5-phosphonovalerate (APV or AP5) (Davies et al 1980, Evans et al 1982), facilitated the separation of EAA receptors into NMDA and non-NMDA receptor subtypes.

It has proven to be more difficult to subdivide the non-NMDA receptor subtypes, largely because potent specific antagonists have been slow in development. Non-NMDA receptors were initially separated into kainate and quisqualate subtypes on the basis of the differential sensitivity of these agonists to a range of weaker, less selective antagonists (Davies et al 1979), and this classification is still commonly used. In contrast to the traditional EAA receptors which produce membrane depolarization when they are activated, there is recent evidence for a novel type of EAA receptor which is coupled to phosphatidyl inositol metabolism (Sladeczek et al 1988). The fact that it regulates inositol triphosphate formation, as do many growth factors, has led to speculation that it might play a role in synaptic growth and plasticity. It is activated by quisqualate and glutamate, and is currently being called the metabotropic receptor (Young and Fagg 1990). In order to eliminate any confusion with the receptor at which quisqualate has a depolarizing action, the traditional quisqualate receptor is in the process of being renamed for its most selective agonist, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) (Monaghan et al 1989). A final class of non-NMDA receptor is named for its most selective agonist, 2-Amino-5-phosphonobutanoate (APB or AP4). The APB receptor is believed to be a presynaptic glutamate autoreceptor, however its mechanism of action is poorly understood (Collingridge and Lester 1989).

Therefore, five EAA receptor subtypes have been classified to date: the NMDA, kainate, quisqualate (or AMPA), metabotropic and APB receptors.

As the characterization of EAA receptors progressed, various investigators sought to map out their distribution in the CNS. Early autoradiographic studies using [^{3}H]glutamate as a radioligand were plagued by the involvement of glutamate in the intermediary metabolism of all cells (Fagg and Foster 1983). By the mid 1980's, however, it became possible to describe discrete populations of [^{3}H]glutamate binding sites in the brain with the appropriate pharmacology for NMDA, kainate and quisqualate receptors (Greenamyre et al 1985).

Most of the radioligand binding work has focused on the brain, and much less is known about the distribution of EAA receptor subtypes in the spinal cord. In 1984 Greenamyre and his colleagues reported that high levels of [^{3}H]glutamate were bound to the substantia gelatinosa of the spinal cord in all segments, while glutamate binding in the ventral horn was scarce. Deschesne et al (1990) produced monoclonal and polyclonal antibodies to kainate receptors and examined the distribution of the receptor using both immunocytochemical and autoradiographic techniques. They reported that kainate receptors are found mainly in the dorsal part of the grey matter of the frog spinal cord, with very sparse staining/binding in the ventro-lateral regions. An autoradiographic study of the distribution of NMDA, kainate and quisqualate receptors in the human spinal cord (Jansen et al 1990) showed all three receptors to be densely concentrated in lamina II of the dorsal horn, with much lower levels in the other laminae. Preliminary autoradiographic work on lumbar spinal tissue of mice has also shown a concentration of NMDA receptors in the substantia gelatinosa, along with the medial border of the dorsal horn and in lamina X (Gonzalez et al 1990). A monoclonal antibody that binds to NMDA receptors has been developed this year (Potter and Moskal 1990), and it will no doubt prove to be a useful tool in future investigations of NMDA receptor distribution.

The anatomical experiments described above would seem to suggest that EAA

receptors are located primarily in the dorsal part of the spinal cord. The spinal cord has received more attention from investigators using electrophysiological techniques, and their work points to EAA actions throughout both dorsal and ventral horns. Armed with the first selective NMDA receptor antagonists, Watkins' group began a decade ago to study excitatory synaptic transmission in the cord. In 1981 they reported that APV blocked the responses of cat spinal neurons to aspartate and to dorsal root stimulation (Davies et al 1981). They further characterized the actions of APV the following year, with a study of isolated frog and rat spinal cords (Evans et al 1982). They looked at evoked response in ventral roots following dorsal root stimulation, and found that APV blocked the later (polysynaptic) components of the response, with relatively little effect on the initial (monosynaptic) component. Returning to the cat spinal cord *in vivo*, Davies and Watkins (1982) found that iontophoretically-applied APV was an effective antagonist of NMDA-induced responses in dorsal horn cells and Renshaw cells, and that polysynaptic excitation of the dorsal horn cells was more susceptible to depression by APV than was monosynaptic excitation. Thus, it appeared that the depression of polysynaptic responses was related to NMDA receptor antagonism. In order to determine whether EAAs were also involved in monosynaptic responses, they used a wider spectrum of antagonists. Davies and Watkins (1983) applied APV and two non-selective antagonists (*cis*-2,3-piperidine dicarboxylate (PDA) and δ-D-glutamylglycine (δDGG)) iontophoretically onto cat dorsal horn neurons. All three antagonists could depress the polysynaptic excitation produced by stimulation of low threshold afferent fibres. The non-selective antagonists, which could reduce the sensitivity of the neurons to kainate and quisqualate as well as NMDA, also suppressed the monosynaptic response. When they compared the effects the three antagonists had on synaptic responses with their abilities to depress responses induced by and L-glutamate, it seemed that actions were mediated mainly by NMDA receptors, while non-NMDA receptors made a greater contribution to L-glutamate actions.

The notion was beginning to emerge that non-NMDA receptors mediated monosynaptic responses to afferent input, whereas NMDA receptors were involved in the interneuronal pathways responsible for polysynaptic responses. This view, first suggested by the work of Watkins' group, has been supported by other investigators working with the anesthetized spinal cat (Peet et al 1983), isolated neonatal rat spinal cord (Jahr and Yoshioka 1986, Gerber and Randic 1989), and isolated hamster spinal cord (Bagust et al 1989). It has been strengthened by recent studies encompassing new antagonists. Morris (1989) reported that 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a relatively selective antagonist at kainate and quisqualate receptors, blocked the first action potential in dorsal horn neurons in the isolated neonatal rat spinal cord evoked by stimulation of myelinated primary afferents. The short latency, small latency variation and ability to follow repetitive stimulation suggested that the first responses were evoked monosynaptically. Later bursts of action potentials were blocked by 3-((-)-2-carboxypiperzin-4-yl)propyl-1-phosphonate (CPP), the most potent and selective NMDA antagonist developed to date. Polc (1985, 1987) reported that NMDA receptors are involved in the polysynaptic component of ventral root reflexes in unanesthetized spinal cats. Intravenous infusions of CPP blocked the late component of the reflex and left the monosynaptic component unaffected. Interestingly, Polc's work also showed that intravenous infusions of the NMDA receptor antagonists 2-amino-7-phosphonoheptanoate (APH) and CPP depressed spontaneous gamma motoneuron activity recorded in the ventral roots, whereas intravenous NMDA infusions evoked heightened excitability of gamma motoneurons recorded in the ventral roots. This suggested that the NMDA receptors might mediate the background activation of gamma motoneurons in spinal cats.

Evidence that primary afferents release a transmitter acting at a non-NMDA receptor is not confined to the spinal cord. Chandler (1989) reported that non-selective EAA antagonists such as kynurenate suppressed monosynaptic transmission from afferents in the

mesencephalic branch of the trigeminal nerve to jaw-closer motoneurons, whereas APV had almost no effect. The possibility that non-NMDA receptors mediate the monosynaptic responses to afferent inputs has spurred interest in the neurotransmitter acting at the primary afferent synapse. L-glutamate would seem to be the most likely candidate among the endogenous acidic amino acids, given its distribution in the substantia gelatinosa and presumed preference by non-NMDA receptors as described above. However, when Roberts and Keen (1974) sectioned the dorsal roots in rats and allowed 30 days to pass for degeneration of the afferent terminals , they found no change in the glutamate content of the dorsal half of the spinal cord. Jahr and Jessell (1985) examined this issue in the synapses formed between dorsal root ganglia and dorsal horn neurons in culture. They recorded intracellularly from dorsal horn neurons which exhibited a monosynaptic response to stimulation of the dorsal root ganglia. The medium contained high divalent cation concentrations to block NMDA receptors. Under these conditions, L-glutamate, kainate and quisqualate were each sufficient to excite the dorsal horn neurons. Their results led the authors to state decisively that L-glutamate mediates the fast excitatory postsynaptic potential (EPSP) at these sensory neuron synapses. Schneider and Perl (1988) were less convinced of L-glutamate's importance. They recorded intracellularly from neurons responding to dorsal root volleys in a horizontal slice preparation of the hamster dorsal horn. They found that less than a third of the neurons sampled were depolarized by either bath applied L-glutamate or L-aspartate and that those excited by L-glutamate were generally confined to the superficial dorsal horn. It is important to note that it was not possible to establish that the EPSPs resulting from dorsal root volleys were monosynaptic, so it is possible that the neurons in their sample did not receive direct inputs from afferents. Nevertheless, they concluded that while L-glutamate might be responsible for the central synaptic excitation by a substantial number of primary afferents, more than half of the fast EPSPs generated by primary afferents in the dorsal horn involve other mediators.

Although the identities of the endogenous ligands at EAA receptors are still a matter of debate, there is no doubt that EAA receptors are intimately involved in spinal excitatory pathways. The NMDA receptor complex in particular has properties which allow it to make contributions to a diverse range of activities. In 1982 MacDonald and Wojtowicz reported that L-glutamate, , NMDA and other EAAs (but not kainate) could depolarize cultured mouse spinal neurons by means of either decreases or increases in membrane conductance. The decrease in membrane conductance was seen only at potentials near resting values, while the increase in membrane conductance appeared when the neurons were tonically depolarized. It was soon established that activation of the NMDA receptor was responsible for inducing a region of negative slope conductance in the current-voltage relationship of the postsynaptic membrane (Mayer and Westbrook 1984). Ascher and his colleagues showed that the voltage sensitivity of NMDA-mediated responses was due to a voltage-dependant block of the associated ion channels by magnesium ions (Nowak et al 1984). This feature means that the responsiveness of the postsynaptic neuron to NMDA receptor activation can be modulated by other inputs: since Mg^{2+} ions block the NMDA channel most effectively at more negative membrane potentials, NMDA receptor-mediated EPSPs will be enhanced when the membrane is depolarized by the simultaneous arrival of other synaptic inputs (MacDermott and Dale 1987). Another interesting property is that a Ca^{2+}-dependant repolarizing conductance might be activated by the accumulation of intracellular calcium during NMDA receptor activation. Together with the negative slope depolarizing conductance, the repolarizing conductance could generate rhythmic shifts in the membrane potential (Flatman et al 1986). MacDonald (1984) reported that iontophoretically-applied L-aspartate induced regenerative "bursts" in cultured spinal neurons in the presence of tetrodotoxin. Such pacemaker-like oscillations make NMDA receptors attractive as possible participants in the production of rhythmic behaviours.

Our current understanding of the role of EAAs in spinally-organized rhythmic activity

owes a great deal to the innovative vertebrate preparations introduced by groups in Stockholm and Bristol ten years ago. *In vitro* preparations of the lamprey spinal cord (Cohen and Wallen 1980) and the paralysed Xenopus frog embryo (Roberts et al 1981) were shown to exhibit locomotor rhythms and were established as legitimate models for detailed studies of spinal locomotor circuitry. The isolated nature of the preparations reduced the number of variables with which investigators had to contend and permitted detailed cellular studies previously possible only with invertebrates (McClellan 1987). Poon (1980) opened the door to EAA studies with her demonstration that bath application of DL-homocysteate and D-glutamate could elicit movements indistinguishable from normal swimming in the myotome-spinal cord lamprey preparation. Grillner and his colleagues (Grillner et al 1981) found that bath-applied NMDA induced fictive locomotion (rhythmic ventral root activity) in the lamprey cord, and that the burst frequency increased over a wide range with increasing dose (Brodin et al 1985). NMDA receptor antagonists blocked NMDA-induced rhythmic activity (Grillner et al 1981) and spontaneous fictive swimming (Brodin and Grillner 1985a). Alford and Grillner (1990) found that bath-applied NMDA could induce locomotion in the isolated lamprey spinal cord in the presence of CNQX and 6,7-dinitroquinoxaline-2,3-dione (DNQX), which block non-NMDA receptors, suggesting that NMDA receptor activation is sufficient to induce locomotion. Taken together, these results provided strong evidence that transmitters activating NMDA receptors participate in the spinal rhythm generating system.

The discovery that NMDA receptors participated in rhythmic motor activity generated interest in the possible contributions of NMDA receptor-induced membrane potential oscillations. First it was necessary to determine whether any neurons rhythmically active during locomotion showed NMDA receptor-induced oscillations when isolated from synaptic inputs. Sigvardt et al (1985) demonstrated this with intracellular recordings from neurons in 4-15 segment sections of lamprey spinal cord. Bath application of NMDA continued to elicit large amplitude membrane potential oscillations in some neurons after elimination of sodium-

dependant action potentials with tetrodotoxin. The oscillations disappeared after NMDA washout, NMDA-receptor blockade by APV, or after Mg++ was removed from the bathing fluid (Grillner and Wallen 1985, Wallen and Grillner 1987). Brodin and Grillner (1986) postulated that if the membrane potential oscillations observed in single cells after tetrodotoxin were important during NMDA-induced fictive locomotion, the locomotor activity should be altered when the region of negative slope conductance was eliminated by removing Mg^{2+} ions from the bathing solution. They found that NMDA-induced locomotion was considerably more unstable in Mg^{2+}-free solutions at low rates of activity, while a more regular locomotor pattern was seen at higher rates. They concluded that NMDA-induced membrane oscillations have a synchronizing effect on the locomotor network at low frequencies, with other neuronal mechanisms being more important at higher frequencies.

McClellan and Farel (1985) looked at EAA involvement in rhythmic motor activity from an ontogenetic standpoint. They studied the effects of EAAs in the bullfrog tadpole, which displays a developmental progression from undulatory swimming to pedal locomotion. Injection of NMDA into spinal tadpoles or bath application to isolated tadpole spinal cords activated motor patterns that paralleled development: swimming in early tadpoles, stepping or wiping in later stage tadpoles. The authors could not be sure whether NMDA caused specific activation of the spinal motor networks or simply a general increase in the excitability of spinal neurons, but the fact the NMDA activated coordinated motor output rather than non-specific excitation was indirect evidence for the former possibility.

Meanwhile, Roberts' group in Bristol was attempting to characterize for the first time the pre- and postsynaptic elements of a synapse utilizing an EAA as neurotransmitter. In the initial experiments, Dale and Roberts (1984) reported that L-glutamate, NMDA and kainate (but not quisqualate) applied to the bathing medium could each evoke fictive swimming in spinal frog embryos. They recorded intracellularly from motoneurons and found that the motoneurons exhibited phasic activity superimposed on a tonic level of depolarization. This

was the same pattern of activity as seen in intact embryos during naturally-induced swimming. The tonic component of motoneuron activity during naturally-induced swimming could be blocked by adding non-selective EAA antagonists to the bath (Roberts et al 1985). Motoneurons appeared to possess all three traditional types of EAA receptor, since NMDA, kainate and quisqualate were able to depolarize them in the presence of tetrodotoxin (Dale and Roberts 1984). With this groundwork established, Dale and Roberts went on to record unitary EPSPs in motoneurons evoked by extracellular stimulation of single axons or intracellular stimulation of interneurons. They found two components to the EPSP: a slow component mediated by NMDA receptors and a fast component mediated by kainate/quisqualate receptors (Dale and Roberts 1985). The authors showed that the slow NMDA-mediated component could summate with repetitive stimulation to produce the tonic depolarization seen in swimming. The fast component of the EPSP could account for the phasic activity which was superimposed on the tonic excitation. They suggested that their findings could be explained by a class of pre-motor interneuron which releases an EAA transmitter acting at both NMDA and kainate/quisqualate receptors. During swimming the interneurons making EAA-dependant synapses on motoneurons had rhythmic "motoneuron-like" activity themselves, and appeared to receive similar EAA-mediated synaptic inputs to those received by motoneurons (Dale and Roberts 1985).

When Dale and Grillner (1986) undertook focal extracellular stimulation of single axons with intracellular recording from motoneurons in the lamprey, they found motoneuronal EPSPs that were almost identical in time course, shape and pharmacology to those seen in the Xenopus embryo. The EPSP consisted of an NMDA-mediated slow component and a kainate/quisqualate receptor-mediated fast component. Bath application of APV blocked the slow component of the EPSP, whereas application of the selective kainate- and quisqualate-receptor antagonists CNQX and DNQX blocked the fast component (Alford and Grillner 1990). As with the frog embryo, the EPSPs could be attributed to rhythmically

firing interneurons releasing a transmitter acting at both NMDA and kainate/quisqualate receptors (Dale 1986). Evidence for this proposal accumulated in subsequent studies with paired interneuron-motoneuron intracellular recordings (Buchanan and Grillner 1987, Buchanan et al 1987, 1989). The technique of intracellular penetrations of neuron pairs has recently been extended to a study of the synapses made by descending fibres from the brainstem onto spinal neurons by Ohta and Grillner (1989). They reported that fast-conducting reticulospinal neurons generated monosynaptic EPSPs in motoneurons, excitatory and inhibitory interneurons. The chemical component of the EPSPs displayed the familiar NMDA and kainate/quisqualate receptor-mediated phases. The authors suggested that the reticulospinal neurons were well-suited to act as the brainstem output neurons for the initiation of locomotion, and they showed an example of one of the neurons phasically modulated during fictive locomotion. The studies which have combined stimulation of the presynaptic neuron with intracellular recording of the postsynaptic neuron have therefore provided conclusive evidence that EAA receptors are located at a variety of sites in the spinal circuitry for locomotion.

The marked similarities which have come to light concerning the generation of locomotion in such different animals as the frog embryo and the lamprey suggest that some of the mechanisms might also be conserved in higher animals (Dale 1986). In an isolated preparation of the chick spinal cord, for instance, bath application of NMDA increased the frequency of spontaneous rhythmic ventral root bursting in a dose-dependant manner, while APV had a depressive effect (Barry and O'Donovan 1987). Smith and Feldman (1985) introduced an *in vitro* brainstem-spinal cord preparation of the neonatal rat, which made it possible to test the findings observed in lower vertebrates in a mammal. It quickly became apparent that bath application of NMDA to the spinal cord partition could elicit stepping-like movements (Smith et al 1986) with interlimb coordination (Smith and Feldman 1987) when the limbs were left attached. NMDA-induced rhythmic activity in the spinal cord was

completely suppressed by bath application of APV (Kudo and Yamada 1987). Smith et al (1988) showed that L-aspartate and L-glutamate could induce locomotion when administered together with an acidic amino acid uptake inhibitor, dihydrokainate (DHK). They also reported that application of DHK by itself elicited locomotion in a dose dependant fashion, thereby demonstrating the involvement of endogenous EAAs in the generation of rhythmic motor patterns. EAAs have also been implicated in respiratory patterns. McCrimmon et al (1989) found that APV and other EAA antagonists blocked spontaneously occurring inspiratory motoneuronal discharges, suggesting that endogenous activation of EAA receptors is important in determining their pattern of discharge.

In contrast to the many detailed studies of EAA involvement in rhythmic activity which have been possible with *in vitro* preparations, work *in vivo* has often consisted of qualitative observations of the behavioural manifestations of EAA agonists and antagonists administered in awake animals. Intrathecal administration of high doses of NMDA through a chronically implanted cannula in the lumbar cord in awake rats (Raigorodsky and Urca 1987) and awake mice (Aanonsen and Wilcox 1986, Urca and Raigorodsky 1988) produced biting of the hindquarters, scratching and vocalization: indications of activation of pain pathways. APV reversed the effects of NMDA and produced dose-dependant analgesia and motor impairment of the hindlimbs when administered alone (Raigorodsky and Urca 1987). Cahusac et al (1984) reported significant postural and locomotor paralysis as measured by the paw-placement test and rotarod performance following intrathecal administration of APV in awake rats. Intraperitoneal or intracerebroventricular injections of APV depressed muscle tone in a genetically spastic strain of rats (Turski et al 1985), and intravenous administration of a variety of EAA antagonists depressed decerebrate rigidity in rats (Shinozaki et al 1989).

In studies designed to study locomotion more specifically, decerebrate animals have been used since they offer the experimenter a greater degree of control over the animals' behaviour. Studies on the brainstem control of locomotion have demonstrated that stepping

can be induced by injections of L-glutamate into the medial reticular formation or pontomedullary locomotor strip in decerebrate cats (Noga et al 1988), or by injections of NMDA into the mesencephalic locomotor region (MLR) in decerebrate rats and cats (Garcia-Rill et al 1990). To date, there have been no studies which have examined *in vivo* the role of EAAs in the generation of locomotion at the spinal level.

The experiments described in this thesis were designed to address this issue: specifically to investigate whether NMDA receptors play a role in the spinal circuitry for locomotion *in vivo*. The aim was to determine whether some of the *in vitro* findings on the involvement of EAAs in spinally-organized rhythmic activity were applicable in a more physiological setting, while at the same time eliminating some of the variables inherent in the use of awake, behaving animals. To this end we used precollicular postmammillary decerebrate cats; with this preparation the experimenter can turn locomotion on and off at will in a reasonably reproducible fashion by stimulating the MLR in the midbrain, and can be assured that spontaneous locomotion will not occur. Drugs were infused intrathecally in the lumbar region of the spinal cord and their effects were observed either on hindlimb locomotion on a treadmill or on fictive hindlimb locomotion in paralysed cats. Intrathecal administration of APV blocked MLR-induced hindlimb stepping on the treadmill and in fictive locomotion. Fictive hindlimb locomotion could be induced in resting cats by intrathecal administration of NMDA along with the uptake blocker DHK.

METHODS

The data presented in this study were obtained from 22 cats weighing 2.4 to 4.0 kg. Animals were initially anesthetized with halothane carried in a mixture of 70% nitrous oxide and 30% oxygen and delivered through a face mask. The trachea was intubated for direct administration of the anesthetic. A cannula filled with heparinized saline was inserted into the left common carotid artery and connected to a pressure transducer for blood pressure monitoring. The right common carotid artery was dissected free from the surrounding tissue and a ligature was looped around it in order to permit temporary occlusion of the artery during decerebration. The right external jugular vein and occasionally the left cephalic vein were cannulated for administration of fluids. A bicarbonate/glucose buffer solution (.84 g sodium bicarbonate, 5 g glucose in 100 ml water) was infused at 5 ml/hr throughout the experiment, and dextran (Travenol) was administered as necessary to maintain blood pressure at or above 80 mmHg. Each animal was given 2 mg dexamethasone (Hexadrol phosphate, Organon) intravenously to minimize tissue swelling. Body temperature was monitored via an esophageal temperature probe and maintained at 36-39°C by a heating pad placed underneath the surgery table and a brood lamp placed over the animal.

Treadmill locomotion experiments

In the 9 experiments in which the cat walked on a treadmill, electromyographic (EMG) recordings were obtained from the following muscles: biceps brachii (BI) and triceps brachii (TRI) in both forelimbs, and lateral head of gastrocnemius (LG) and tibialis anterior (TA) in both hindlimbs.

A laminectomy was performed, removing the 13th thoracic, 5th lumbar and 6th lumbar vertebrae. A 3-0 silk suture was gently passed underneath the exposed thoracic spinal

cord. At the caudal end of the exposed lumbar region, a small hole was made in the dura mater and the arachnoid slightly lateral to the dorsal root entry zone on the left and right sides. Thin, flexible tubing for intrathecal drug infusions was inserted bilaterally through the holes, into the subarachnoid space. The tubing was passed rostrally underneath the dorsal roots to approximately the region of the L4 spinal segment. The position of the tubing and the extent of fluid diffusion were checked at the end of each experiment by infusions of fast green dye.

Animals were placed in a frame over a treadmill and were supported by a sling under the abdomen. The head was placed in a stereotaxic head-holder, and the cranium was removed between the coronal suture and cerebellar tentorium. A precollicular, postmammillary decerebration was performed with a blunt spatula, following which anesthesia was discontinued. After a 1 hour recovery period, locomotion was induced by electrical stimulation (0.5-1.0 ms pulses, 15-20 Hz, 40-220 uA) of the mesencephalic locomotor region (MLR) as previously described by Jordan et al (1979). EMGs were amplified, band-pass filtered (30-3000 Hz), rectified, and integrated before being digitized by a Masscomp computer for analysis.

Fictive locomotion experiments

In order to monitor fictive locomotion in paralysed cats (13 experiments), various muscle nerves were dissected free, cut and used to monitor motor activity. Cleidobrachialis in the left and right forelimbs, and the sartorius (SART) and quadriceps femoris (QUAD) branches of the left femoral nerve were inserted into recording nerve cuffs. The right femoral nerve was cut in order to maintain symmetry with the left side. The sciatic nerves were placed in custom-made boats filled with mineral oil maintained at 38°C with a feedback-controlled heating lamp. The anterior biceps (AB), semitendinosus (ST), lateral and medial heads of gastrocnemius (LG and MG) and tibialis anterior (TA) branches of the sciatic

nerve in both hindlimbs were placed on bipolar electrodes.

The thoracic spinal cord was exposed and looped with a suture as in the treadmill locomotion experiments. A lumbar laminectomy (L3-L7 vertebrae) was performed, and cannulae for intrathecal drug infusions were inserted as described above.

Animals were transferred to a spinal frame with legs pendant. They were supported by two vertebral clamps and hip bars. A back pool was formed and filled with mineral oil maintained at 38°C with a feedback-controlled heating lamp. Anesthesia was discontinued following a precollicular, postmammillary decerebration. After a 1 hour recovery period, animals were paralysed with 8-16 mg gallamine triethiodide (Flaxedil, Rhone-Poulenc) given intravenously, with additional doses administered as necessary. Artificial ventilation was adjusted to maintain end tidal CO_2 between 2.5 and 5%. Locomotion was induced by MLR stimulation, and the ENGs were amplified, band-pass filtered (30-3000 Hz), rectified and integrated, then digitized on a Masscomp computer for analysis.

Drug trials

The suture around the thoracic cord was tightened prior to each drug trial just sufficiently to occlude the subarachnoid space. This was done to confine the spread of intrathecally administered fluids to the lumbar region of the spinal cord. In some experiments a hole was cut in the dura and arachnoid slightly caudal to the thoracic tie in order to allow excess fluid to drain out after drug infusions.

DL-2-amino-5-phosphonovaleric acid (APV, Sigma), dihydrokainic acid (DHK, Sigma), L-(+)-glutamic acid (Baker) and N-methyl-D-aspartic acid (NMDA, Sigma) were dissolved in an artificial cerebrospinal fluid (CSF) (Elliott's Solution A, Elliott & Jasper, 1949) for intrathecal administration. In most cases a volume of 0.5 mL was infused through each intrathecal cannula. Control intrathecal infusions of Elliott's Solution A were done in order to test whether or not the vehicle itself affected locomotion.

RESULTS

The control infusions of a volume of artificial (CSF) equal to the fluid volume infused during drug trials did not affect MLR-induced hindlimb locomotion. This indicates that the effects seen after drug infusions cannot be attributed to an action of the artificial CSF itself, or to an increase in the pressure on the spinal cord at around the time of injection. The fact that locomotion could be elicited after the ligature was tightened suggests that blood flow was not impaired. Injections of fast green dye through the intrathecal cannulae after each experiment showed that the diffusion of the intrathecally administered fluids was confined to the lumbar and sacral spinal cord. The dye was usually distributed throughout the subarachnoid space around the lumbar cord within 2-3 minutes after infusion, however in some cases the distribution was uneven.

Effects of intrathecal APV

After the control infusions of artificial CSF, the threshold MLR stimulation strength for locomotion was recorded. This represented the lowest current strength which could induce quadrupedal stepping within approximately 20 seconds of the start of the stimulation in treadmill locomotion experiments. In fictive locomotion experiments the threshold for bipedal hindlimb stepping was measured, since the attempts to record forelimb ENG activity were often unsuccessful. When the selective NMDA receptor antagonist APV was administered intrathecally, the MLR stimulation strength needed to elicit hindlimb treadmill or fictive stepping started to increase within 15-20 minutes. The threshold continued to rise gradually, until there was no hindlimb activity at stimulation currents of up to 220 uA (Figure 1), 30 to 100 minutes after APV administration. APV blocked locomotion in a total of 5 treadmill and 6 fictive locomotion experiments. In 1 treadmill experiment, administration of

4 doses of APV ranging from 1-10 mM had very little effect on hindlimb locomotion.

The deterioration in the quality of the hindlimb locomotion followed a consistent pattern: the amplitude of the EMG or ENG bursts gradually decreased, but the frequency of stepping remained constant (Figure 2). When watched on the treadmill, the steady decline in the vigour of the hindlimb steps was quite striking. First the hindlegs would undergo smaller excursions of the limb during the swing phase, and the ankle would fail to extend so that the animal stepped on its toes rather than footpads during the stance phase. Then the dorsum of the foot would be dragged along the treadmill throughout the step cycle. As the strength of the muscle contractions continued to decline, the legs would increasingly be pulled behind the animal by the moving treadmill. Just before all hindlimb activity ceased, weak contractions of the thigh muscles could be seen through the skin, still occurring at the same frequency as the forelimb steps. In the two fictive locomotion experiments in which SART and QUAD ENGs were recorded, SART activity persisted the longest.

Recovery of hindlimb locomotion should be possible with a competitive antagonist, and this was seen in 1 treadmill locomotion experiment (Figure 3) and 1 fictive locomotion experiment (Figure 4) 3-4 hours after APV administration, with stimulation of the same MLR site. In the treadmill locomotion experiment, the hindlimbs resumed walking at the same frequency as the forelimbs (forelimb locomotion was not monitored in the fictive locomotion experiment).

In the treadmill experiments, there was usually no change in forelimb locomotion after APV administration, which suggests that the loss of hindlimb locomotion was not due to a decline in the effectiveness of the MLR site, or to a general deterioration in the health of the animal. In the instances in which forelimb locomotion became weaker, it is possible that a small quantity of the drug leaked past the tie, or that the decrease in propriospinal input as the hindlimbs stopped locomoting affected forelimb stepping.

Effects of intrathecal NMDA, L-glutamate and DHK

a) *treadmill locomotion experiments:* During the series of treadmill locomotion experiments, attempts were made in 3 cats to induce hindlimb stepping with NMDA (0.1 mM - 10 mM) in the absence of MLR stimulation. The first priority in the treadmill experiments was to block MLR-induced locomotion with APV. Thus two of the animals described in this subsection had undergone an APV (1 mM) trial several hours before NMDA infusions were started. In one case MLR stimulation produced good hindlimb locomotion before NMDA was administered; in the other case there was no locomotion with a variety of MLR stimulation sites. In both of these animals there was an immediate increase in hindlimb muscle tone within 1 minute of NMDA (0.1-10 mM) administration. There were periods of hindlimb flexion in which the legs were folded up against the abdomen or over the sides of the frame, which initially lasted 1 minute or more then gradually became shorter. The periods of flexion were occasionally interrupted by brief episodes of high frequency oscillations of one hindleg or the other. The hindlimb flexion was accompanied by forelimb extension. In the third animal, the brainstem was swollen and hemorrhaging, making it impossible to induce locomotion with MLR stimulation. Two hours before NMDA infusions, this animal was spinalized by cooling the exposed segment of the thoracic cord with crushed frozen Elliott's Solution A and tightening the suture tied around the cord. Similar effects were seen to those described above, after intrathecal NMDA (0.1-1 mM) administration, except that there were no forelimb effects.

b) *fictive locomotion experiments*: NMDA (1-5 mM) was administered intrathecally to 4 paralysed cats. As in the treadmill experiments, the first priority was to test the effect of APV on MLR-induced locomotion. In 3 of the cats, there was little or no MLR-induced locomotion from the start, and therefore no APV was administered prior to the NMDA. One of these animals was spinalized as described in the preceding section, 20 minutes before NMDA infusion. In the fourth cat MLR-induced stepping was blocked with APV, and when

there was no recovery after four hours it was decided to test the effects of NMDA. In all 4 cats, NMDA infusions resulted in occasional single bursts in the flexor ENGs which lasted for 2-10 seconds each. In the cat which had earlier received APV there were also brief runs of rhythmic activity in the left SART or right TA ENGs (Figure 5a).

Intrathecal infusions of 5 mM glutamate alone (1 cat), 5 mM glutamate with 5 mM DHK then 10 mM glutamate with 10 mM DHK (1 cat), or 5 mM DHK alone (1 cat) resulted in isolated single bursts of activity (Figure 5b) or else had no effect at all.

The cat which received the doses of glutamate with DHK did not show an immediate response. However, when 5 mM NMDA was administered 20 minutes later, spontaneous fictive locomotion (no MLR stimulation) appeared in several hindlimb nerves. It had not been possible to elicit MLR-induced locomotion in this animal. NMDA was subsequently found to consistently induce locomotion in four more fictive locomotion experiments when DHK was intrathecally administered either at the same time as NMDA, or 20-60 minutes prior to NMDA. When the order of infusion was reversed (NMDA, then DHK 30 minutes later), the resulting locomotion was not as vigorous. We felt confident that the locomotion could be attributed to drug effects, since with the postmammillary level of decerebration used in this study, the animals did not exhibit spontaneous rhythmic motor activity before the drug trials.

The fictive locomotion elicited by NMDA and DHK administration ranged in frequency from 0.9 to 5.9 steps/second. Locomotion could be maintained for up to 30 minutes after a given dose of NMDA and DHK, and the locomotion increased in frequency (Figure 6). At the lower stepping frequencies (below 3.5 Hz), the ENGs demonstrated flexor/extensor alternation in each limb, and alternation between limbs (Figure 7). At high stepping frequencies the quality of the locomotion deteriorated.

In one experiment, locomotion induced by intrathecal adminstration of 5 mM DHK followed by 5 mM NMDA was blocked by a 10 mM dose of APV infused 30 minutes later (Figure 8). The amplitude of the ENG bursts first started to decrease approximately 15

minutes after APV administration, comparable to the time course of the effects of APV on MLR-induced locomotion.

Attempts were made to reproduce these results with comparable doses of NMDA and DHK in 2 treadmill locomotion experiments. In both cases non-specific excitation occurred, with long periods of hindlimb flexion, intermittent periods of vigorous shaking of the leg and trunk muscles, and sporadic episodes of 2-4 step cycles (Figure 9).

In two of the fictive locomotion experiments, the locomotion induced by NMDA and DHK was occasionally interrupted by episodes of high frequency rhythmic activity (Figure 10). The frequency (7 Hz) is faster than that reported for fictive scratch by Deliagina and colleagues (1975), and they described a pattern of very long flexor bursts alternating with short extensor bursts which is not apparent here. Fictive paw shake has not yet been characterized, however the frequency and sequence of nerve activations (particularly the coactivation of QUAD and TA) seems to be in agreement with the pattern seen in the paw-shake responses of spinal cats before and after hindlimb deafferentation (Smith et al 1985, Koshland and Smith 1989).

FIGURE 1

The lowest MLR stimulation strength (threshold) which could elicit treadmill locomotion in all four limbs was tested before and after intrathecal administration of 1 mM APV. The dashed arrows signify that no response could be elicited at the indicated current. In this example, 100 minutes after APV infusion, both hindlimbs dragged on the treadmill at 200 uA, which was the maximum MLR stimulation strength tested.

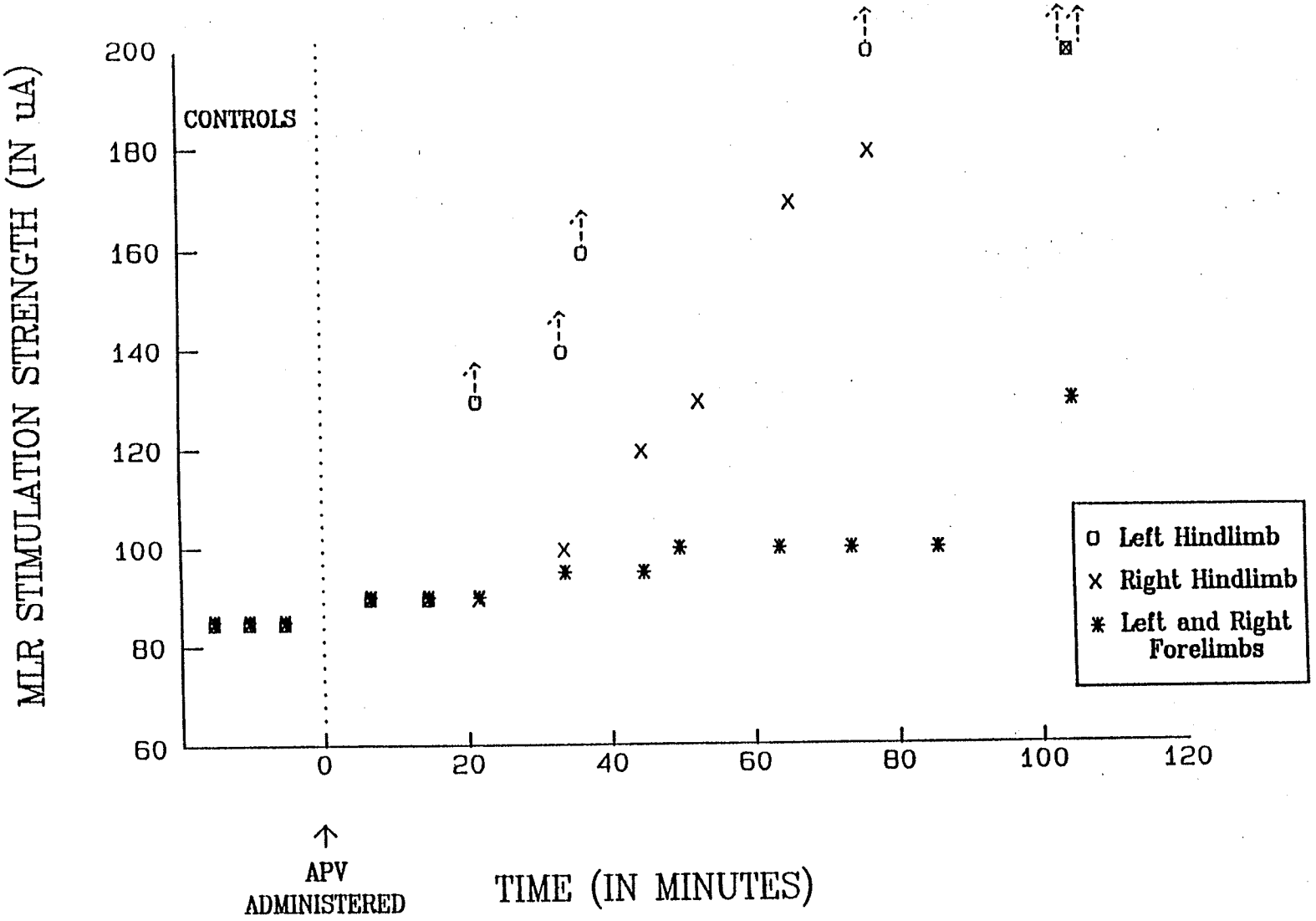

MLR STIMULATION STRENGTH (IN uA)
200
180
160
140
120
100
80
60
CONTROLS
0
20
40
60
80
100
120
APV
ADMINISTERED
TIME (IN MINUTES)
Left Hindlimb
Right Hindlimb
Left and Right
Forelimbs

FIGURE 2

Early effects of APV on hindlimb stepping on the treadmill. (A) Control. Locomotion was elicited with 110 uA MLR stimulation following intrathecal administration of the vehicle, Elliot's Solution A. (B) Locomotion was elicited with 110 uA MLR stimulation 30 minutes after intrathecal administration of 1 mM APV. In this and subsequent figures, the scale of the abscissa for each waveform in the drug trials is adjusted to match the scale of the corresponding waveform in the control period. Note in this figure that the frequency of hindlimb locomotion is not altered by APV, but the amplitude of each burst is decreased.

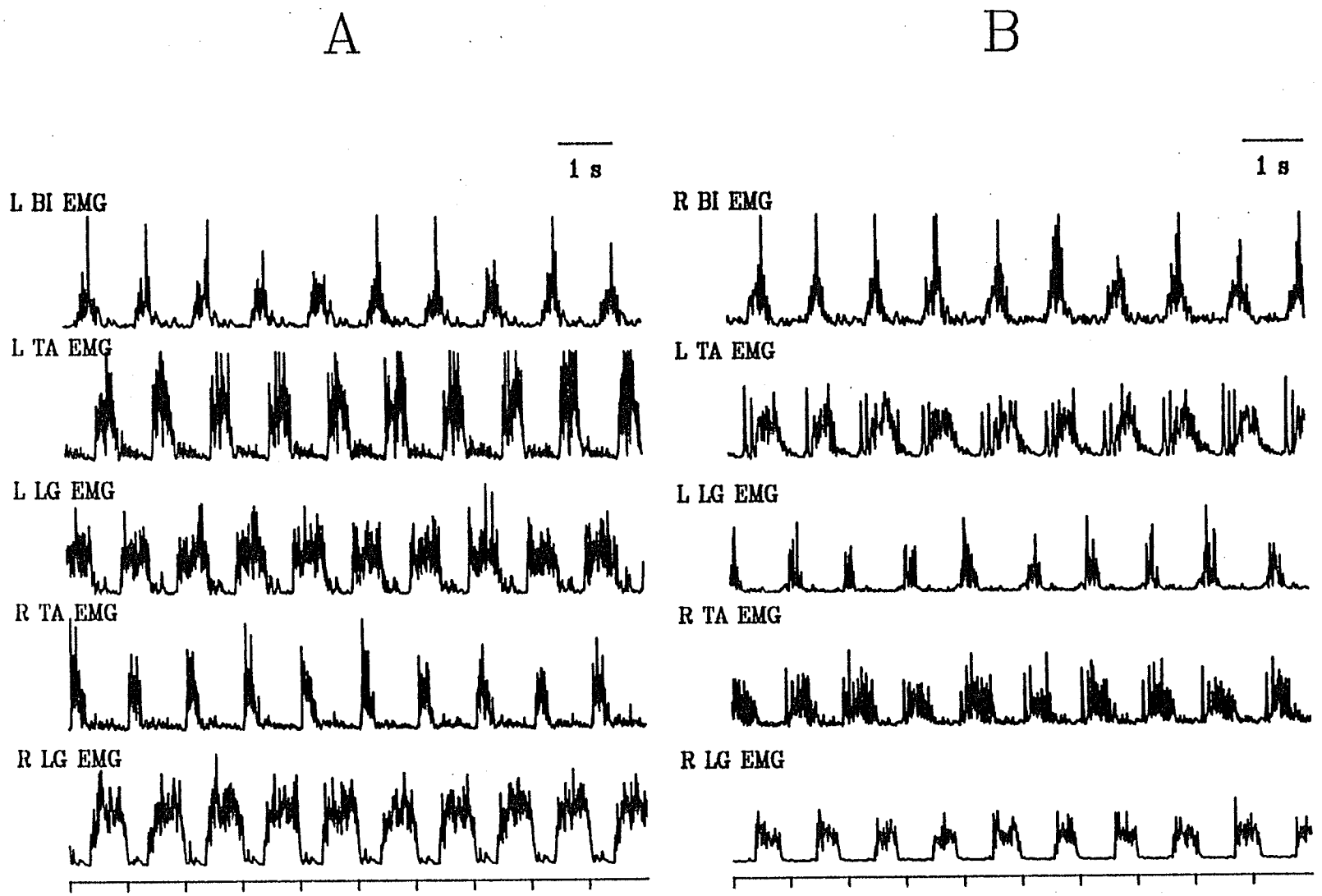

A
B
1 s
1 s
L BI EMG
L TA EMG
L LG EMG
R TA EMG
R LG EMG
R BI EMG
L TA EMG
L LG EMG
R TA EMG
R LG EMG

FIGURE 3

Demonstration of recovery of hindlimb treadmill locomotion following intrathecal administration of 1 mM APV. (A) Control. Quadrupedal locomotion with 110 uA MLR stimulation. (B) No hindlimb activity with 120 uA MLR stimulation, 47 minutes after APV. (C) Hindlimb locomotion returned with 90 uA MLR stimulation, 183 minutes after APV.

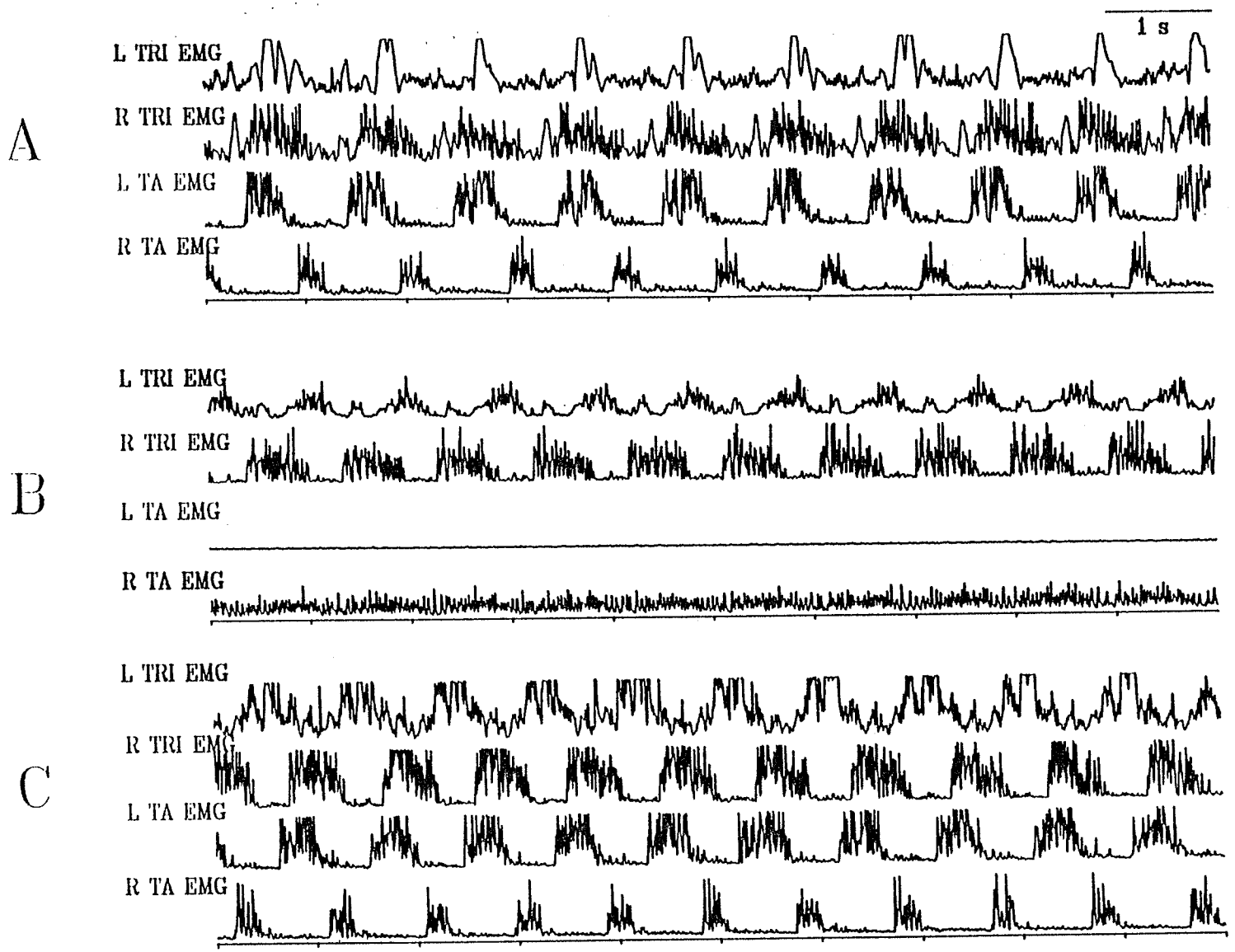

1 s
A
L TRI EMG
R TRI EMG
L TA EMG
R TA EMG
B
L TRI EMG
R TRI EMG
L TA EMG
R TA EMG
C
L TRI EMG
R TRI EMG
L TA EMG
R TA EMG

FIGURE 4

Demonstration of recovery of hindlimb fictive locomotion following administration of 0.5 mM APV. Forelimb locomotion was not monitored in this experiment. (A) Control. Hindlimb locomotion with 80 uA MLR stimulation. (B) Hindlimb locomotion could not be elicited with 150 uA MLR stimulation, 75 minutes after APV. (C) Stepping partially returned with 150 uA MLR stimulation, 3 hours and 45 minutes after APV.

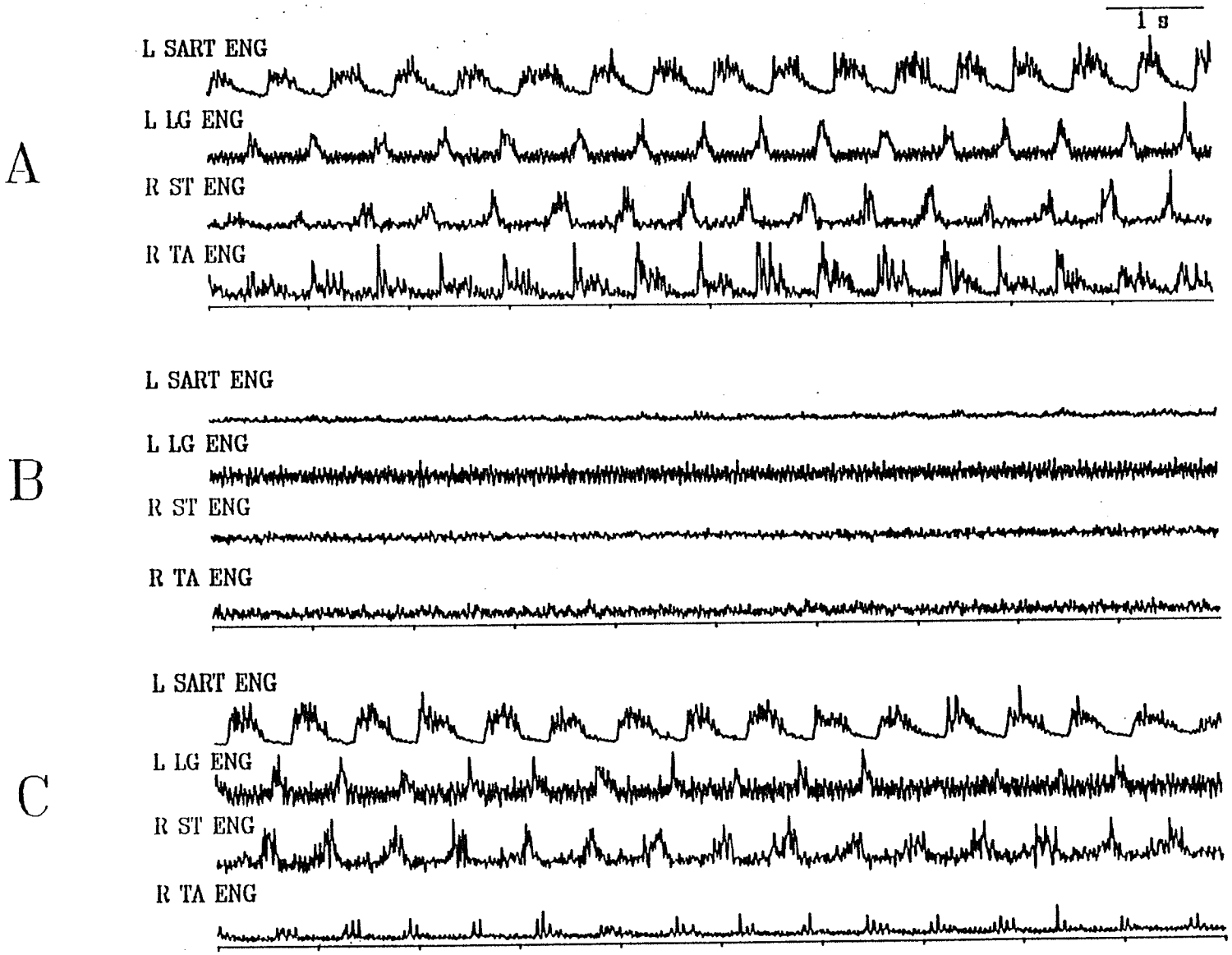

1 s
A
L SART ENG
L LG ENG
R ST ENG
R TA ENG
B
L SART ENG
L LG ENG
R ST ENG
R TA ENG
C
L SART ENG
L LG ENG
R ST ENG
R TA ENG

FIGURE 5

Intrathecal administration of 5 mM NMDA (A) in a fictive locomotion preparation resulted in occasional brief periods of oscillations in one nerve at a time. Intrathecal administration of 5 mM glutamate with 5 mM DHK (B) in a fictive locomotion preparation produced isolated bursts of activity in single nerves.

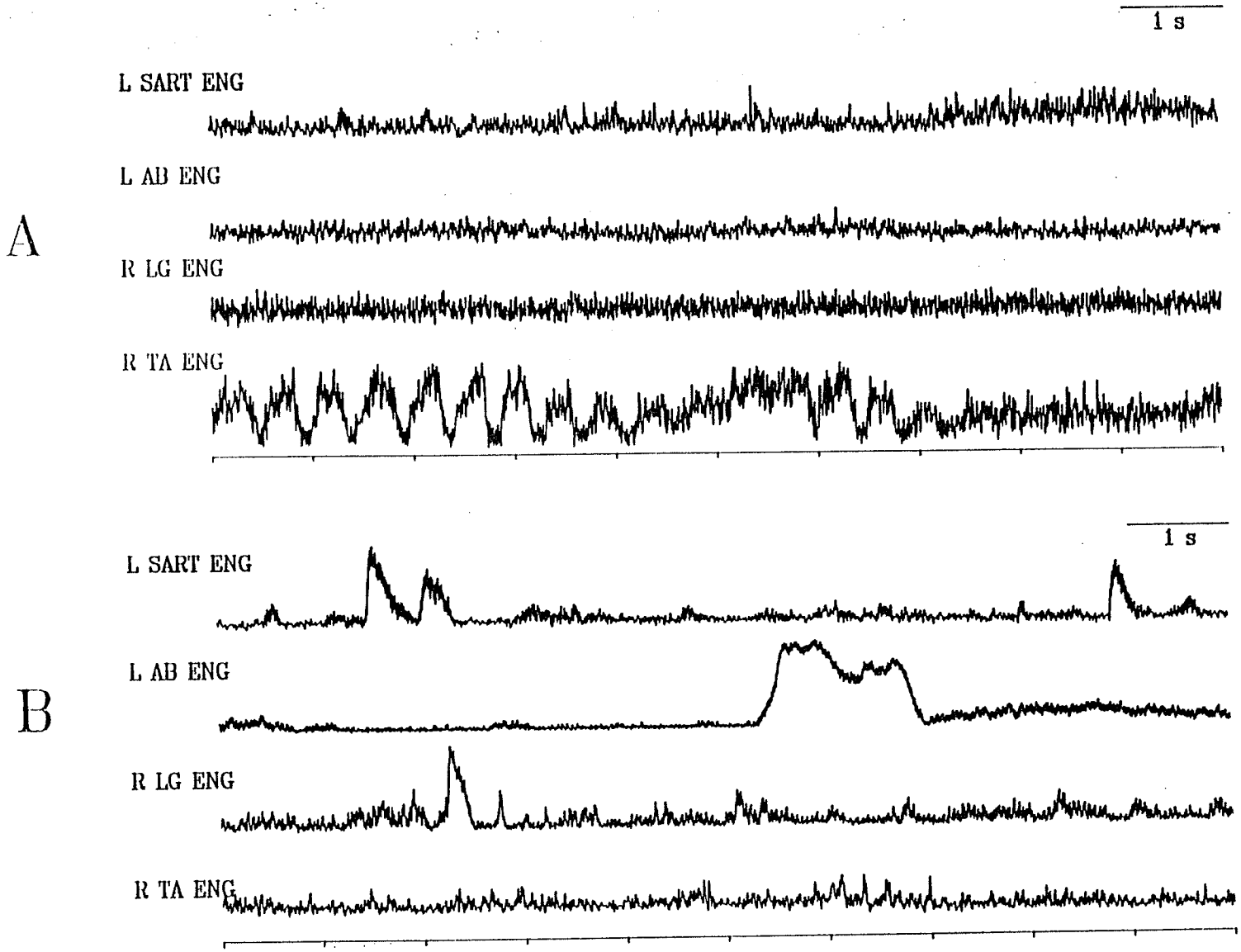

1 s
L SART ENG
L AB ENG
A
R LG ENG
R TA ENG
1 s
L SART ENG
L AB ENG
B
R LG ENG
R TA ENG

FIGURE 6

Spontaneous fictive hindlimb locomotion (ie. without MLR stimulation) was evident 10 minutes after administration of 2.5 mM NMDA with 2.5 mM DHK (A), and had doubled in frequency 13 minutes later (B).

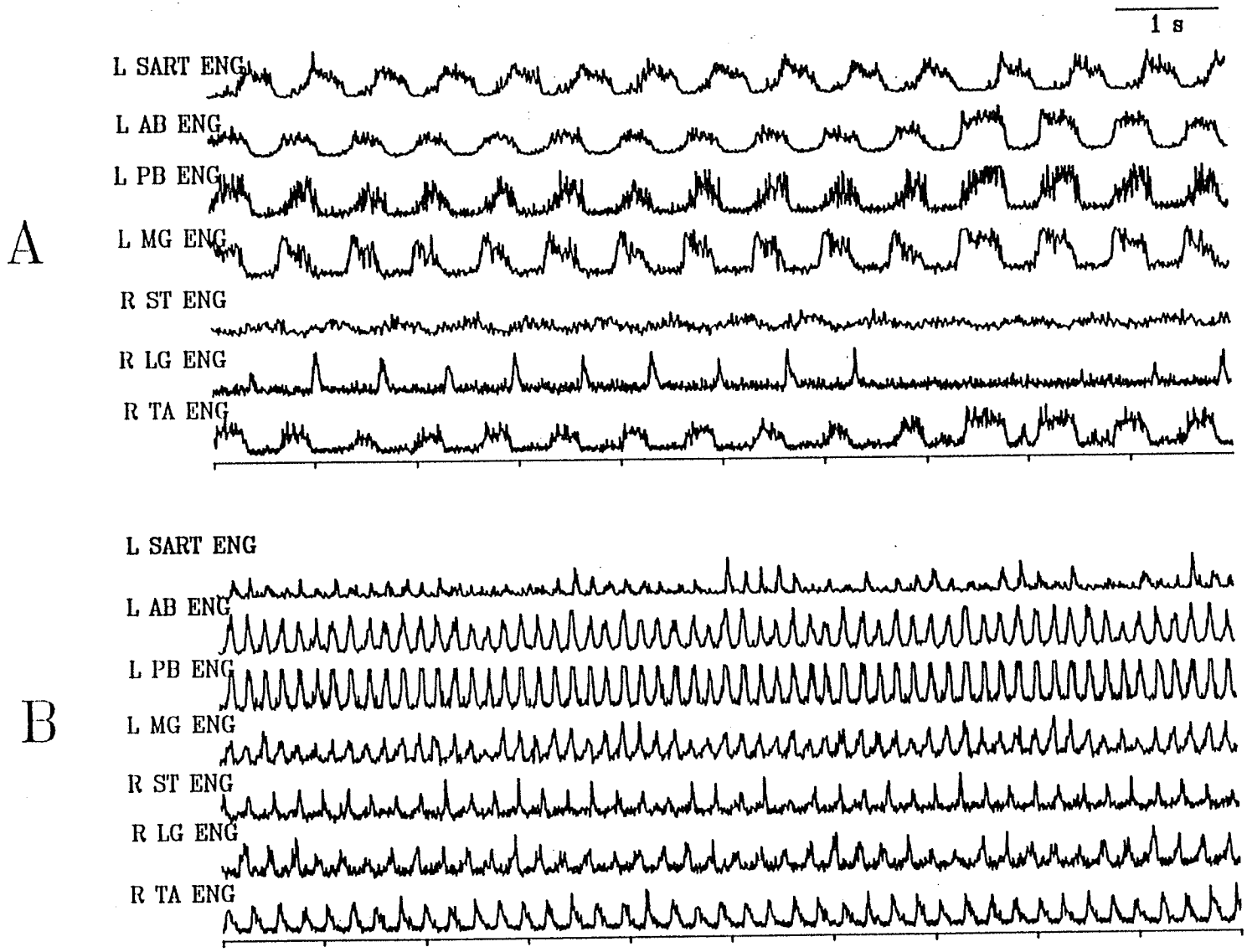

1 s
A
L SART ENG
L AB ENG
L PB ENG
L MG ENG
R ST ENG
R LG ENG
R TA ENG
B
L SART ENG
L AB ENG
L PB ENG
L MG ENG
R ST ENG
R LG ENG
R TA ENG

FIGURE 7

The records for each hindlimb nerve represent rasters of 16 sequential steps during ten seconds of fictive hindlimb locomotion induced by intrathecal administration of 2.5 mM NMDA with 2.5 mM DHK. The onset of left AB activity was used as the trigger, and the stepping frequency was 1.4 Hz. Note that intralimb flexor/extensor alternation is maintained, as well as interlimb coordination.

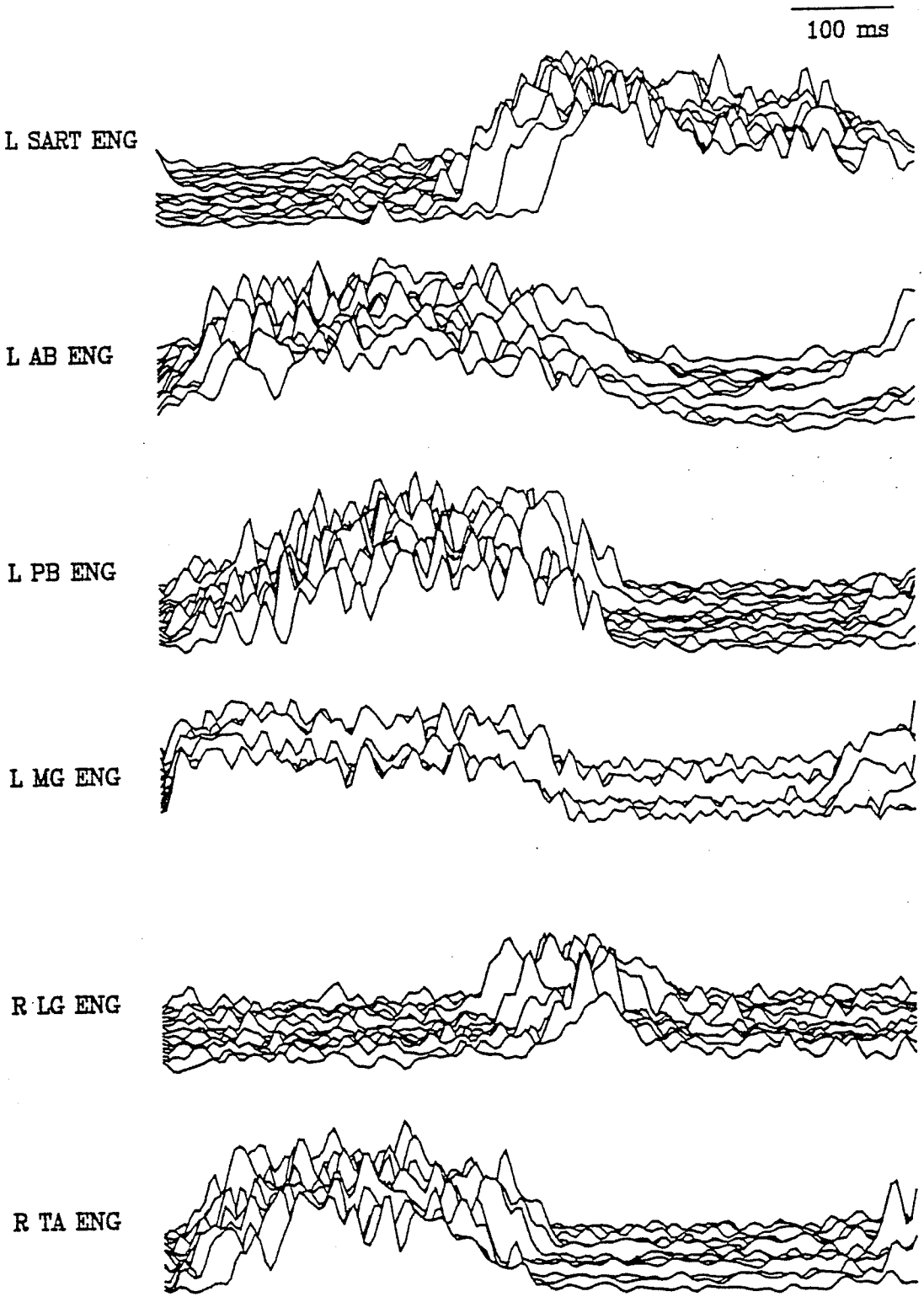

100 ms
L SART ENG
L AB ENG
L PB ENG
L MG ENG
R LG ENG
R TA ENG

FIGURE 8

(A) Fictive hindlimb locomotion induced by intrathecal administration of 5 mM DHK and 5 mM NMDA. (B) 24 minutes after 10 mM APV. The APV was given 30 minutes after the dose of DHK and NMDA.

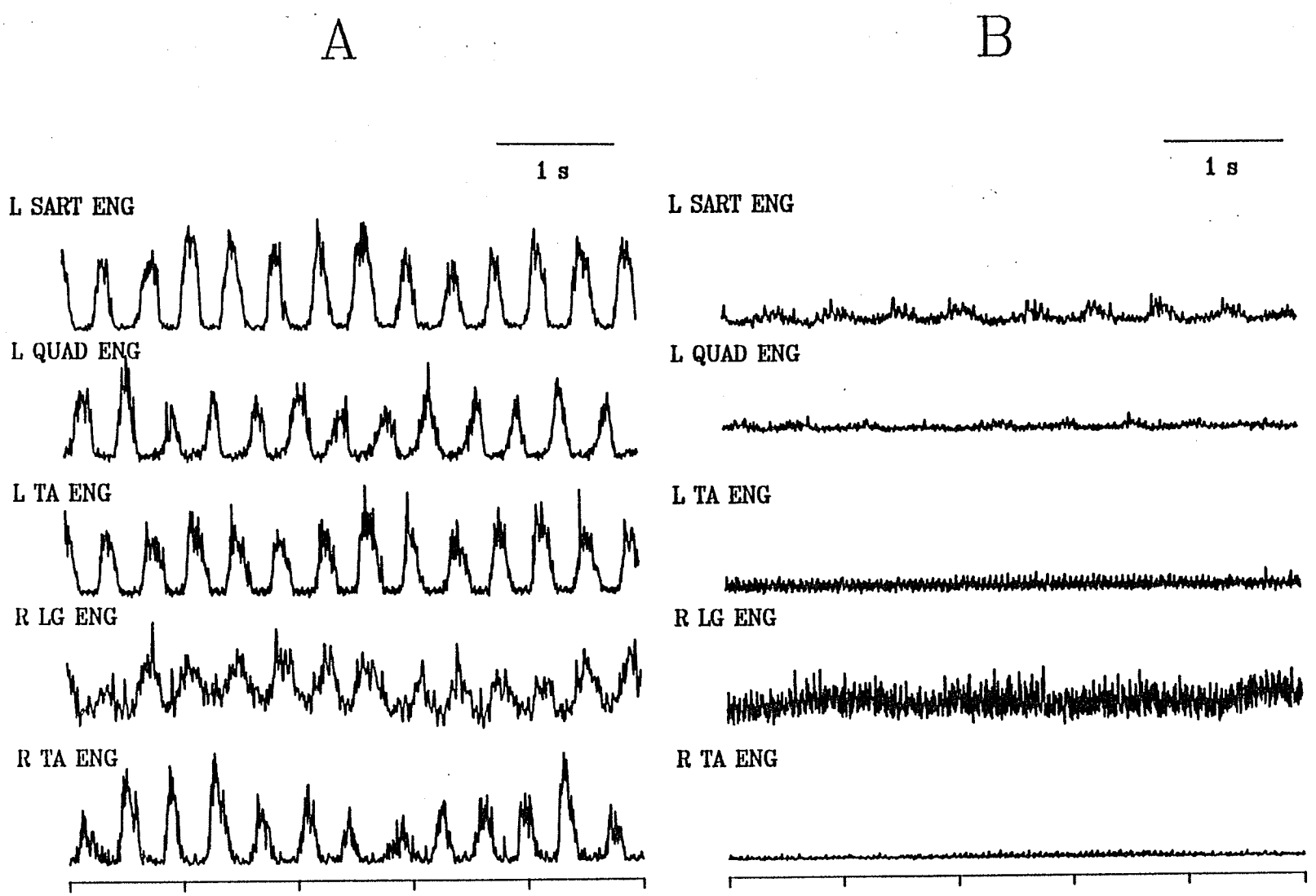

A
B
1 s
1 s
L SART ENG
L QUAD ENG
L TA ENG
R LG ENG
R TA ENG
L SART ENG
L QUAD ENG
L TA ENG
R LG ENG
R TA ENG

FIGURE 9

A few steps were seen in a treadmill locomotion preparation 6 minutes after intrathecal administration of 1 mM NMDA + 1 mM DHK.

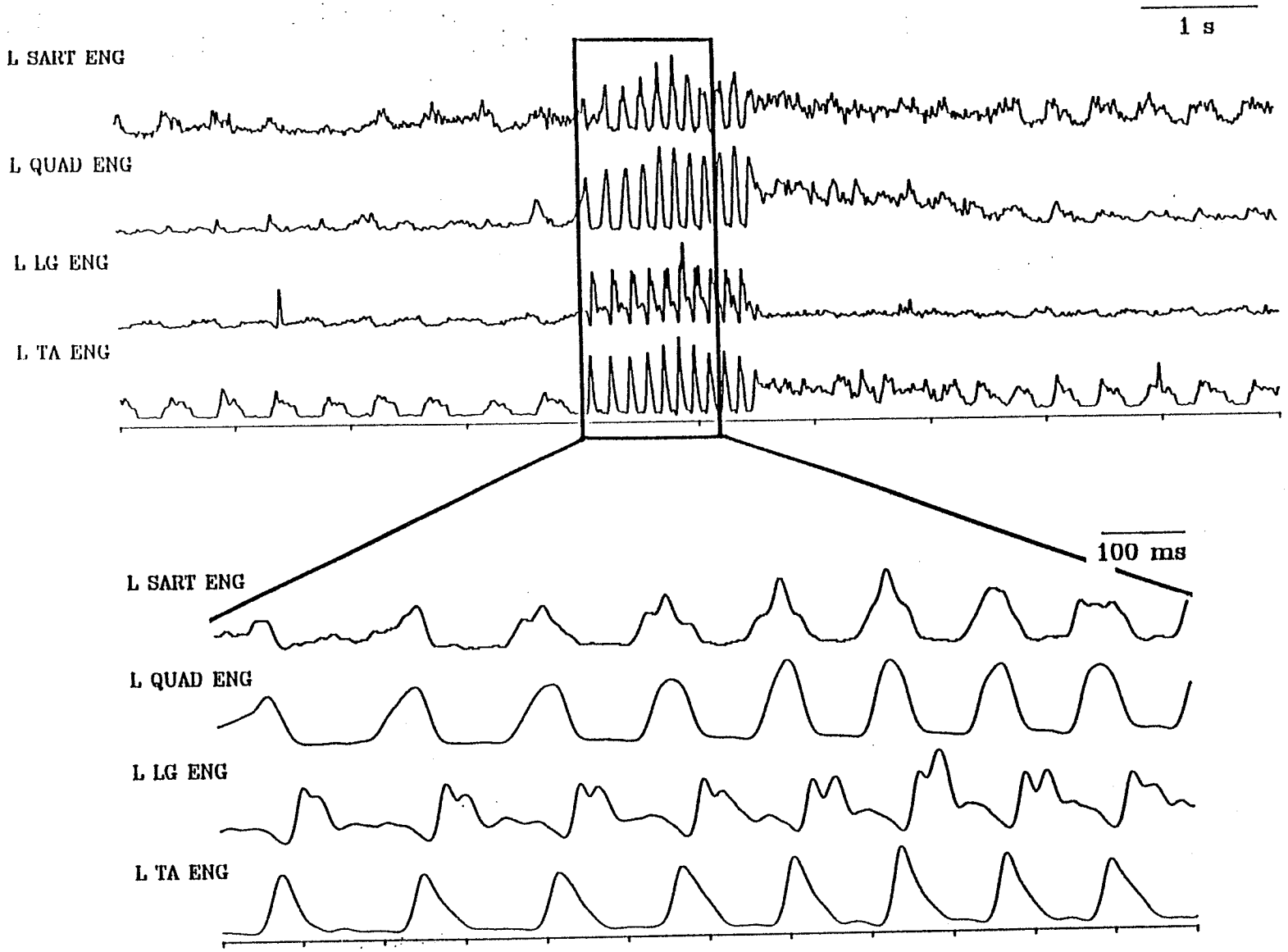
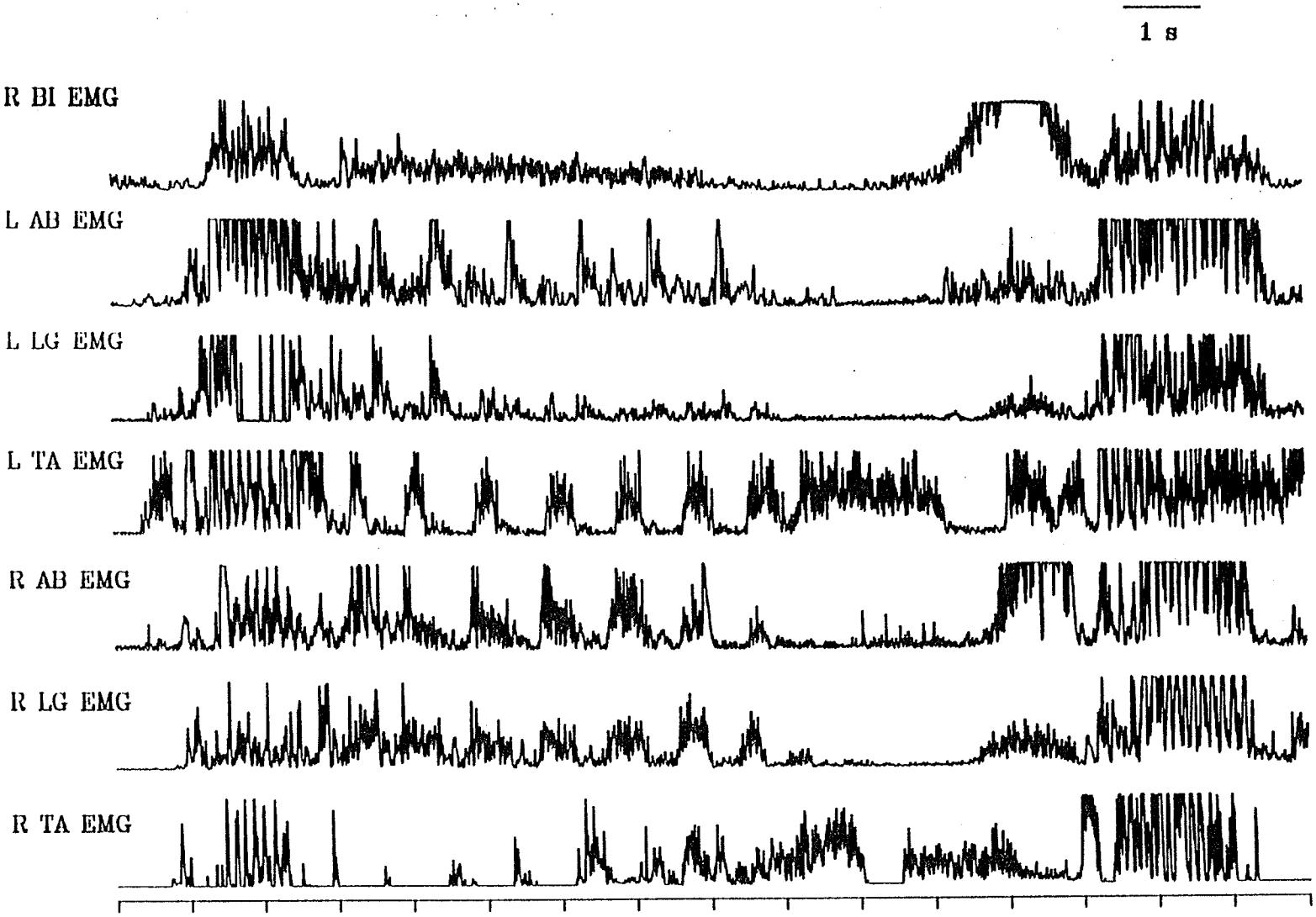

1 s
L SART ENG
L QUAD ENG
L LG ENG
L TA ENG
100 ms
L SART ENG
L QUAD ENG
L LG ENG
L TA ENG

FIGURE 10

This figure illustrates a spontaneous fictive paw shake episode seen 11 minutes after intrathecal administration of 5 mM NMDA. The animal had previously received two doses of glutamate + DHK.


1 s
R BI EMG
L AB EMG
L LG EMG
L TA EMG
R AB EMG
R LG EMG
R TA EMG

DISCUSSION

The results of these experiments indicate that NMDA receptor activation plays a role in the spinal systems responsible for locomotion in the adult decerebrate cat. The study can be divided into two complementary components: the blockade of MLR-induced locomotion by intrathecal administration of an EAA antagonist, and the induction of locomotion with an EAA agonist. The two components will first be discussed individually, and then some general observations and speculations will be made in light of previous findings by other investigators.

The protocol we used in the EAA antagonist experiments represents an *in vivo* adaptation of the *in vitro* vertebrate preparations in which the fluid bathing the rostral portion of the nervous system (entire head, brainstem or rostral segments of spinal cord) is separated by a partition from the fluid bathing the caudal portion. This arrangement allows the effects of various substances to be tested on the caudal portion, during locomotion driven by the rostral portion. Our blockade of brainstem-induced hindlimb locomotion with APV applied to the lumbar spinal cord corresponds to observations that APV in the spinal cord partition blocks locomotion induced by substance P or bicuculline applied to the brainstem partition in the neonatal rat (Smith et al 1986). It has parallels with Dale's (1986) finding that APV in the partition bathing the caudal segments of the isolated lamprey spinal cord blocks locomotion induced by applying NMDA to the rostral segments.

Although our preparation is theoretically similar to the *in vitro* preparations, in practice there are a number of additional variables with which to contend. For instance, the concentration of APV bathing the spinal cord could not be estimated, since the volume of CSF around the lumbar cord was unknown, and the amount of APV which penetrated the spinal cord was not known. Diffusion distances in the adult cat are greater than in the newborn rat

or lamprey, and there might be a time delay for the drug to penetrate the central portions of the cord. The 15-20 minute delay between the time APV was administered and the time the first effects on hindlimb locomotion were seen (Figure 1) might represent the time required for APV to reach its site of action.

The return of hindlimb locomotion 2-3 hours after it was blocked (Figures 3 and 4) is consistent with the competitive nature of APV's actions. With each trial of MLR stimulation the endogenous ligand at the NMDA receptor would be expected to be released, and as the concentration of APV declined with time, the endogenous transmitter would become more successful at competing for the binding site.

Before any conclusions about EAA transmission can be drawn from our antagonist experiments, the specificity of APV must first be established. APV is a potent antagonist of NMDA receptors, and has no known effects at other EAA receptors (see Introduction). It produces no depression of cholinergic transmission (Davies and Watkins 1982) and a recent report has demonstrated that it does not antagonize substance P-, carbachol-, noradrenaline- or 5-hydroxytryptamine-induced depolarization of motoneurons (Childs et al 1988). Since it only blocks NMDA receptors, the ability of APV to completely block rhythmic activity when applied to the spinal cord would suggest that activation of NMDA receptors on spinal neurons is necessary for the production of locomotion in the decerebrate cat.

The second component of the experiments involved the attempt to induce hindlimb locomotion in a resting animal by intrathecally administering EAA agonists. Although it has been reported that bath application of NMDA by itself to the spinal cord *in vitro* elicits locomotion in the lamprey (Grillner et al 1981), Xenopus embryo (Dale and Roberts 1984), chick (Barry and O'Donovan 1987) and newborn rat (Kudo and Yamada 1987), NMDA did not induce treadmill or fictive stepping in the decerebrate cat (Figure 5). Looking at the *in vitro* studies cited here, the concentration range of NMDA capable of producing rhythmic motor activity is often quite narrow: 10-30 uM in the chick and rat, 30-40 uM in the frog

embryo. Therefore, the possibility that we did not see locomotion because we were not in the effective concentration range cannot be ruled out. A wider dose range would have to be tested before concluding that NMDA receptor activation is not sufficient to induce locomotion.

It is not surprising that we were unable to elicit locomotion with L-glutamate alone. L-Glutamate and L-aspartate are subject to rapid inactivation by high affinity uptake processes in glial cells and nerve terminals (Fagg and Lane 1979), which reduces their potency (Garthwaite 1985). The ability of L-glutamate to induce locomotion *in vitro* was enhanced if the bathing fluid was rapidly circulated over the preparation, or if it was coadministered with a high affinity uptake blocker such as DHK (Brodin and Grillner 1985b, Smith et al 1988). Coadministering DHK did not allow us to elicit locomotion with L-glutamate with the limited number of doses tried; however, higher doses would have to be tested before stating with certainty that the combination of L-glutamate and DHK does not induce locomotion in the cat *in vivo*.

The fact that we could induce locomotion when the animal was treated with DHK followed by NMDA, or with DHK and NMDA administered together, was unforeseen. The first possible explanation to be considered is that DHK was blocking the uptake of NMDA. NMDA is not synthesized endogenously, and it is not susceptible to high affinity uptake systems (Balcar and Johnston 1972, Balcar et al 1987, Johnston et al 1979, Roberts and Watkins 1975). On this basis, blockade of the uptake systems by DHK should not affect NMDA levels. It should be noted that Skerritt and Johnston (1981) found a low affinity uptake system for NMDA which is inhibited by DHK in rat brain slices. The uptake system was relatively inefficient, however, and the authors believed that the transportation rate was too slow to affect the time course of excitation induced by NMDA. In the cat spinal cord DHK potentiates the responses of and L-glutamate but has little or no effect on NMDA responses (Lodge et al 1979, 1980). Therefore, it would seem unlikely that the dramatic

difference in the effects of NMDA with DHK as compared to without DHK were due to any effect on NMDA inactivation by uptake processes. A second possible explanation is that DHK, being structurally related to kainate, might have agonist actions at EAA receptors, which when combined with the actions of NMDA are sufficient to induce rhythmic activity; DHK has been shown to be a very weak excitant of spinal neurons (Johnston et al 1974). In a study of uptake processes in the isolated lamprey spinal cord, Brodin and Grillner (1985b) reported that in high doses DHK administered alone could sometimes elicit fictive locomotion, and they could not exclude the possibility that it was due to direct excitation. In our experiments DHK administered alone did not produce any response at a higher concentration than the dose which could produce locomotion in combination with NMDA. One way to test whether it is the uptake blocking or direct effects of DHK which are more important would be to substitute an uptake blocker which does not produce direct excitation. One possibility is *p*-chloromercuriphenylsuphonate (pCMS), but it would have to be taken into account that this compound also blocks the uptake of inhibitory amino acids (Curtis et al 1970). It would be interesting to determine whether intrathecal NMDA + DHK infusion can induce locomotion in chronic spinal cats, since this would have possible implications for spinal-cord injured humans.

From the experiments performed in this study, at least one thing seems clear. In the adult decerebrate cat, the activation of NMDA receptors on lumbar spinal neurons is necessary for the production of hindlimb locomotion. Since our only window on spinal cord activity is the output messages carried out through the motor nerves, it is difficult to draw more specific conclusions than this. However, the *in vitro* work published in the last decade has demonstrated that various populations of spinal neurons have EAA receptors, and it is interesting to speculate on their possible contributions to the results obtained in the present study.

1) The first site of action which will be examined is the output stage: the motoneurons.

In the lamprey and frog embryo, motoneurons have NMDA receptors which are responsible for the tonic depolarization component of motoneuron activity during locomotion, and non-NMDA receptors which mediate the phasic excitation component (see Introduction). Could the selective blockade of NMDA receptors at the motoneuron level explain our findings with APV? In our experiments APV depressed the amplitude of the ENG/EMG bursts without disrupting the rhythm. It can be hypothesized that as APV diffused into the ventral horn it gradually depressed the depolarization of the motoneurons, until eventually even the activation of other receptors would be insufficient to bring the membrane to its firing threshold. In this case, pattern generation would still be active, but locomotion would be blocked at the output stage.

It is less easy to explain all the results of the agonist experiments solely at the motoneuron level. The tonic responses most commonly seen when NMDA was applied by itself could be due to activation of NMDA receptors on the motoneurons. Since NMDA receptors can induce membrane potential oscillations, it is possible that the occasional periods of rhythmic activity induced by NMDA alone could also be explained by activation of NMDA receptors only on the motoneurons, although it is unlikely that the motoneurons travelling in the nerve would all be oscillating in phase with each other in the absence of some common input. The ability of NMDA alone to induce rhythmic ventral root activity in the *in vitro* vertebrate preparations argues against the likelihood of only motoneuronal NMDA receptors being activated, and the appearance of coordinate activity in different muscles when the hindlimbs are left intact in these preparations (Kudo and Yamada 1987) excludes the possibility of NMDA directly activating motoneurons in a periodic fashion. The same argument applies in our results with NMDA and DHK: it is highly unlikely that these two compounds could produce the coordinate activation of different nerves (Figure 7) if they were activating only the motoneurons.

2) The next site of action concerns the sensory input to the spinal cord. The apparent

concentration of NMDA receptors in the dorsal horn in autoradiographic studies makes this an option worth considering. The behavioural studies described in the Introduction point to a role for NMDA receptors in pain transmission, and the vigorous hindlimb flexion elicited by NMDA administration in our experiments could be a response to the activation of neurons in pain pathways. As for the results of the antagonist experiments, the possibility that the loss of locomotion was due entirely to a decrease in spinal cord excitability because of sensory depression cannot be ruled out. The attribution of NMDA effects to dorsal horn actions seems more plausible than for APV, if the time delays before the onset of drug effects are considered. NMDA administration elicited a response within 1-2 minutes, whereas the first effects of APV only became apparent after 15 minutes or more. This suggests that NMDA had to diffuse a shorter distance to reach its site of action. Since the intrathecal cannulae were inserted underneath the dorsal roots, the drugs might have reached the superficial laminae of the dorsal horn first.

A possible role for DHK also arises in this context. In trying to explain why NMDA and DHK can induce locomotion while NMDA by itself cannot, an obvious possibility is that DHK can directly or indirectly activate a population of receptors which are inaccessible to NMDA. DHK increases extracellular glutamate levels in the spinal cord (Brodin and Grillner 1988), which could result in increased activation of both NMDA and non-NMDA receptors. Therefore, the primary afferent synapse might be an important site of action for DHK for two reasons: firstly, many investigators believe it uses glutamate serves as a transmitter here, and secondly, its EAA receptors are primarily of the non-NMDA subtypes (see Introduction). Second order neurons would not be excited by NMDA, but they would be excited indirectly by DHK, through its effect on extracellular glutamate levels. It can be postulated that the reason NMDA + DHK administration induces locomotion where NMDA does not is that the presence of DHK permits the additional activation of the neurons which receive monosynaptic excitation from primary afferents. One way to test this hypothesis would be to attempt to

induce locomotion with NMDA and DHK after degeneration of primary afferent terminals following chronic dorsal root section.

The subject of sensory input to the spinal cord arises when considering the differences in the stepping induced by NMDA + DHK in the treadmill locomotion preparation versus the fictive locomotion preparation. While fictive stepping progressed smoothly over several minutes, treadmill stepping episodes were very brief, and were interspersed with periods of non-specific excitation (Figure 9). On the treadmill, the sensory feedback from the moving limbs on the treadmill might have disrupted the locomotor rhythm which was established when NMDA + DHK was infused. The normal effects produced by sensory input in the spinal cord might have been altered by the presence of NMDA and DHK in two ways. Firstly, some of the spinal neurons receiving sensory input might have been partially depolarized by NMDA + DHK, so that less sensory input than usual was required to bring them to their threshold potential. Thus, sensory input might have an abnormally strong effect. Secondly, if the depolarization produced by NMDA + DHK was prolonged, some neurons would undergo accommodation, and sensory input would thus have an abnormally weak effect. Either way, sensory feedback would produce unusual responses, which might be sufficient to disrupt locomotion.

3) The third site of action to be considered is the synapse of descending terminals onto spinal neurons, based on evidence in the lamprey that reticulospinal neurons release a transmitter which activates NMDA and non-NMDA receptors (Ohta and Grillner 1989). If APV blocked this site in our experiments, it would mean that the descending drive to the spinal circuitry for locomotor pattern generation would be hampered. In other words, the spinal locomotor system would be "turned off". In order to distinguish blockade at the input stage from blockade at the output stage (the motoneurons), it would be necessary to have a reliable monitor of the pattern generator activity.

It is conceivable that activation only of the neurons which receive monosynaptic input

from the descending terminals could account for the production of locomotion with NMDA and DHK. For instance, these neurons might require concurrent occupation of NMDA and non-NMDA receptors in order to be depolarized to their threshold potential. The NMDA receptors on these neurons might be completely blocked by Mg^{2+} at the resting membrane potential, accounting for our inability to induce locomotion with NMDA alone. When DHK is also present, however, the increase in glutamate levels could depolarize the neurons sufficiently through non-NMDA receptor activation to relieve the voltage-dependant blockade. Actions at this site could explain our finding that the frequency of locomotion could gradually increase after NMDA and DHK were administered (Figure 6). Increasing the strength of MLR simulation can increase the frequency of locomotion (Garcia-Rill 1990), presumably through an increase in the release of endogenous neurotransmitter by the descending nerve terminals. In an analogous manner, the presence of exogenous transmitter would build up as NMDA and DHK gradually diffused into the area.

So far three possible sites of action for EAA receptors in the spinal circuitry for locomotion have been dealt with: sensory input, descending input from higher centres, and the final output. Given the extensive involvement of EAAs in excitatory neurotransmission, it seems highly improbable that the drug effects we saw were restricted to only one of these populations of neurons. It is more likely that the responses were due to ensemble effects on a wide range of spinal neurons. Certainly, the occasional period of paw-shake seen after NMDA and DHK administration (Figure 10) indicates that the effects were not restricted to the locomotor circuitry. The notion of widespread actions is particularly relevant in the case of DHK. If, as it is suspected, glutamate is one the endogenous transmitters mediating excitatory transmission in the CNS, then the rise in extracellular glutamate after DHK administration would probably cause many neurons to be somewhat depolarized. This gives rise to new possibilities to explain our agonist results.

Suppose that the NMDA receptors on all neurons except motoneurons are fully

blocked by Mg^{2+} at their resting membrane potential, so that when NMDA is administered by itself, it only affects motoneurons, resulting in random, uncoordinated excitation. When DHK is administered, glutamate inactivation is blocked, so the concentration of extracellular glutamate rises. One possible consequence is that some non-NMDA receptors will be activated. The possibility of glutamate-mediated activation of non-NMDA receptors was previously raised in the context of specific excitation of spinal neurons receiving sensory or descending inputs. In the present context, the non-NMDA receptors would be activated on a wide range of neurons. The resulting depolarization would relieve the voltage-dependant blockade of NMDA receptors, giving NMDA access to populations of neurons which were not able to respond to it before, such as perhaps interneurons in the locomotor circuitry. A second possible consequence of increased glutamate levels is that glutamate could activate inhibitory interneurons through their non-NMDA receptors, thereby increasing glycine release. Glycine occupies an allosteric site on the NMDA receptor (Thedinga et al 1989) and potentiates responses to NMDA (Johnson and Ascher 1987), so it is possible that increased extracellular glycine levels might enhance NMDA receptor activation. However, although it has been shown that NMDA receptors on cultured neurons cannot be activated in glycine-free medium (Sands and Barish 1989), in more physiological preparations it is likely that the glycine site on the NMDA receptor is fully occupied (Collingridge and Lester 1989). Therefore, any increase in glycine release would be unlikely to have any effect on EAA receptor function in our experiments.

The finding that APV administration blocked locomotion induced by NMDA + DHK (Figure 8) would suggest that, just as with locomotion induced by MLR stimulation, the activation of NMDA receptors is a necessary component. Therefore, regardless of any other EAA receptors that might be activated when the NMDA/DHK combination is administered, they cannot induce locomotion without the participation of NMDA receptors. It should be noted that this was only tested once, and the APV was given 30 minutes after NMDA + DHK,

by which time in other experiments the locomotion was deteriorating anyway, so the loss of locomotion might have been coincidental.

This discussion has raised a number of possible explanations for the results of the experiments in this study. Trying to determine the mechanism of drug action by observing only the output of the spinal cord is like trying to figure out how a Polaroid camera works by looking at the snapshot it puts out. In order to narrow down the list of possibilities, it would be necessary to record inside the spinal cord from neurons whose role in the locomotor system is known.

Nevertheless, the experiments described here suggest that the *in vitro* findings on the participation of NMDA receptors in locomotion might have *in vivo* analogies. The most convincing evidence that EAAs can act physiologically as neurotransmitters comes from the intracellular recordings of pre- and post-synaptic neuronal pairs *in vitro* (Headley and Grillner 1990). The challenge is to produce equally convincing evidence *in vivo*.

REFERENCES

Aanonsen LM, Wilcox GL (1986) Phencyclidine selectively blocks a spinal action of N-methyl-D-aspartate in mice. Neurosci Lett 67:191-197.

Alford S, Grillner S (1990) CNQX and DNQX block non-NMDA synaptic transmission but not NMDA-evoked locomotion in lamprey spinal cord. Brain Res 506:297-302.

Bagust J, Kerkut GA, Rakkah NIA (1989) Differential sensitivity of dorsal and ventral root activity to magnesium and 2-amino-5-phosphonovalerate (APV) in an isolated mammalian spinal cord preparation. Brain Res 479:138-144.

Balcar VJ, Johnston GAR (1972) Glutamate uptake by brain slices and its relation to the depolarization of neurones by acidic amino acids. J Neurobiol 3:295-301.

Balcar VJ, Schousboe A, Spoerri P, Wolff JR (1987) Differences between substrate specificities of L-glutamate uptake by neurons and glia, studied in cell lines and primary cultures. Neurochem Int 10:213-217.

Barry MJ, O'Donovan MJ (1987) The effects of excitatory amino acids and their antagonists on the generation of motor activity in the isolated chick spinal cord. Devel Brain Res 36:271-276.

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

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

Brodin L, Grillner S (1986) Effects of magnesium on fictive locomotion induced by activation of N-methyl-D-aspartate (NMDA) receptors in the lamprey spinal cord in vitro. Brain Res 380:244-252.

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

Brodin L, Tossman U, Ohta Y, Ungerstedt U, Grillner S (1988) The effect of an uptake inhibitor (dihydrokainate) on endogenous excitatory amino acids in the lamprey spinal cord as revealed by microdialysis. Brain Res 458:166-169.

Buchanan JT, Brodin L, Dale N, Grillner S (1987) Reticulospinal neurones activate excitatory amino acid receptors. Brain Res 408:321-325.

Buchanan JT, Grillner S (1987) Newly identified "glutamate interneurons" and their role in locomotion in the lamprey spinal cord. Science 236:312-314.

Buchanan JT, Grillner S, Cullheim S, Risling M (1989) Identification of excitatory

interneurons contributing to generation of locomotion in lamprey: structure, pharmacology, and function. J Neurophysiol 62:59-69.

Cahusac PMB, Evans RH, Hill RG, Rodriquez, Smith DAS (1984) The behavioural effects of an N-methylaspartate receptor antagonist following application to the lumbar spinal cord of conscious rats. Neuropharmacology 23:719-724.

Chandler SH (1989) Evidence for excitatory amino acid transmission between mesencephalic nucleus of V afferents and jaw-closer motoneurons in the guinea pig. Brain Res 477:252-264.

Childs AM, Evans RH, Watkins JC (1988) The pharmacological selectivity of three NMDA antagonists. Eur J Pharmacol 145:81-86.

Cohen AH, Wallen P (1980) The neuronal correlate of locomotion in fish. "Fictive swimming" induced in an in vitro preparation of the lamprey spinal cord. Exp Brain Res 41:11-18.

Collingridge GL, Lester RAJ (1989) Excitatory amino acid receptors in the vertebrate central nervous system. Pharmacol Rev 40:143-210.

Curtis DR, Duggan AW, Johnston GAR (1970) The inactivation of extracellularly administered amino acids in the feline spinal cord. Exp Brain Res 10:447-462.

Curtis DR, Phillis JW, Watkins JC (1959) Chemical excitation of spinal neurones. Nature 183:611-612.

Curtis DR, Phillis JW, Watkins JC (1960) The chemical excitation of spinal neurones by certain acidic amino acids. J Physiol 150:656-682.

Dale N (1986) Excitatory synaptic drive for swimming mediated by amino acid receptors in the lamprey. J Neurosci 6:2262-2675.

Dale N, Grillner S (1986) Dual-component synaptic potentials in the lamprey mediated by excitatory amino acid receptors. J Neurosci 6:2653-2661.

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

Dale N, Roberts A (1985) Dual-component amino-acid-mediated synaptic potentials: excitatory drive for swimming in Xenopus embryos. J Physiol 363:35-59.

Davies J, Evans RH, Francis AA, Watkins JC (1979) Excitatory amino acid receptors and synaptic excitation in the mammalian central nervous system. J Physiol (Paris) 75:641-654.

Davies J, Francis AA, Jones AW, Watkins JC (1980) 2-Amino-5-phosphonovalerate (2APV), a highly potent and specific antagonist at spinal NMDA receptors. Br J Pharmacol 70:52-53P.

Davies J, Francis AA, Jones AW, Watkins JC (1981) 2-Amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation.

Neurosci Lett 21:77-81.

Davies J, Watkins JC (1982) Actions of D and L forms of 2-amino-5-phosphonovalerate and 2-amino-4-phosphonobutyrate in the cat spinal cord. Brain Res 235:378-386.

Davies J, Watkins JC (1983) Role of excitatory amino acid receptors in mono- and polysynaptic excitation in the cat spinal cord. Exp Brain Res 49:280-290.

Dechesne CJ, Oberdorfer MD, Hampson DR, Wheaton KD, Nazarali AJ, Goping G, Wenthold RJ (1990) Distribution of a putative kainic acid receptor in the frog central nervous system determined with monoclonal and polyclonal antibodies: evidence for synaptic and extrasynaptic localization. J Neurosci 10:479-490.

Deliagina TG, Feldman AG, Gelfand IM, Orlovsky GN (1975) On the role of central program and afferent inflow in the control of scratching movements in the cat. Brain Res 100-297-313.

Duggan AW (1974) The differential sensitivity to L-glutamate and L-aspartate of spinal interneurones and Renshaw cells. Exp Brain Res 19:522-528.

Elliott KAC, Jasper HH (1949) Physiological salt solutions for brain surgery: studies of local pH and pial vessel reactions to buffered and unbuffered isotonic solutions. J Neurosurg 6:140-152.

Evans RH, Francis AA, Jones AW, Smith DAS, Watkins JC (1982) The effects of a series of phosphonic α-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. Br J Pharmacol 75:65-75.

Fagg GE, Foster AC (1983) Amino acid neurotransmitters and their pathways in the mammalian central nervous system. Neuroscience 9:701-719.

Fagg GE, Lane JD (1979) The uptake and release of putative amino acid neurotransmitters. Neuroscience 4:1015-1036.

Flatman JA, Schwindt PC, Crill WE (1986) The induction and modification of voltage-sensitive responses in cat neocortical neurons by N-methyl-D-aspartate. Brain Res 363:62-77.

Garcia-Rill E, Kinjo N, Atsuta Y, Ishikawa Y, Webber M, Skinner RD (1990) Posterior midbrain-induced locomotion. Brain Res Bull 24:499-508.

Garthwaite J (1985) Cellular uptake disguises action of L-glutamate on N-methyl-D-aspartate receptors. Br J Pharmacol 85:297-307.

Gerber G, Randic M (1989) Participation of excitatory amino acid receptors in the slow excitatory synaptic transmission in the rat spinal dorsal horn in vitro. Neurosci Lett 106:220-228.

Gonzalez DL, Fuchs JL, Droge MH (1990) The distribution of N-methyl-D-aspartic acid receptor binding in neonatal and adult mouse lumbar spinal cord. Soc Neurosci Abst 16:90.

Greenamyre JT, Olson JMM, Penney JB, Young AB (1985) Autoradiographic characterization of N-methyl-D-aspartate, quisqualate and kainate-sensitive glutamate binding sites. J Pharmacol Exp Ther 233:254-263.

Greenamyre JT, Young AB, Penney JB (1984) Quantitative autoradiographic distribution of L-[^{3}H]-glutamate binding sites in rat central nervous system. J Neurosci 4:2133-2144.

Grillner S, McClellan A, Sigvardt K, Wallen P, Wilen M. (1981) Activation of NMDA-receptors elicits "fictive locomotion" in lamprey spinal cord in vitro. Acta Physiol Scand 113:549-551.

Grillner S, Wallen P (1985) The ionic mechanisms underlying N-methyl-D-aspartate receptor-induced, tetrodotoxin-resistant membrane potential oscillations in lamprey neurons active during locomotion. Neurosci Lett 60:289-294.

Headley PM, Grillner S (1990) Excitatory amino acids and synaptic transmission: the evidence for a physiological function. Trends Pharmacol Sci 11:205-211.

Jahr CE, Jessell TM (1985) Synaptic transmission between dorsal root ganglion and dorsal horn neurons in culture: antagonism of monosynaptic excitatory postsynaptic potentials and glutamate excitation by kynurenate. J Neurosci 5:2281-2289.

Jahr CE, Yoshioka K (1986) Ia afferent excitation of motoneurones in the *in vitro* newborn rat spinal cord is selectively antagonized by kynurenate. J Physiol 370: 515-530.

Jansen KLR, Faull, RLM, Dragunow M, Waldvogel H (1990) Autoradiographic localisation of NMDA, quisqualate and kainic acid receptors in human spinal cord. Neurosci Lett 108:53-57.

Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529-531.

Johnston GAR, Curtis DR, Davies J, McCulloch RM (1974) Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid. Nature 248:804-805.

Johnston GAR, Kennedy SME, Twitchin B (1979) Action of the neurotoxin kainic acid on high affinity uptake of L-glutamic acid in rat brain slices. J Neurochem 32:121-127.

Jordan LM, Pratt CA, Menzies JE (1979) Locomotion evoked by brain stem stimulation: occurrence without phasic segmental afferent input. Brain Res 177:204-207.

Koshland GF, Smith JL (1989) Mutable and immutable features of paw-shake responses after hindlimb deafferentation in the cat. J Neurophysiol 62:162-173.

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

Lodge D, Curtis DR, Johnston GAR, Bornstein JC (1980) In vivo inactivation of quisqualate: studies in the cat spinal cord. Brain Res 182:491-495.

Lodge D, Johnston GAR, Curtis DR, Bornstein JC (1979) Kainate neurotoxicity and glutamate inactivation. Neurosci Lett 14:343-348.

MacDermott AB, Dale N (1987) Receptors, ion channels and synaptic potentials underlying the integrative actions of excitatory amino acids. Trends Neurosci 10:280-284.

MacDonald JF (1984) Substitution of extracellular sodium ions blocks the voltage-dependant decrease of input conductance evoked by L-aspartate. Can J Physiol Pharmacol 62:109-115.

MacDonald JF, Wojtowicz JM (1982) The effects of L-glutamate and its analogues upon the membrane conductance of central murine neurones in culture. Can J Physiol Pharmacol 60:282-296.

Mayer ML, Westbrook GL (1984) Mixed-agonist action of excitatory amino acids on mouse spinal cord neurones under voltage clamp. J Physiol 354:29-53.

Mayer ML, Westbrook GL (1987) The physiology of excitatory amino acids in the vertebrate central nervous system. Prog Neurobiol 28:197-276.

McClellan AD (1987) In vitro CNS preparations: unique approaches to the study of command and pattern generation systems in motor control. J Neurosci Met 21:251-264.

McClellan AD, Farel PB (1985) Pharmacological activation of locomotor patterns in larval and adult frog spinal cords. Brain Res 332:119-130.

McCrimmon DR, Smith JC, Feldman JL (1989) Involvement of excitatory amino acids in neurotransmission of inspiratory drive to spinal respiratory motoneurons. J Neurosci 9:1910-1921.

McLennan H, Hicks TP, Hall JG (1981) Receptors for the excitatory amino acids. In: Amino Acid Neurotransmitters, (FV DeFeudis, P Mandel, eds), pp 213-221. New York: Raven Press.

Monaghan DT, Bridges RJ, Cotman CW (1989) The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 29:365-402.

Morris R (1989) Responses of spinal dorsal horn neurones evoked by myelinated primary afferent stimulation are blocked by excitatory amino acid antagonists acting at kainate/quisqualate receptors. Neurosci Lett 105:79-85.

Noga BR, Kettler J, Jordan LM (1988) Locomotion produced in mesencephalic cats by injections of putative transmitter substances and antagonists into the medial reticular formation and the pontomedullary locomotor strip. J Neuroscience 8:2074-2086.

Nowak L, Bregestovski P, Ascher P (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462-465.

Ohta Y, Grillner S (1989) Monosynaptic excitatory amino acid transmission from the posterior rhombencephalic reticular nucleus to spinal neurons involved in the control of

locomotion in lamprey. J Neurophysiol 62:1079-1089.

Peet MJ, Leah JD, Curtis DR (1983) Antagonists of synaptic and amino acid excitation of neurones in the cat spinal cord. Brain Res 266:83-95.

Polc P (1985) 2-Amino-7-phosphonoheptanoic acid depresses gamma motoneurons and polysynaptic reflexes in the cat spinal cord. Eur J Pharmacol 117:387-389.

Polc P (1987) NMDA receptors mediate background and excessive activity of gamma-motoneurones in the spinal cord. Eur J Pharmacol 144:113-115.

Poon, MLT (1980) Induction of swimming in lamprey by L-DOPA and amino acids. J Comp Physiol 136:337-344.

Potter PE, Moskal JR (1990) B_6B_{21}, a monoclonal antibody that binds to NMDA receptors, modulates norepinephrine release from hippocampal slices in a glycine-like manner. Soc Neurosci Abst 16:88.

Raigorodsky G, Urca G (1987) Intrathecal N-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems. Brain Res 422:158-162.

Roberts A, Dale N, Evoy WH, Soffe SR (1985) Synaptic potentials in motoneurons during fictive swimming in spinal Xenopus embryos. J Neurophysiol. 54:1-10.

Roberts A, Kahn JA, Soffe SR, Clarke JDW (1981) Neural control of swimming in a vertebrate. Science 213:1032-1034.

Roberts PJ, Keen P (1974) Effect of dorsal root section on amino acids of rat spinal cord. Brain Res 74:333-337.

Roberts PJ, Watkins JC (1975) Structural requirements for the inhibition for L-glutamate uptake by glia and nerve endings. Brain Res 85:120-125.

Sands SB, Barish ME (1989) A quantitative description of excitatory amino acid neurotransmitter responses on cultured embryonic Xenopus spinal neurons. Brain Res 502:375-386.

Schneider SP, Perl ER (1988) Comparison of primary afferent and glutamate excitation of neurons in the mammalian spinal dorsal horn. J Neurosci 8:2062-2073.

Shinozaki H, Ishida M, Goto Y (1989) Depression of decerebrate rigidity in the rat by antagonists of excitatory amino acids. Neuropharmacology 28:593-598.

Sigvardt KA, Grillner S, Wallen P, Van Dongen PAM (1985) Activation of NMDA receptors elicits fictive locomotion and bistable membrane properties in the lamprey spinal cord. Brain Res 336:390-395.

Skerrit JH, Johnston GAR (1981) Uptake and release of N-methyl-D-aspartate by rat brain slices. J Neurochem 36:881-885.

Sladezek F, Recansens M, Bockdert J (1988) A new mechanism for glutamate receptor action: phosphoinositide hydrolysis. Trends Neurosci 11:545-549.

Smith JC, Feldman JL (1985) Motor patterns for respiration and locomotion generated by an *in vitro* brainstem-spinal cord from neonatal rat. Soc Neurosci Abst 11:24.

Smith JC, Feldman JL (1987) Neural control system for mammalian locomotion *in vitro*. Soc Neurosci Abst 13:825.

Smith JC, Feldman JL, Schmidt BJ (1988) Neural mechanisms generating locomotion studied in mammalian brain stem-spinal cord in vitro. FASEB J 2:2283-2288.

Smith JC, Garcia-Rill E, Feldman JL (1986) Chemical activation of mammalian locomotion *in vitro*. Soc Neurosci Abst 12:386.

Smith JL, Hoy MG, Koshland GF, Phillips DM, Zernicke RF (1985) Intralimb coordination of the paw-shake response: a novel mixed synergy. J Neurophysiol 54:1271-1281.

Stone TW, Burton NR (1988) NMDA receptors and ligands in the vertebrate CNS. Prog Neurobiol 30:333-368.

Thedinga KH, Benedict MS, Fagg GE (1989) The N-methyl-D-aspartate (NMDA) receptor complex: a stoichiometric analysis of radioligand binding domains. Neurosci Lett 104:217-222.

Turski L, Schwarz M, Turski WA, Klockgether T, Sontag K-H, Collins JF (1985) Muscle relaxant action of excitatory amino acid antagonists. Neurosci Lett 53:321-326.

Urca G, Raigorodsky G (1988) Behavioral classification of excitatory amino acid receptors in mouse spinal cord. Eur J Pharmacol 153:211-220.

Watkins JC (1981) Pharmacology of excitatory amino acid transmitters. In: Amino Acid Neurotransmitters, (FV DeFeudis, P Mandel, eds), pp 205-212. New York: Raven Press.

Watkins JC, Krogsgaard-Larsen P, Honore T (1990) Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. Trends Pharmacol Sci 11:25-33.

Wallen P, Grillner S (1987) N-methyl-D-aspartate receptor-induced, inherent oscillatory activity in neurons active during fictive locomotion in the lamprey. J Neurosci 7:2745-2755.

Young AB, Fagg GE (1990) Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches. Trends Pharmacol Sci 11:126-133.