LOCOMOTION INDUCED BY INTRATHECAL DRUG ADMINISTRATION IN CHRONIC SPINAL CATS

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Note to the reader: All figure numbers, figure titles and figure legends are located on the left hand page facing the appropriate figure.

Abstract

Available evidence suggests that vertebrate locomotion is patterned at the level of the spinal cord and requires signals from the brainstem to activate the central pattern generator (CPG) for locomotion (Grillner, 1975). Pharmacological, biochemical and anatomical evidence support norepinephrine (NE) (Grillner,1975), dopamine (DA) (Commissiong and Neff, 1979) and serotonin (5-HT) (Viala and Buser, 1971; Martin et al., 1978) as neurotransmitter candidates for release of the spinal CPG. In this study the effects of Ne, DA and 5-HT on locomotion and hind limb extensor tonus were examined utilizing the technique of intrathecal drug administration. In addition, the feasibility of intrathecal drug injection as a means for the initiation of locomotion in spinal animals was determined.

The spinal cords of cats (n=19) were transected in the lower thoracic region. A cannula utilized for drug administration was inserted in the subarachnoid space of the lumbosacral region. Walking abilities were assessed by the ratings of independent observers and standard kinematic techniques (Goslow et al., 1973). Hind limb extensor tonus was assessed using a force plate.

Norepinephrine (1.0 mM and 10.0 mM) was effective in producing rhythmic locomotor movements and increasing hind limb extensor tonus. Well developed steps were produced by cats after administration of NE 10.0 mM. This stepping had some characteristics of both the walk and gallop gaits observed in intact cats.

Both DA and 5-HT were much less effective in producing increases in walking ability and hind limb extensor tonus. Intrathecal drug injection was found to be an effective means of introducing drugs into the CNS to induce locomotion in spinal animals.

TABLE OF CONTENTS

List of Tables

List of Figures

Abbreviations

α	- alpha
β	- beta
BLS	- bulbar locomotor region
CA	- catecholamines
CID	- chronic intrathecal drug study
CNS	- central nervous system
CPG	- central pattern generator
CSF	- cerebral spinal fluid
DA	- dopamine
DBH	- dopamine-beta-hydroxylase
DCT	- diethyldithio carbamate
E1	- first extension component of step cycle
E2	- second extension component of step cycle
E3	- third extension component of step cycle
EMG	- electromyogram
F	- flexion component of step cycle
fps	- frames per second
FRA	- flexion reflex afferents
HRP	- horseradish peroxidase
5-HT	- 5-hydroxytryptamine (serotonin)
5-HTP	- 5-hydroxytryptophan
i.v.	- intravenous
i.m.	- intramuscular
Kg	- kilogram

Abbreviations (cont'd)

kg sec	- kilogram seconds
ul	- microliters
L	- lumbar
L-DOPA	- L-3,4-dihydroxyphenylalanine
LH	- left hind limb
MAO	- monoamine oxidase
mg	- milligrams
ml	- milliliter
MLR	- mesencephalic locomotor region
mm	- millimeter
mM	- millimolar
msec	- millisecond
m/sec	- meters per second
NE	- norepinephrine
6-OH-DA	- 6-hydroxydopamine
PLS	- pontine locomotor region
RH	- right hind limb
SD	- standard deviation
SEM	- standard error of the mean
sec	- second
τ	- tau
T	- thoracic
TH	- tyrosine hydroxylase
WBI	- weight bearing index

Definitions

Decapitate animals: An animal whose brain stem has been transected at the caudal end of the medulla. The decapitate preparation discussed in this paper received a transection at the caudal end of the rhomdoid fossa (Sherrington,1910).

Decerebrate animals: An animal whose brain stem has been transected at the rostral end of the midbrain. In this paper the precollicular postmammillary decerebrate preparation was discussed. The line of transection extends from the rostral end of the superior colliculi to the caudal extent of the mammillary bodies (Grillner and Shik, 1973).

Spinal animals: An animal whose spinal cord has been transected (Sherrington, 1910). The levels of the transections discussed in this paper are given in the text.

Introduction

The precursors of the monoamines norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (serotonin or 5-HT), have been observed to induce locomotion in acute spinal animals (animals with complete spinal transections) (Jankowska et al., 1967; Budakova, 1973). In contrast, the effects of those three monoamines themselves on locomotion in spinal animals has not been evaluated. This is primarily due to the inability of these drugs to cross the blood-brain barrier (Weil-Malherbe et al., 1961; Fuxe and Hillarp, 1964; Douglas, 1980). In this study, the effects of these monoamines on locomotion in the chronic spinal cat preparation were studied.

The inability of these drugs to cross the blood-brain barrier was circumvented by administering the drugs through a cannula implanted in the subarachnoid space of the lumbosacral region of the spinal cord (intrathecal administration). This method of drug administration has been utilized successfully for NE administration in studies of the effects of NE on antinociception in intact cats and rats (Reddy and Yaksh, 1980). In addition, intrathecal injections of NE and DA were used to study the effects of catecholamines on the flexor reflex of intact cats (Dhawan and Sharma, 1970). In this latter study DA (50-200 micrograms / 0.5 milliliters of normal saline) did not facilitate the flexor reflex while NE (40-80 micrograms/ 0.5 milliliters of normal saline) produced a marked facilitation of the flexor reflex within 3 to 5 minutes. These effects lasted for 90 to 120 minutes. These doses also did not produce any change in peripheral

blood pressure. In addition, it was unlikely that the NE effects were produced by vascular effects in the spinal cord since a vasoconstrictor (vasopressin) and a vasodilator (histamine) did not modify the flexor reflex. Both H -NE and H -DA have been shown to enter the central nervous system (CNS) when administered intraventicularly (Glowinski and Axelrod, 1966). Studies with H -NE administered intraventricularly in rats illustrate that the blood-brain barrier also was effective in preventing NE from diffusing out of the CNS into the peripheral circulation (Glowinski and Axelrod, 1966). These studies indicate that the effects observed after intrathecal injection of NE into the CNS were central in origin.

In this current study the effects of intrathecal monoamine drug injections on the locomotion of chronic spinal cats with transections in the thoracic region were examined. The purpose of this study was to learn whether monoamines could initiate walking and increase weight support in chronic spinal cats that were incapable of hindlimb walking and/or supporting their hindquarter weight. In addition, chronic cats that were capable of walking and weight support were also given monoamines. These animals then were examined to learn whether monamines would increase the animals' abilities. Another important aspect of this study was to evaluate the method of intrathecal injection for therapeutic use.

The evaluation techniques utilized in this study were to assess locomation rating assessments were by independent

observers and standard kinematic techniques (Goslow et al., 1973; Wetzel et al.,1976). The drug effects on hind limb weight support and extensor tonus were assessed by a force plate technique developed for this study.

Review of the Literature

Since the 1870's investigators have observed stepping movements in spinal mammals particularly rodents and carnivores (see Table 1). These studies indicated that a mechanism which could be stimulated to produce locomotion was present in the isolated spinal cord. Ranson and Hinsey (1930) observed poor stepping movements in the hind limbs of chronic spinal cats transected as far caudal as the third lumbar (L_3) spinal level. Stepping by the midlumbar transected spinal animals was rare, however. No stepping was demonstrated in cats with lesions below this level. Spinal cats with lesions above twelfth thoracic level (T_{12}) were more likely to produce hind limb stepping (Ranson and Hinsey,1930). Ranson and Hinsey's, (1930) study, therefore, indicated that the lumbosacral region of the spinal cord was necessary to produce well developed stepping movements in the hind limbs of spinal cats. The studies quoted in table 1 give a brief history of the investigation of locomotion in spinal animals. Three major points brought out by these investigations were: 1. Spinal primates do not produce spinal locomotion (Eidelberg, 1980; Eidelberg, 1981). 2. Animals transected shortly after birth tend to produce better-developed stepping which requires less peripheral stimulation for its production (Schurrager and Dykman, 1951; Grillner, 1973, Stelzner et al., 1975; Forssberg et al, 1980a). 3. Stepping quality and the ease of evoking stepping tend to increase with time after transection (Ranson and Hinsey, 1930; Shurrager and Dykman, 1951; Grillner, 1973; Eidelberg et al.,1980; Stelzner et al.,1975; Forssberg et al., 1980a

TABLE I Locomotion Investigations in Spinal Animals

Investigators	Animal Preparation	Locomotor Abilities	Time Period for Acquisition of Ability after Spinalization
Eidelberg,1980	Macaque monkeys (transection T_6-T_8)	unable to produce stepping on a treadmill with peripheral stimulation	animals observed for 2 - 4 months
Graham Brown 1914-15	foetal kittens (transection in thoracic region)	-vague stepping movements stimulated by the transection	-occur immediately after transection
Shurrager and Dykeman,1951	kittens (transection T_{12} - L_3). Surgery performed 2 days to 12 weeks after birth. Kittens were exercised with electrical stimulation to hind limbs.	a)rhythmical stepping movements b)could walk with hind limbs maintaining balance. c)kitten's hind limbs stepped in the air when the kitten was held in a vertical position (Freusberg's phenomenon)	a) occur a few hours after transection b) This was observed as kittens grew older. Increases in ability ceased when electrically stimulated exercise was stopped. c) observed as kittens grew older (kittens were observed for 1 to 4 months.)

Table 1 cont'd

Investigators	Animal Preparation	Locomotor Abilities	Time Period for Acquisition of Ability after Spinalization
Grillner,1973	8 kittens (lower thoracic transection) Surgery performed 7 - 16 days after birth	a) spontaneous walking and galloping with balance maintained observed in 1 kitten	a) observed 6 to 7 weeks after spinalization
		b) stepping when the animals'feet touch a moving treadmill	b) This was observed in all 8 kittens. In some kittens it was noted as early as 1 to 2 days after surgery.
Forssberg et al., 1980a	14 kittens (transection in lower thoracic region) Surgery performed 6 to 17 days after birth.	a) Freusbergs phenomenon	a) observed 1 to 2 days after transection.
		b) Walking,trotting and galloping on a moving treadmill and walking on the ground (overground) when tail held for balance.	b) observed as kittens grew older
Smith et al, 1982	12 kittens (transection T_{11} to T_{13}) Surgery performed 2 weeks after birth	a) 11 of the 12 demonstrated spontaneous stepping with weight support and good joint range of motion. No difference was found between 7 kittens that were exercised and 5 that were not	a) kittens rated 4 months after surgery
		b) Freusberg's Phenomenon observed in all kittens	b) no time frame given

Table 1 (cont'd)

Investigators	Animal Preparation	Locomotor Abilities	Time Period for Acquisition of Ability after Spinalization
Smith et al., 1982	9 kittens (transection T_{11} to T_{13}) Surgery performed 12 weeks after birth. Exercise performed by 5 kittens.	a) 2 of 5 kittens exercised demonstrated spontaneous walking with weight support and good joint range of motion	a) kittens rated 4 months after surgery
		b) all other kittens walked without weight support and some spontaneous steps or steps with stimulation at base of tail	b) kittens rated 4 months after surgery.
		c) Freusberg's Phenomenon observed in all kittens	c) no time frame given
Sherrington, 1910	adult cats (decapitate preparation where lesion performed at caudal end of rhomdoid fossa in medulla)	-stepping occurs with stimulation (electrical or manual) of perineum, skin at base of tail, pinna, and back of neck	no time frame given

Table 1 (cont'd)

Investigators	Animal Preparation	Locomotor Abilities	Time Period for Acquisition of Ability after Spinalization
Graham Brown, 1911	decerebrate adult cats (Transected later T_{11} to T_{13})	locomotion in hind limbs generated by the action of the cordotomy	-noted immediately after transection
Graham Brown, 1914	decerebrate adult cats (Transection in lower thoracic region) Transection performed during narcosis progression (stepping that is induced during certain levels of anesthesia)	stepping continued after transection	noted during first few minutes after transection
Ranson and Hinsey,1930	27 adult cats (Transections T_4 to L_6)	stepping with pinching of perineum or tip of tail in animals with lesions at L_3 or rostral (Stepping was better in cats with lesions above T_{12})	Trace of stepping movements and poor stepping movements were observed during the first 3 days after surgery. In cats transected in the thoracic region stepping quality increased until sacrifice three weeks later.

Table 1 (cont'd)

Investigators	Animal Preparation	Locomotor Abilities	Time Period for Acquisition of Ability after Spinalization
Ten Cate,1962	5 adult cats (Transection T_8 - T_{11})	walking observed when cat in a walker for support. Freusberg's Phenomenon also present	weeks to months after transection
Eidelberg et al, 1980	6 adult cats (Transection in mid-thoracic region)	inconsistent stepping on moving treadmill	-observed 1 to 6 weeks after transection
Shurrager and Dykman,1951	puppy (transected at L_1) Surgery performed at 5 weeks after birth	-could make several hops and return to a crouched position	observed as animal grew older (puppy was observed for a period of 95 days after spinalization).
Freusberg,1874; Goltz and Freusberg, 1874	dogs (Transected in lower thoracic region)	a) stepping noted with stimulation in anal region	a) observed 3 days postspinalization The amount of stepping and quality increased with time after transection
		b) Freusberg's Phenomenon	b) observed as early as the first few days after transection
Sherrington, 1910	dog (Transected in thoracic region)	Freusberg's Phenomenon	observed a few weeks after transection

Table 1 (cont'd)

Investigators	Animal Preparation	Locomotor Abilities	Time Period for Acquisition of Ability after Spinalization
Kellogg et al., 1946	5 dogs (Transection L_1 to L_3)	a few spontaneous stepping movements noted in the hind limbs of one dog	time period after transection not given
Ten Cate, 1964	adult rabbits (transected in lower thoracic region)	could make alternating stepping movement and occasionally synchronous bilateral hind limb movements when moving in a walker	observed at least one week after spinalization
Stelzner et al., 1975	17 neonatal rats (Transected in mid-thoracic region). Surgery performed 0 to 5 days after birth	-stepping (waddling gait) and hopping of hind limbs not co-ordinated with forelimbs	This was observed at 13 to 15 days of age. Amount and rapidity of stepping increased between 15 to 20 days of age
Stelzner et al., 1975	10 weanling rats transected in the mid-thoracic region 21 - 26 days after birth	stepping or hopping observed only rarely when all hind limb joints were flexed	observed during first three weeks after transection.
Meisel and Rakerd, 1982	3 neonatal rats (Transection T_8 to T_{10}) Surgery performed 13 days after birth	stepping when tail was pinched	Rats were tested when 115 days old Stepping was of a faster rate in the neonates than in similar rats transected as adults.

Table 1 (cont'd)

Investigators	Animal Preparation	Locomotor Abilities	Time Period for Acquisition of Ability after Spinalization
Meisel and Rakerd,1982	3 adult rats (Transection T_8 to T_{10}) Surgery performed 53 - 54 days after birth	stepping when tail was pinched	Rats were tested when 103 to 104 days old. Stepping was of a slower rate in the adult rats than in similar rats transected as neonates

Ten Cate, 1962 and 1964; Smith et al., 1982).

Investigators have reported also that chronic dogs, puppies (Freusberg, 1874; Philippson, 1907; Sherrington, 1910; Kellogg et al., 1946; Shurrager and Dykman, 1951), cats and kittens (Ranson and Hinsey, 1930; Shurrager and Dykman, 1951; Grillner, 1973; Forssberg et al., 1980a, Smith et al., 1982) transected in the thoracic and high lumbar spinal regions demonstrated increased hind limb extensor tone and in some cases the ability to stand. The animals transected when adult could stand for periods as long as one minute. These animals, however, were unable to make postural adjustments (Kellogg et al., 1946; Ranson and Hinsey, 1930). Animals transected just after birth were capable of standing for prolonged periods and in some cases making postural adjustments (Shurrager and Dykman, 1951; Grillner, 1973; Forssberg, 1980a, Smith et al., 1982).

The stepping and standing abilities mentioned above appeared days, weeks or even months after transection (see Table 1). To obtain stepping in acutely transected animals, however, pharmacological manipulation is usually necessary.

Pharmacological agents are hypothesized to stimulate a network of cells in the spinal cord which generates rhythmic locomotor movement in a spinal animal's limbs (Jankowska et al., 1967; Grillner and Zangger, 1979). This proposed network of cells is termed the central pattern generator for locomotion (CPG). Several models for the locomotor CPG have been formulated, but the details will not be discussed in this review. The reader is directed to Lungberg (1980)

and Grillner (1975) for these details.

Prior to the intravenous (i.v.) injection of the noradrenergic and dopaminergic precursor L-3-4-dihydroxy - phenylalanine (L-DOPA) a short latency discharge (attributed to polysynaptic reflexes) was recorded from motor nerves of hind limb muscles when high threshold muscle afferents and cutaneous afferents (flexion reflex afferents) were stimulated (Anden et al., 1966a). These recordings were performed in acute spinal cats immobilized with curare. When flexion reflex afferents (FRA) were stimulated after the administration of L-DOPA this short latency reflex became depressed and a long latency massive discharge (late reflex discharge) appeared (Anden et al., 1966a). These effects were potentiated by the monoamine oxidase (MAO) inhibitor nialamide (Anden et al., 1966b) and also were found to be dependent on the dose of L-DOPA utilized (Baker and Anderson, 1970a). Jankowska and coworkers (1967) also observed these changes in the FRA induced polysynaptic reflex discharge in acute spinal cats after administration of L-DOPA and nialamide. In addition, they recorded rhythmic alternating discharges in flexor and extensor motorneurons in these animals. These discharges were described as "spinal stepping" (Jankowska et al., 1967). Baev (1977) demonstrated that a combination of L-DOPA and the MAO inhibitor iproniazid not only produced a stepping-like discharge pattern in flexor motor nerves of spinal cats, but could also produce a gallop-like discharge pattern. Viala and coworkers (1974) again with L-DOPA and nialamide treated spinal cats and also

rabbits recorded both the late reflex discharge and locomotor-like pattern of bursting in the hind limb motor nerves during FRA stimulation. These investigators concluded that both phenomena developed simultaneously during the action of L-DOPA. For this reason they are likely closely related and belong to the same category of activities.

In intact rabbits under light anesthesia L-DOPA produced spontaneous discharges in both flexor and extensor nerves--the extensor bursts being more predominant (Viala and Buser, 1969). In acute spinal rabbits treated with L-DOPA and nialamide, alternating discharge activity was produced in the motor nerves to symmetrical homologous (limbs of the same girdle--hindlimbs or forelimbs) flexors or extensors of the two hind limbs (Viala and Buser, 1971). In the decerebrate rabbit, alternating and synchronous discharges were produced in the nerves to symmetrical homologus muscles after the above pharmacological treatment (Viala and Baser, 1971). In both these animal preparations the extensor nerve bursts were again predominant.

Work by Grillner (1969) and Budakova (1973) demonstrated that stepping could be induced in the hind limbs of acute thoracically transected spinal cats after administration of L-DOPA and nialamide. These animals, however, could walk only when their feet were placed on a moving treadmill or when stimulated by other external stimuli. Other workers (Halbertsma et al., 1976; Miller and Van der Meche, 1976) observed coordinated stepping in all four limbs in cervically

transected acute spinal cats when the animal's limbs were placed on a moving treadmill. These animals had been treated with L-DOPA, nialamide and R04-4602/1 (a peripheral decarboxylase inhibitor). All of these investigators noted that the L-DOPA animals were capable of following the speed of the treadmill. The L-DOPA and nialamide treatment was not effective in producing stepping in spinalized macaque monkeys. However, this lack of response may have been due to the prolonged spinal shock observed in the monkey (Eidelberg, 1980).

A method of action of L-DOPA in the spinal cord was hypothesized by Anden and coworkers (1966a and 1966b). These investigators suggested that L-DOPA increased the synthesis of transmitter from the terminals of a descending noradrenergic pathway. Credence was given to this view when Forssberg and Grillner (1973) were able to evoke stepping in acute spinal cats with an i.v. injection of the alpha (α) adrenergic agonist, clonidine. These animals did not show spontaneous stepping, but did step when their feet were placed on a moving treadmill belt. Clonidine administration in chronic spinal cats does modify the locomotor pattern by increasing step length, producing better foot placement, increasing weight support and increasing the duration of flexor and extensor muscle bursts (Barbeau and Rossignol, 1982).

Apomorphine (i.v. administration), a dopaminergic agonist, showed a marked tendency to disrupt the locomotor pattern of chronic spinal cats by inducing sustained flexion

of the animals' hind limbs (Barbeau and Rossignol,1982). In addition, D-amphetamine, which causes a release of adrenergic mediators, produced locomotor-like bursting activity in the hind limbs of acute spinal rabbits (Viala and Buser, 1971). This activity was similar to the type produced by L-DOPA in acute spinal rabbits (Viala and Buser, 1971). These D-amphetamine effects were potentiated when the animals were pretreated with nialamide. Amphetamine was, however, ineffective in producing the locomotor bursting activity in chronic spinal rabbits (6 days postspinalization) (Viala and Buser, 1971). The concentration of NE in chronic spinal rabbits was markedly decreased 5 to 7 days after transection (Anden et al, 1964a; Magnusson and Rosengren,1963). It is possible that the lack of effect by D-amphetamine in the chronic spinal rabbit was due to this decrease.

Although very few studies on locomotion have been executed utilizing antagonists and synthesis inhibitors, the effects of L-DOPA on the depression of the short latency FRA polysynaptic reflex has been examined extensively by these means.

The depression produced by L-DOPA on the short latency FRA reflex was inhibited in animals pretreated with the decarboxylase inhibitor, NSD 1015 (Anden et al., 1966b). The drug NSD 1015 inhibits the decarboxylation of L-DOPA which is an essential step in the systhesis of both NE and DA. It appears that the effects of L-DOPA are to be mediated through its synthesis products, DA or NE, and not by L-DOPA directly.

The enzyme that converts DA to NE (dopamine-B-hydroxylase) can be inhibited by diethyldithiocarbamate (DCT) (Jurna and Lundberg, 1968). When DCT was administered prior to L-DOPA in acute spinal cats, the subsequent L-DOPA injection still produced the depression of the short latency FRA reflex. However, when a second administration of L-DOPA was given after the animal recovered from the first injection, the depression of the short latency FRA reflex (the L-DOPA effect) did not occur. The investigators interpreted these results in the following manner. During the first administration of L-DOPA, the DA which was synthesized displaced stored NE. The displaced NE produced the short latency reflex depression. However, during the second L-DOPA administration the NE stores were depleted, therefore, no NE was available to produce the short latency FRA reflex depression.

Anden and coworkers (1966b) demonstrated that the effects of L-DOPA on the FRA induced reflexes were reversed by the adrenergic blocker, phenoxybenzamine. Chlorpromazine (α blocker) also produced a partial reversal of the effects. The reversal was not observed when the β-adrenergic blocker, nethalide, was adminstered. Other workers (Baker and Anderson, 1970b) also demonstrated a partial restoration of the short latency FRA reflexes (reversal of the L-DOPA effects) with the administration of α blockers (phenoxybenzamine, chlorpromazine and ethobutamoxane). The β-blocker, pronethalol, failed to show any significant antagonism of the L-DOPA effects.

Commissiong and Neff (1979) discussed evidence that dopamine was a mediator of the L-DOPA motor effects. First of all, L-DOPA is converted into both DA and NE. Secondly, as discussed earlier, after administration of the decarboxylase inhibitor NSD 1015 which inhibits the synthesis of DA, the effects of L-DOPA on the short latency FRA reflex were inhibited (Anden et al., 1966b). Thirdly, also mentioned previously, the effects of L-DOPA were still observed after the administration of a dopamine-B-hydroxylase inhibitor (Jurna and Lundberg, 1968). In addition, NE and DA depletion studies in the rat indicate that there are seperate stores of NE and DA in the spinal cord (Commissiong et al., 1978a). Administration of benzatropin and 6-hydroxydopamine (6-OH-DA) selectively depletes noradrenergic neurons while desipramine and 6-OH-DA, selectively depletes dopaminergic neurons (Commissiong et al.,1978a). When benzatropin and 6-OH-DA were administered to rats, NE and not DA was depleted from the spinal cords. However, when desipramine and 6-OH-DA was given to rats, DA and not NE was depleted from the spinal cords. If the sole source of DA in the spinal cord was the NE-containing neurons then both NE and DA would deplete in parallel. Since NE and DA are depleted selectively from the spinal cord, there must be separate neuronal stores of NE and DA in the spinal cord (Commissiong et al., 1978a).

Besides L-DOPA, locomotor effects have been attributed to another monoamine precursor. This drug is 5-hydroxytryptophan (5-HTP) the precursor to serotonin

(5-HT). The serotonergic precursor has been shown to induce spontaneous rhythmic bursts in predominantly flexor muscle nerves of lightly anesthetised intact rabbits. This effect was inhibited by the antiserotonergic drugs methysergide and lysergide (Viala and Buser, 1969). In curarized spinal rabbits, 5-HTP produced rhythmic alternating discharges in flexor and extensor muscle nerves. As in the intact rabbit treated with 5-HTP, the flexor bursts predominated. The bursting activity in the spinal rabbits was alternating between symmetrical homologous muscles on the opposing hind limbs. This differed from the decerebrate rabbit treated with 5-HTP where discharges in symmetrical homologus muscles were both alternating and synchronous, (Viala and Buser, 1971).

When 5-HTP was administered to chronic spinal rats transected as adults, an increase was observed in the spontaneous rhythmic EMG activity of a monitored quadriceps muscle. In addition, locomotor ability also increased (Bedard et al.,1979; Barbeau et al.,1981). The increased EMG activity was first observed after 5-HTP administration four days after transection. A progressive increase in the amount of spontaneous activity after 5-HTP was observed as time after transection increased. The spontaneous EMG activity was inhibited by injection of the serotonergic antagonists cyproheptadine, pizotilin and SQ-10631 and the antagonist chlorpromizine but not phenoxybenzamine (Barbeau et al.,1981). In addition, prior injection of the L-aromatic decarboxylase inhibitor Ro-4-4602, (which inhibits the

synthesis of 5-HT from 5-HTP) depressed the post 5-HTP response (Barbeau et al.,1981). Drugs which can mimic serotonin (quipazine, fenfluramine, D-lysergic acid diethylamide (LSD)) all increased the spontaneous EMG activity in the chronic rat (Barbeau et al.,1981). These investigators did not observe the increased EMG response after the administrations of L-DOPA, apomorphine hydrochloride, clonidine or acetycholine (Bedard et al., 1979; Barbeau et al.,1981).

In chronic spinal cats, the 5-HT agonist quipazine (i.v.administration), tended to increase extensor tone at rest. In addition, the amplitude of the EMG bursts in extensor muscles during treadmill walking was also increased (Barbeau and Rossignol, 1982).

The pharmacological evidence appears to support a hypothesis that the monoamines NE and 5-HT are involved in locomotion. They have been considered as putative neuro - transmitters for the release of the spinal locomotor generator. The possibility that DA is involved in locomotor generation has not yet been ruled out, however.

The hypothesis that monoamines are involved in locomotion is strengthened by biochemical, anatomical and electrophysiological studies. Biochemical and histological studies confirm that all three monoamines are present in the mammalian spinal cord and brainstem as, are their synthetic and degradative enzymes. Binding studies in rat spinal cord confirm the existence of spinal cord $\alpha 1$, and $\alpha 2$ adrenergic receptors (Jones et al.,1982). Other work by Jones (1981)

gives evidence for adenosine dependent cyclic AMP formation in rat spinal cord mediated by a receptor which is coupled to the alpha adrenergic receptor. In addition, work by Jones and Alcantara (1982) suggest that synaptic supersensitivity exists in the lumbar spinal cord of rats following a complete spinal cord transection in the thoracic region. An observation supporting this hypothesis was a 3 to 4 fold increase in NE-stimulated cyclic AMP accumulation in the lumbar cord (below transection) verses the cervical cord (above the transection). The enhanced NE-stimulated cyclic AMP response was observed as early as 3 days after transection (Jones and Alcantara, 1982). This time correlates with the increase of spontaneous flexor reflex activity which also was observed as early as three days after spinal cord transection (Jones and Alcantara, 1982). These investigators also noted that NE uptake and NE steady state concentrations decreased after spinal transection (Jones and Alcantara,1982). Changes in locomotor performance after the administration of apomorphine to chronic spinal cats gives evidence for the presence of DA receptors in the spinal cord (Barbeau and Rossignol, 1982). Apomorphine which is a DA agonist induced periods of sustained flexion which interrupted locomotion in chronic spinal cats (Barbeau and Rossignol,1982). In addition, the 5-HT agonist quipazine, increased the intensity of extensor mucle contractions in the hind limbs of chronic spinal rats (Bedard et al.,1979) and cats (Barbeau and Rossignol,1982). The response of chronic

spinal animals to quipazine represents supporting evidence for the presence of 5-HT receptors in the spinal cord.

The enzymes for synthesis of monoamines are present in the mammalian spinal cord and brainstem. The enzymes necessary for the synthesis of the catecholamines (CA) are as follows (Mayer,1980; Anden,1965):

compound - tyrosine------→ L-DOPA------→ DA------→ NE

converting enzyme	tyrosine hydroxylase (TH)	DOPA decarboxylase	dopamine- - hydroxylase (DBH)

The presence of the converting enzymes for the synthesis of each step have been confirmed by immunohistochemical techniques or assays for specific activity. These studies were carried out by the following investigators:

a) Ziven and coworkers (1976) in rabbit spinal cord for TH.

b) Anden (1965) in rabbit and cat spinal cord and Karoum et al.,(1981) in rat spinal cord for dopa decarboxylase.

c) Ziven and coworkers (1976) in rabbit spinal cord, Karowm et al. (1981) and Westlund et al. (1983) rat spinal cord and brainstem for DBH

d) Pickel et al.,(1976) localized typtophan hydroxylase,which converts 5-HTP to 5-HT,in the raphe nuclei of the rat. These nuclei are the major source of 5-HT for the spinal cord (Dahlstrom and Fuxe, 1965; Bowker et al., 1981 a and b)

The degradative processes of monoamines have been studied in monkey spinal cord (Kessler et al.,1976). The principle metabolites of DA and NE are homovanillic acid and 3 - methoxy-4-hydroxyphenylethylene glycol, respectively. The concentrations of these metabolites were investigated during

perfusion of the subarachnoid space of the monkey spinal cord. The perfusate was injected into a catheter at the lumbar region and collected from a catheter in the cervical region. A continuous flow of perfusate was maintained rostrally past the catheter to prevent supraspinal cerebral spinal fluid from descending into the spinal subarachnoid space (Kessler et al., 1976). Both of the catecholamines' metabolites were found in the perfusate (Kessler et al.,1976). This indicates the presence of catecholamine degradative enzymes in the spinal cord. In addition, the 5-HT metabolite, 5-hydroxy-3-indoleacetic acid has been found in the spinal cerebral spinal fluid of cats. A ligature placed around the thoracic region of the spinal cord prevented mixing of spinal and cisternal CSF during determinations of the 5-HT metabolite (Zivkovic and Bulat,1971).

All three monoamines (NE,DA and 5-HT) are present in all regions of the cat, rat and rabbit spinal cord. Their content is alway higher in the grey matter than in the white matter (Anden,1965; Anderson and Holgerson,1966; Zivin et al.,1975; Commissiong and Sedgwick, 1975; Raw et al.,1977; Oliveras et al, 1977; Fleetwood-Walker and Coote,1981).

In the cervical and lumbar regions of rat, rabbit and cat spinal cords, the NE concentration was greatest in the ventral horns, although it was present in the central region and dorsal horns (Anderson and Holgerson, 1966; Ziven et al.,1975; Fleetwood-Walker and Coote,1981). Dahlstrom and

Fuxe (1965) using catecholamine fluorescence techniques observed NE terminals to be uniform in appearance throughout the spinal grey matter of rat spinal cord. These terminals were most numerous in the most dorsal laminae of the dorsal horns, the lateral sympathetic column and ventral horn. In the ventral horns NE terminals appeared to be in contact with motorneurons. Westlund and coworkers (1983) observed the same distribution of NE terminals throughout the spinal grey matter of the rat. These investigators utilized antibodies to the NE synthesizing enzyme DBH in their study. They also noted that NE terminals were in contact with motorneurons in the ventral horn (Westlund et al.,1983). Dahlstrom and Fuxe (1965) noted that the cat spinal cord had the same distribution of NE terminals as the rat.

The concentrations of DA were lower than that of NE in the cat and rabbit spinal cords (Zivin et al., 1975; Rawe et al., 1977; Anden, 1965; Fleetwood-Walker and Coote, 1981). Rawe and coworkers, (1977) found DA concentrations to be 8 - 19% of mean tissue concentrations at the same segment in the cat spinal cord. In both cat and rabbit, the DA concentration was greatest in the central region of the grey matter (Zivin et al.,1975; Fleetwood-Walker and Coote,1981).

The concentrations of 5-HT in cat, rabbit and rat spinal cord are greater than the NE concentrations in the spinal cord. Anderson and Holgerson (1966) found no difference between 5-HT concentrations in the dorsal and ventral halves of the spinal grey matter in the cat. These investigators used a

bioassay method for their determinations. Oliveras and coworkers (1977), however, demonstrated that 5-HT was greater in the ventral horn of the cat than in the dorsal. In Oliveras' studies spectrofluorometric methods were utilized. Work in rabbit and rat also show the concentration of 5-HT to be greater in the ventral horn than in the dorsal horn (Zivin et al, 1975). The distribution of 5-HT concentration found by Oliveras et al.,(1977) and Zivin et al.,(1975) coincides with monoamine fluorescence histochemical results of rat spinal cord (Dahlstrom and Fuxe, 1965). Histochemical results demonstrated that 5-HT terminals followed a distribution which was similar to NE terminals in the spinal cord. The 5-HT fibers were less numerous than NE fibers in the dorsal horn, however, there were dense concentrations of 5-HT terminals in the lateral sympathetic column and ventral horn. Their terminals in the ventral horn were in contact with motorneurons. The distribution of 5-HT terminals in the cat spinal cord was similar to that observed in the rat (Dahlstrom and Fuxe, 1965).

Release of both NE and 5-HT from the spinal cord has been demonstrated. The spinal cords from mice (in vitro) pretreated with nialamide normally released NE into a bathing solution. However, when the rostral ends of the cords were stimulated the amount of NE in the bathing solution increased (Anden et al.,1965). Intrathecal perfusion of a potassium solution into the spinal cord subarachnoid space of cats and rats (pretreated with MAO inhibitor) in vivo produced an increase of both NE and 5-HT in the perfusate (Yaksh and

Tyce, 1980). In addition, in similar in vivo perfusion experiments in cats by Yaksh and Tyce (1981) the concentrations of both monoamines were observed to increase during sciatic nerve stimulation.

The concentrations of CA and 5-HT become markedly reduced below the level of a complete spinal cord transection in rabbits and rats 7 to 14 after surgery (Dahlstrom and Fuxe, 1965; Magnasson and Rosengren,1963; Carlson et al, 1963; Anden et al.,1964a; Haggendal and Dahlstrom, 1973). In cats the period for 5-HT and the monoamine sythesizing enzyme, dopa decarboxylase, to disappear is longer than the one observed for rats and rabbits being 14 to 21 days (Anden et al, 1964b, Anderson, 1972; Oliveras et a.,1977). Since the concentration of monoamines and their synthesizing enzymes decreased significantly after a spinal transection, investigators reason the major source of monoamines to the spinal cord to be of supraspinal origin (Dahlstrom and Fuxe, 1965).

The descending monoaminergic fibers to the rat spinal cord project mainly through the anterior and lateral funiculi of the spinal cord (Dahlstrom and Fuxe, 1965). The fibers running to the lateral sympathetic column in the rat appear to descend in the superficial and anterior part of the dorsolateral funiculus (Carlsson et al., 1964). The descending NE fibers to the ventral horn appear to run mainly in the border zone between the anterior and lateral funiculi in the rat. The descending serotonergic projections to the

ventral horn run in the medial portion of the anterior funiculi (Dahlstrom and Fuxe, 1965; Steinbusch, 1981). In the cat the concentration of NE was found to be highest in the ventral funiculus. After a spinal transection NE accumulates above the lesion in the ventrolateral and dorsolateral regions of the lateral funiculus. This indicates the possible presence of descending pathways in this region (Fleetwood-Walker and Coote, 1981). Dopamine was found to accumulate in only the central superficial region of the lateral funiculus of the spinal cord after a complete transection. This finding was interpreted as a distinct DA fiber tract in this region of the spinal cord (Fleetwood-Walker and Coote, 1981).

Dahlstrom and Fuxe (1964) stated that the major source of monoamine terminals to the spinal cord appeared to be the monoamine containing cell groups of the brainstem. These workers were the first to identify catecholamine containing cell groups in the brainstem. They enumerated the cell group from A1 to A12 moving caudal to rostral in the brainstem. The A1 - A12 terminology will be utilized throughout this review.

Westlund and coworkers (1983) utilized a combination of DBH immunohistochemical labelling and horseradish peroxidase (HRP) retrograde labelling in their study of NE projections to the rat spinal cord. These investigators found that the major source of noradrenergic projections to the spinal cord were three groups of cells in the pontine region. These three cell groups had been identified previously by Dahlstrom

and Fuxe (1964) as groups A7, A6 and A5. The major nucleus found within the A6 cell group is the locus coeruleus (Dahlstrom and Fuxe,1964). The major nuclei within area A7 include the locus subcoeruleus, the medial and lateral parabrachial nuclei and the Kolliker-Fuse nucleus (Westlund et al.,1983; Dahlstrom and Fuxe,1964). The third cell group, A5, is located in the ventrolateral reticular formation adjacent to the superior olivary nucleus (Westlund et al., 1983; Dahlstrom and Fuxe, 1964). Westlund and coworkers observed noradrenergic projections from each of the nuclei mentioned above in groups A7, A6 and A5. In addition, the majority of the noradrenergic projections to the spinal cord in the rat originated from the locus coeruleus and subcoeruleus regions (Westlund et al., 1983).

Nygren and Olson (1977) lesioned the locus coeruleus and subcoeruleus regions bilaterally in rats. Analysis of the spinal cords 10 days after the surgical procedure showed that most of the CA terminals throughout the spinal grey matter disappeared. The grey matter least effected by this procedure was the central grey of the thoracic region including the sympathetic lateral column. Confirmation of these observations came from Commissiong and coworkers (1978b) in their analysis of rat thoracic cord. These investigators observed a loss of ventral horn catecholamine terminals and some loss of dorsal horn CA terminals in the rat thoracic spinal cord after bilateral lesions of the locus coeruleus and subcoeruleus regions. The lateral sympathetic

cell column terminals were unaffected by this lesioning. In addition, Commissiong et al., (1978b) gave evidence that the descending fibers from the lesioned region were noradrenergic. Utilizing gas chromatographic-mass-spectrometric methods they revealed that NE was significantly reduced in the thoracic region of the rat spinal cord after the lesioning. The DA levels were uneffected by the locus coeruleus and subcoeruleus lesioning (Commissiong et al, 1978b). In other experiments (Karum et al., 1980) unilateral lesions of the locus coeruleus and subcoeruleus were performed in the rat. After a unilateral lesion a bilateral loss of NE was observed from the spinal cord (Karum et al, 1980). This indicated that the NE projections from locus coeruleus and subcoeruleus were bilateral. In addition, after a unilateral locus coeruleus and suboceruleus lesion histological assessment of rat spinal cord demonstrated a reduction in CA containing terminals in the ventral horn of the spinal cord bilaterally with no loss of terminals from the lateral sympathetic column in the thoracic region (Commissiong, 1981a). The crossing of the locus coeruleus-subcoeruleus projection fiber appears to be at a spinal level since a bilateral loss of CA-containing terminals was noted below the level of a unilateral hemisection of the thoracic spinal cord (Commissiong, 1981a).

Nygren and Olson (1977) noted a decrease in catecholamine containing fibers in the white matter of the rat spinal cord after bilateral locus coeruleus-subcoeruleus lesions. The decrease was in the ventral funiculus and the ventral portion

of the lateral funiculus (Nygren and Olson, 1977). Radioautographic studies by Pickel and coworkers (1974) in the rat illustrated the presence of a unilateral coerulospinal projection in the ventral spinal cord.

In the cat, several investigators have identified catecholamine containing cell groups in areas corresponding to A5, A6, and A7 (Dahlstrom and Fuxe terminology). In the A6 area, locus coeruleus, catecholamine cells were discovered by Poitras and Parent (1978), Chu and Bloom (1974) and Jones and Moore (1974). Nucleus cuniformis has also been shown to contain CA containing cells (Poitras and Parent, 1978). Catecholamine groups in A7 have been observed in the subcoeruleus nucleus, and medial and lateral parabrachial nuclei (Poitras and Parent, 1978; Chu and Bloom, 1974). In addition, another nucleus in the A7 group, the Kolliker-Fuse nucleus, has also been shown to contain CA (Stevens et al., 1982). Poitras and Parent (1978) have observed catecholamine containing cells in the A5 area. These include CA-containing cells near the superior olivary nucleus, cells dorsal and ventral to the mensencephalic tract of the trigemenal nerve, cells in the dorsal part of the principle nucleus of the trigeminal nerve and cells dorsal to the genu of the facial nerve (Poitras and Parent 1978).

Kuypers and Maisky (1975) using HRP retrograde tracing techniques have demonstrated descending projections from the locus coeruleus and subcoeruleus nuclei in the cat. These projections appeared to be mainly ipsilateral (Kuypers and

Maisky, 1975). In addition, Tohyama and coworkers (1979b) also have utilized HRP retrograde labelling techniques in the cat. After injection of HRP into the ventrolateral funiculus, these investigators observed retrograde labelled cells in the following nuclei: nucleus cuniformis, locus coeruleus, locus subcoeruleus, Kolliker-Fuse nucleus and the medial and lateral parabrachial nuclei (Tohyama et al.,1979b). All of these nuclei contain cells with catecholamines (Poitras and Parent, 1978; Chu and Bloom, 1974; Jones and Moore, 1974). In addition, HRP retrograde labelled cells have been observed in the A5 area of the cat (Kuypers and Maisky, 1975) after a spinal injection of HRP. These labelled cells were located at the level of the motor nucleus of the fifth nerve.

To ascertain whether descending fibers from cat catecholamine cell groups actually contain catecholamines, combined retrograde tracing and catecholamine labelling techniques are required. This type of study was performed by Stevens and coworkers (1982). These investigators utilized a combination of Evans Blue fluorescent dye and catecholamine histofluorescence to study the ponto-spinal catecholaminergic pathways. The major descending catecholamine pathway from the cat brainstem appeared to originate from the Kolliker-Fuse nucleus. In addition, they observed that the vast number of catecholamine containing cells of the locus coeruleus, subcoeruleus and medial parabrachial nucleus did not project to the lumbar spinal cord. Stevens and coworkers (1982) did not mention whether they observed double labelled cells in

the A5 pontine region.

Catecholamine-containing cells have been observed in the medulla of the rat (Dahlstrom and Fuxe, 1964; Westlund et al., 1983). These areas were termed A1-A4 by Dahlstrom and Fuxe (1964). The largest of these CA-containing areas are A1 and A2. The cells in group A1 lie in a region extending from the pyramidal decussation to the inferior olivary complex. Some A1 cells are situated lateral to the nucleus reticularis lateralis (Dahlstrom and Fuxe, 1964). The cells of group A2 lie in the region of the nucleus tractus solitarii, dorsal motor nucleus of the vagus and the nucleus commisuralis (Dahlstrom and Fuxe, 1964).

Westlund and coworkers (1983) did not find cells in the rat medulla which were labelled by both the retrograde tracer, HRP, and the antibody to the NE synthesizing enzyme DBH. Dahlstrom and Fuxe (1965) however, found that some CA containing cells in the medulla became swollen and displayed increased fluoresence 2 to 4 days after a total transection of the spinal cord. Most of these cells were located in area A1 with some cells in A2. It is likely that the swollen appearance of these cells was due to reaction from axotomy at the spinal level (Dahlstrom and Fuxe, 1965). Forty percent less of the medullary CA-containing cells became swollen when only the ventral and ventral lateral funiculi were cut. This would indicate that the medullary CA cells do not project solely through the ventral and ventrolateral funiculi (Dahlstrom and Fuxe, 1965).

Catecholamine-containing cells in the cat medulla have been demonstrated by Poitras and Parent (1978) and Blessing and coworkers (1980). These CA-containing cells could be divided generally into two groups, a ventral and a dorsal group. The ventral group is located near the lateral reticular nucleus of the medulla in a region dorsolateral to the pyramids (Poitras and Parent, 1978; Blessing et al.,1980). Poitras and Parent found CA-containing cells within the lateral reticular nucleus while Blessing and coworkers (1980) found them scattered around this nucleus. The ventral catecholamine area corresponds with the A1 area of the rat (Blessing et al, 1980). The dorsal group cells were found in or ventral to the nucleus of the hypoglossal nerve (Poitras and Parent, 1978). Blessing and coworkers (1980) did not find CA-containing cells in or ventral to the hypoglossal nucleus. These investigators observed the dorsal CA cells to be mainly within the nucleus tractus solitarius. The dorsal CA-containing area described by Blessing and coworkers (1980) corresponds with area A2 in the rat (Dahlstrom and Fuxe, 1965).

Using injections of the retrograde tracer, HRP, into the spinal cord, investigators have found retrograde labelled cells in the solitary nucleus (Kuypers and Maisky, 1975). It is possible that some of the dorsal CA-containing cells described by Blessing and coworkers (1980) lie in this region. In addition, HRP labelled cells have been observed around the lateral reticular nucleus (Kuypers and Maisky, 1975; Tohyama et al., 1979a). This area was described by

both Poitras and Parent (1978) and Blessing and coworkers (1980) as containing the ventral medullary CA-containing cells. The fibers from the lateral reticular nucleus appear to descend in the dorsolateral funiculus (Tohyama et al., 1979a). Double labelling techniques with both a retrograde tracer and a catecholamine marker would be necessary to confirm the existence of catecholamines within the descending medullary pathways.

Studies utilizing HRP retrograde tracing techniques in combination with catecholamine fluorescence in rabbits revealed double labelled cells in the region of the locus coeruleus (A6), subcoeruleus (A7) and near the motor nucleus of the trigeminal nerve and on the lateral border of the superior olive (A5) in the rabbit. In addition some double labelled cells were observed in the ventral medullary catecholamine group (A1) (Blessing et al, 1981).

In the rat, a dopaminergic pathway has been traced recently by Hokfelt and coworkers (1979). These authors utilized a combination of retrograde tracing and catecholamine histochemical techniques. The retrograde tracer, prinuline,was injected into the spinal cord. To ascertain whether catecholamine cells were dopaminergic adjacent sections were processed with the antibody to DBH. Cells in the periventricular region at the border between the mesencephalon and the hypothalamus contained dual labelled cells. The adjacent sections were not labelled by DBH during histochemical processing (Hokfelt, et al.,1979).

The anatomy of the serotonin descending systems has been studied in the cat and rat also. In the rat, 5-HT fiber projections have been observed from areas B1- B3 in the medulla and pons (Dahlstrom and Fuxe,1965). The areas B1- B3 refer mainly to the raphe nuclear region. Most of the cells of group B1 lie in the nucleus raphe pallidus, cells of group B2 are from nucleus raphe obscurus and cells of group B3 lie in the nucleus raphe magnus and among the fibers of the medial lemniscus lateral to the pyramidal tract (Dahlstrom and Fuxe, 1964). Bowker and coworkers (1981a and b) utilized a combination of immunohistochemical and HRP retrograde labelling techniques to study descending serotonergic projections in the rat. These investigators found cells labelled with both HRP and serotonin antiserum in the following medullary nuclei: raphe obscurus, raphe pallidus, raphe magnus, nucleus reticularis ventralis, and the nucleus reticularis gigantocellularis. Of the 5-HT cells located in the medullary nuclei, 73.4% were identified as having projections to the spinal cord (Bowker et al., 1981b). None of the 5-HT containing cells in the pons were labelled with HRP (Bowker et al., 1981b). There were, however, some cells in the midbrain labelled by both methods (Bowker et al., 1981b). These cells were located in the mesencephalic reticular formation. A few cells also were found within the nucleus raphe dorsalis and among fibers of the medial lemniscus in the mesencephalon (Bowker et al., 1981b). The percentage (36.4%) of 5-HT containing mesencephalic cells that sent projections to the spinal cord was smaller than the

percentage of spinally projecting 5-HT cells of the medulla (Bowker et al., 1981b).

In the cat as in the rat, 5-HT containing cells were located in the midbrain, pons and medulla (Poitras and Parent, 1978). Serotonergic cell groups in the midbrain were found in the caudal regions in the following nuclei: rostral portion of the nucleus raphe dorasalis in the periaqueductal grey, the intermediate part of the nucleus linearis, and the rostral part of nucleus centralis superior (Poitras and Parent, 1978). Kuypers and Maisky (1975) did not observe retrograde HRP labelled cells in any of these areas in cats. Tohyama and coworkers (1979a), however, noted retrograde labelled cells in the mesencephalic reticular formation just lateral to the periaqueductal grey after injection of HRP in the anterior spinal funiculus in cats.

Injections of HRP in the cervical spinal segments labelled cells in the nucleus raphe dorsalis and the nucleus raphe linearis intermedius (Tohyama et al., 1979b). These fibers appear to descend in the anterior funiculus (Tohyama et al., 1979b). Combined labelling studies would be necessary to confirm that the mesencephalic descending fibers contain 5-HT, however.

Most of the major 5-HT containing cell groups in the cat pons and medulla are located in and around the raphe nuclear group (Poitras and Parent, 1978). The nuclei containing the 5-HT labelled cells listed from rostral to caudal are : nucleus raphe dosalis, nucleus centralis superior, nucleus

raphe magnus, nucleus raphe pallidus, nucleus raphe obscurus, nucleus reticularis tegmenti pontis, nucleus paragigantocellularis, cells near the lateral reticular nucleus (Poitras and Parent, 1978).

Descending fibers labelled with HRP were noted in the lateral reticular nucleus of the cat (Kuypers and Maisky, 1975; Tohyama et al., 1979a). However, combined labelling techniques are required to identify the type of transmitter contained in these cells.

Several investigators have located retrograde labelled cells in the raphe nuclei after injection of HRP into the spinal cord of the cat (Kuypers and Maisky, 1975; Martin et al., 1978; Tohyama et al., 1979b). A great number of labelled cells were observed in nuclei raphe pallidus and obscurus but not in the nucleus raphe magnus after injection of HRP into the ventral funiculus (Toyama et al., 1979b). In contrast more labelled cells appeared in nucleus raphe magnus after a dorsolateral funicular injection of HRP (Tohyama et al., 1979b). Martin and coworkers (1978) combined spinal HRP injections with spinal cord lesions in the cat. The work of these investigators is consistent with the results of Tohyama and coworkers (1979b). Martin and coworkers (1978) found most retrograde labelled cells were located in the nucleus raphe pallidus and obscurus when HRP injects of the spinal grey matter occurred below the level of a dorsal and lateral funiculi transection. When HRP was injected below the level of a ventral cord transection, most retrograde labelled cells were observed in the nucleus raphe magnus and nucleus raphe

pontis. The number of labelled cells in the nucleus raphe obscurus and pallidus were greatly reduced in comparison to control animals (Martin et al, 1978). It appears that descending fibers from nucleus raphe pontis and magnus project mainly through the dorsal and lateral funiculi while fibers from nucleus raphe obscurus and pallidus project mainly through the ventral portion of the spinal cord (Tohyama et al., 1979b; Martin et al., 1978). Combined studies with retrograde tracing techniques and 5-HT labelling are required to confirm the presence of 5-HT within the fibers descending from the cat raphe nuclei.

Oliveras and coworkers (1977) performed selective lesions of the nucleus raphe dorsalis and observed no loss of 5-HT from the cat spinal cord. When these investigators performed a partial lesion (40%) of the nucleus raphe magnus, a substancial decrease of 5-HT was observed in the spinal cord. The spinal grey matter most affected by the nucleus raphe magnus lesion was the substancia gelatinosa of the dorsal horns (Oliveras et al., 1977). This evidence supports a hypothesis that the nucleus raphe magnus is a major source of 5-HT to the spinal cord dorsal horns.

The acute spinal cat described earlier is not the only cat preparation incapable of locomotion. Cats whose brains are transected at the rostral end of the brainstem where the line of transection extends from the rostral end of the superior colliculi to the caudal extent of the mammillary bodies (postmammillary-precollicular decerebrate cats) are incapable of spontaneous locomotion (Shik et al., 1966;

Grillner and Shik, 1973). This cat preparation can be made to walk by electrically stimulating the region of the nucleus cuniformis at the caudal extent of the inferior colliculus (Shik et al., 1966). This stimulation region has been termed the mesencephalic locomotor region (MLR)

Grillner and Shik (1973) studied FRA induced reflexes in the postmammillary-precollicular decerebrate cat preparation. These investigators were able to evoke the late reflex discharge during FRA stimulation when the MLR was not stimulated. The FRA stimulation was produced by stimulation of a peripheral nerve at high current strengths. The late reflex discharge evoked by Grillner and Shik (1973) was similar to the type evoked by FRA stimulation in the acute spinal cat treated with L-DOPA (Jankowska et al., 1967; Anden et al., 1966a). When the MLR was stimulated, this late reflex discharge was evoked at lower stimulation strengths of the peripheral nerves (Grillner and Shik, 1973). If the peripheral nerves were stimulated at a low strength without MLR stimulation no late reflex discharge was observed (Grillner and Shik, 1973). Grillner and Shik (1973) concluded that the neuronal network responsible for the late reflex discharges was directly influenced by brainstem stimulation. They had noted that the late reflex discharge facilitated by MLR stimulation displayed characteristics which were similar to the late reflex discharge induced by FRA stimulation in the L-DOPA treated spinal cats (Grillner and Shik, 1973). For this reason, these investigators hypothesized that stimulation of the MLR releases the neuronal mechanism

responsible for locomotion and in parellel lowers the threshold for evoking discharges similar to the type evoked in the spinal cat treated with L-DOPA (Grillner and Shik, 1973). Grillner and Shik (1973) suggested that the MLR responses were mediated through activation of the descending noradrenergic reticulospinal system.

Support was given to the Grillner and Shik's hypothesis by Steeves and coworkers (1975). These investigators observed that CA-containing cells were situated close to the effective MLR stimulation site in the brainstem in the cat. These CA neurons were in fact close enough to the MLR to receive effective stimulation due to spread of electrical current from the stimulation site (Steeves et al., 1975). The nearby CA neurons were located in the locus coeruleus (Steeves et al., 1975). As described earlier the locus coerulus in the cat has catecholaminergic projections which descend into the spinal cord (Stevens et al., 1982). Edwards (1975) observed ipsilateral projections from the nucleus cuniformis to the locus coeruleus (Edwards,1975). The nucleus cuniformis as mentioned previously is located in the region of the MLR (Grillner and Shik,1973). Edward's (1975) findings, however, do not preclude the possibility of synaptic connections between the MLR and other monoamine containing areas.

The projections from the MLR and nucleus cuniformis have been studied by several investigators. An autoradiographic study of the projections of the nucleus cuniformis was

undertaken by Edwards (1975). He used injections of tritiated amino acids into the superior aspect of the nucleus cuniformis to examine anterograde projections. The region injected was rostral to the effective MLR stimulation site (Garcia-Rill, 1983). This author observed both ipsilateral and contralateral projections from this nucleus. The contralateral projections descended through the ventromedial tegmentum of the brainstem and terminated in the following nuclei: reticularis tegmenti pontis, nucleus reticularis pontis caudalis and gigantocellularis, raphe magnus and the dorsal facial nucleus (Edwards, 1975). Catecholamine-containing cells have been observed near only one of the these nuclei, the periventricular grey region dorsal to the genu of the seventh nerve (Poitras and Parent, 1978). However, projections descending to the spinal cord have not been identified from this region (Kuypers and Maisky, 1975; Tohyama et al., 1979a). Serotonergic cells are found in the nucleus raphe magnus of the cat (Poitras and Parent, 1978). This was also one of the regions that received descending contralateral projections from the nucleus cuniformis (Edwards, 1975). The nucleus raphe magnus does have descending spinal projections (Martin et al., 1978; Tohyama et al., 1979b). However, the major spinal projections from nucleus raphe magnus descend in the dorsal half of the spinal cord (Martin et al., 1978; Tohyama et al., 1979b). The pathways necessary for locomotion appear to descend through the ventrolateral funiculi (Steeves and Jordan, 1980; Eidelberg et al., 1981). The induction of

locomotion with MLR stimulation requires intact ventral funiculi (Steeves and Jordan, 1980).

Ipsilateral projections from the nucleus cuniformis were observed to descend to the following nuclei (Edwards,1975): nucleus locus coeruleus, nucleus raphe dorsalis, the central grey matter, reticularis pontis oralis, medial accessory olivary nucleus. The most notable CA-containing nucleus that receives nucleus cuniformis projections was the locus coeruleus (Poitras and Parent, 1978; Edward, 1975). These connections were discussed above. A nucleus which contains both catecholamines and 5-HT also received a projection from the nucleus cuniformis. This nucleus was the lateral reticular nucleus (Poitras and Parent, 1978; Edwards, 1975). The lateral reticular nucleus in turn has been observed to descending projections to the spinal cord of the cat (Kuypers and Maisky, 1975; Tohyama et al., 1979a). However, studies must be done in the cat to confirm that the descending projections from the lateral reticular nucleus are indeed monoaminergic. The nucleus raphe dorsalis contains 5-HT cells (Poitras and Parent, 1978), but this nucleus does not appear to be a major source of 5-HT to the cat spinal cord (Oliveras et al., 1977).

Another autoradiographic study of the projections from the nucleus cuniformis was performed by Steeves and Jordan (in preparation). Unlike Edwards (1975) who injected tritiated amino acids into the rostral nucleus cuniformis, these investigators injected tritiated amino acids into the

effective locomotor stimulation sites in the midbrain of decerebrate cats. This area was in the caudal nucleus cuniformis (Steeves and Jordan, in preparation). In addition tritiated amino acids were also injected into the caudal nucleus cuniformis in intact cats. These authors observed that the projections from the caudal nucleus cuniformis were mainly ipsilateral.Descending fibers projected to the following areas: nucleus reticularis tegmenti pontis (dorsal tegmental reticular nucleus, ventromedial reticular formation in the gigantocellular and magnocellular tegmental fields and nucleus raphe magnus.

The projections noted by Steeves and Jordan to the nucleus raphe magus were ipsilateral. This was unlike the ones noted by Edwards (1975) which were contralateral. However, as mentioned above, the nucleus raphe magnus sends projections to the dorsal half of the spinal cord (Martin et al., 1978; Tohyama et al., 1979b). The pathways necessary for locomotion appeared to descend through the ventral half of the spinal cord (Steeves and Jordan, 1980; Eidelberg et al., 1981).

The most prominent terminations observed in the cats after the injection of tritiated amino acids into the caudal nucleus cuniformis occurred in the tegmental reticular nucleus and the gigantocellular and magnocellular reticular fields (Steeves and Jordan). Monoaminergic cells have not been observed in this region in the cat (Poitras and Parent, 1978). However, Mori and coworkers (1978) have evoked locomotion by stimulation of the tegmental reticular nucleus

and the ventromedial gigantocellular and magnocellular reticular fields. When stimulating in the caudal ventromedial tegmental field concomitantly with stimulation of the MLR, the production of locomotion by the MLR was facilitated (Mori et al., 1978). In addition, Mori and coworkers (1978) noted increased extensor tone in the hind limbs of the decerebrate cat when the caudal ventromedial tegmental field was stimulated. As noted previously, the serotonergic agonist, quipazine, increased extensor tonus in chronic spinal cats (Barbeau and Rossignol, 1982). The raphe nuclei lie medial and immediately adjacent to the ventromedial tegmental field (Poitras and Parent, 1978; Martin et al., 1978). It is possible that the increased extensor tonus described by Mori and coworkers (1978) evoked by stimulation of the ventromedial tegmental field in the decerebrate cat was mediated through a serotonergic pathway involving the raphe nuclei. Nucleus raphe obscurus and pallidus both project to the spinal cord through the ventrolateral funiculi (Martin et al., 1978; Tohyama et al., 1979b). This is the spinal cord region where the locomotor pathways are thought to descend (Steeves and Jordan, 1980; Eidelberg et al., 1981).

In one control cat, Steeves and Jordan (in preparation) injected tritiated amino acids into the rostral nucleus cuniformis. The projections observed in this animal were similar to the ones observed by Edwards (1975). The major differences between the autoradiography of projections from

the superior nucleus cuniformis and the inferior nucleus cuniformis was the labelling of the ipsilateral reticular nucleus and the contralateral gigantocellular tegmental field after injections in the superior cuniform nucleus (Edwards, 1975; Steeves and Jordan,in preparation).

Garcia-Rill and coworkers (1983) also studied projections from the MLR using anterograde tracing methods in cats. The anterograde tracers were injected into the MLR. Prior to sacrifice, the cats received a rostral brainstem transection and the site of injection was stimulated to ensure that the MLR had been injected (Garcia-Rill et al., 1983). The following structures were found to receive projections from the MLR (Garcia-Rill et al., 1983): ipsilateral dorsolateral reticular formation, bilateral Probst's tracts (located medioventrally to the trigeminal nucleus), contralateral ventral aspect of the gigantocellular reticular nucleus.

The MLR projections to Probst's tract are significant. Probst's tract coincides with another important brain stem locomotor region termed the pontine locomotor region (PLR) (Garcia-Rill,1983; Mori et al., 1980). The PLR appears to be part of a locomotor strip extending throughout the brainstem. The caudal extent of this brain stem locomotor strip is located at the ventrolateral border of the most caudal laminal trigeminal nucleus in the dorsolateral funiculus of the first cervical segment (Mori et al., 1980). The caudal locomotor strip appears to extend rostrally through the lateral tegmentum of the medulla. This caudal region of the

locomotor strip is termed bulbar locomotor region (BLR) (Mori et al., 1980). In the pons, the locomotor strip is located in the lateral tegmentum in the paralemniscal tegmental field where it is termed the PLR (Mori et al.,1980). The PLR extends to the region of the MLR in the mesencephalon (Mori et al., 1977; Mori et al.,1980). Probst's tract appears to be medial to CA-containing cells in the lateral pons (Stevens et al., 1982; Poitras and Parent, 1978). Poitras and Parent (1978) observed CA-containing cells in the region dorsal and ventral to the mesencephalic tract of the trigeminal nerve and in the region of the superior olivary nucleus. These catecholamine containing areas coincide with area A5 and the Kolliker-Fuse nucleus of area A7 (Stevens et al.,1982). The Kolliker-Fuse nucleus is thought to provide the major catecholaminergic projection to the lumbar cord (Stevens et al., 1982). Tohyama and coworkers (1979b) found the HRP labelled cells in the Kolliker-Fuse nucleus after injection of HRP into the ventrolateral funiculus. This would indicate that the Kolliker-Fuse nucleus projects to the spinal cord through the ventrolateral funiculus, the spinal cord region where the locomotor pathways descend (Steeves and Jordan, 1980; Eidelberg et al., 1981). In addition, projections from the A5 CA region have been observed by Kuyers and Maisky (1975). It is possible that synaptic connections may exist between the pontine CA regions and Probst's tract.

Garcia-Rill and coworkers (1983) also observed

connections from the region of the MLR to the contralateral ventral aspect of the gigantocellular reticular nucleus in the ventromedial tegmental field. This projection differed from one observed by Steeves and Jordan (in preparation). These investigators noted only an ipsilateral projection to the ventromedial reticular formation in the gigantocellular and magnocellular tegmental fields.

When the results from the autoradiographic studies by Steeves and Jordan (in preparation) and Garcia-Rill and coworkers (1983) are combined, projections from the MLR appear to extend bilaterally to Probst's tract and the gigantocellular tegmental field. Probst's tract is adjacent to the Kolliker-Fuse nucleus which is a major descending CA pathway (Stevens et al., 1982). The gigantocellular tegmental field is adjacent to a major serotonergic nuclear complex (Martin et al., 1978; Poitras and Parent, 1978) which also projects to the spinal cord (Tohyama et al., 1979b; Martin et al., 1978).

Pharmacological, biochemical and anatomical evidence appear to support the hypothesis of release of the spinal locomotor generator (CPG) by a descending monoaminergic system or systems. However, the importance of monoamines in locomotion is seriously questioned in the light of studies utilizing monoamine depletion techniques. Steeves and coworkers (1980) utilizing chemical monoamine depletion techniques decreased NE and 5-HT content from the spinal cord and brainstem of cats. The maximal depletion of monoamines was as follows: NE: 14% of control in lumbar cord, 16% of

control in pons; 5-HT: 19% of control in sacral cord, 25% of controls in medulla (Steeves et al., 1980). Locomotion was evoked using MLR stimulation in cats depleted of both NE and 5-HT (Steeves et al., 1980). In addition, depletion of NE or 5-HT alone did not alter locomotion in otherwise intact animals (Steeves et al., 1980).

The result of monoamine depletion studies does not appear to support the involvement of monoamines in the release of the spinal locomotor CPG. However, it is not known how much depletion of monoamines is actually necessary to cause a functional depletion of the brainstem monoamine systems. Eventhough a significant reduction of NE and 5-HT was produced in the study by Steeves and coworkers (1980), it is possible that residual stores of 5-HT and NE were utilized during locomotion in these animals.

It is important to administer monoamines in a spinal animal preparation to investigate whether monoamines are capable of releasing the spinal locomotor CPG. This study, therefore, was designed to investigate the effects of monoamines on the locomotion of spinal animals. As was mentioned previously intrathecal drug injection was used to administer the monoamines to circumvent the problem of the inability of monoamines to cross the blood brain barrier. Since animals with spinal cord transections at the level of T_{12} are capable of producing hind limb locomotor movements (Ranson and Hinsey, 1930), the lumbosacral enlargement is likely involved in the generation of these movements. For

this reason, the monoamines were injected into the subarachnoid space in the lumbosacral region of the spinal cord. The chronic cat model was utilized in these studies because cats commonly are used in locomotion studies. In addition, in the chronic state, the cat most closely models the human spinal cord injury patient.

A force plate technique was developed to measure the hind limb extensor tonus in the animals since no other adequate quantitative method of analysis could be found. This technique is discussed in detail in the materials and methods section of this paper.

The animals' walking movements were rated by independent observers in this study to avoid experimentor bias. This method has been utilized successfully by other investigators (Smith et al., 1982). In addition to the rating assessment, kinematic analysis of the cat locomotion also was done to obtain detailed information about the movement generated. This kinematic information was utilized to investigate whether the walking obtained after drug injection in the spinal cat had characteristics similar to the intact cat.

In this following section, kinematic studies are reviewed to provide the background information necessary to understand the kinematic analysis. The literature covered will be restricted to hind limb locomotion except during the review of fore-and hind limb coupling patterns.

For study purposes every movement of lifting a limb off the ground, returning it to the ground, and standing on it until it is once again lifted is termed a step cycle (Goslow

et al., 1973). A similar term often used interchangeably with step cycle in the study of quadruped locomotion is the stride. A stride has been defined as a complete set of cyclic movements of all four limbs beginning and ending with the same reference limb (Lockard et al., 1976). A stride actually defines the same time interval and event as the step cycle. The former term stride, however, acknowledges the existence of the simultaneous cycling of all four limbs with each limb beginning and ending at a part of the step cycle different from the reference limb. The step cycle or stride is the basic descriptive element of gait.

Each step cycle is divided classically into two phases, the first being the swing or transfer phase, and the second the stance or support phase (Grillner, 1975). Swing is defined as the interval that commences when the foot leaves the ground and terminates when it returns to the ground. This encompasses the period when the foot is in the air transferring weight forward (Grillner, 1975). Stance consists of the entire interval when the foot is in contact with the ground. This includes the period from initial touch to the moment just prior to lifting (Grillner, 1975).

Several authors have cataloged the types of quadrupeds gaits into different categories. Maybridge (1899) and Hildebrand (1966 and 1976) examined gaits from many different varieties of quadruped while Stuart and coworkers (1973), Miller and Van der Burg (1973), and Miller and coworkers (1975a and b) looked solely at the gaits of the cat. All

these authors noted that gaits first can be divided into two major categories termed symmetrical and asymmetrical gaits (Hildebrand, 1966). Symmetrical and asymmetrical gaits also have been called alternate and inphase stepping respectively (Miller and Van der Burg, 1973; Miller et al., 1975a and b). The former terminology, however, is utilized in this review.

In symmetrical gaits the time interval between right foot contact and left foot contact equals the time interval between left foot contact and right foot contact in a pair of homologus limbs (limbs of the same girdle--hindlimbs or forelimbs) (Hildebrand, 1966 and 1976; Miller et al., 1975b). Hildebrand (1966 and 1976) divided symmetrical gaits into two broad categories based on the duration of the stance phase. The first category was the walk. Walks are gaits whose stance phase phase duration constitutes greater than 50% of the duration of the step cycle (Hildebrand, 1966 and 1976). The second category, the run, has a stance phase shorter than 50% of the duration of the step cycle (Hildebrand, 1966 and 1976). During the walk, the animal is supported by at least one hindlimb and one forelimb throughout the step cycle (Stuart et al., 1973; Hildebrand, 1976). In the run there often are periods of single limb support or no limb support at all (Hildebrand, 1976).

The walks and runs were further subdivided by Hildebrand (1966 and 1976) into the pace, trot and varieties of lateral sequence and diagonal sequence gait patterns. This latter classification is dependent on the time interval between midstance of a hindlimb to the midstance of the ipsilateral

forelimb (Hildebrand, 1966 and 1976). In the trot pattern, the contralateral fore-and hind limbs flex and extend simultaneously. This means that the contralateral fore-and hind limbs are coupled in phase (Hildebrand, 1966 and 1976; Miller et al., 1975a and b). In the pace the ipsilateral fore-and hind limbs are coupled in phase (Hildebrand, 1966 and 1976; Miller et al., 1975a and b). The lateral sequence and diagonal sequence gaits have fore-and hind limb coupling which falls between the two extremes of the trot and pace (Hildebrand, 1966 and 1976). Theoretically all these fore-and hind limb coupling patterns are possible at both walking and running speeds (Hildebrand, 1966 and 1976).

Symmetrical gaits have been observed in intact (Goslow et al., 1973; Stuart et al., 1973; Miller and Van der Burg, 1973; Miller et al., 1975a and b; Engberg and Lundberg, 1969), decerebrate (Miller et al., 1975a and b; Halbertsma et al., 1976) and spinal cats (Forssberg et al., 1980a and b; Grillner, 1973). Miller and coworkers (1975a and b); and Hildebrand (1976) observed that the intact cat preferred the trot gait pattern at both walking and running speeds. The pace, although it was observed normally in cats, was utilized only rarely (Miller et al., 1975a and b). The pace was observed most commonly in normal and decerebrate cats walking on a treadmill at a low speed (< 1.0 m/sec). When a transition between the two gait patterns occurred, it was usually fairly abrupt (Miller et al., 1975a).

In asymmetrical gaits the time interval between

right-left and left-right foot contacts in a homologous pair of limbs is unequal (Hildebrand, 1966; Miller et al., 1975b). Gaits included within the asymmetrical category are the canter, transverse and rotatory gallops and half-bound (Muybridge, 1899; Engberg and Lundberg, 1969; Stuart et al., 1973; Miller et al., 1975b). In addition as in the run, in these gaits the stance phase is less than 50% of the step cycle (Stuart et al., 1973; Goslow et al., 1973; Muybridge, 1899). Goslow and coworkers (1973), in fact, demonstrated during the gallop that the stance phase was less than 40% of the step cycle in freely moving cats. Besides stance duration and the asymmetry of timing within the homologous pair of limbs, these gaits also depend on hind limb and forelimb timing data for their proper classification (Stuart et al., 1973; Muybridge, 1899; Miller et al., 1975b). Another characteristic which these gaits share with the run are periods of single limb support and intervals with no limb support at all (Muybridge, 1899; Stuart et al., 1973; Miller and Van der Burg, 1973; Miller et al., 1975b).

Like the symmetrical gaits, the asymmetrical gaits have been identified in several cat preparations. Included in this tally are intact (Goslow et al., 1973; Stuart et al., 1973; Miller et al., 1975b) decerebrate (Miller et al., 1975a and b; Halbertsma et al., 1976) and spinal cats (Forssberg et al., 1980a and b; Grillner, ;1973). In spinal kittens, galloping was observed even at low treadmill speeds, however, it was produced more often at high speeds (Grillner, 1973; Forssberg et al., 1980a and b). The reports of asymmetrical

gaits in decerebrate and spinal animals indicate that these animals are capable of producing the complex gait patterns seen in intact cats.

Most authors have classified cat gaits into three categories, the walk, trot and gallop (Stuart et al., 1973; Miller et al., 1973; Goslow et al., 1973; Coss et al., 1978; Engberg and Lungberg, 1969; Forssberg et al., 1980a and b; Grillner, 1973). This classification is consistent for intact cats locomoting freely overground (Stuart et al., 1973; Goslow et al., 1973; Engberg and Lundberg, 1969) and intact and spinal cats locomoting on a treadmill (Miller and Van der Burg, 1973; Forssberg et al., 1980a and b; Grillner, 1973). It should be noted that the definition of walk and gallop employed by these investigators is similar to Hildebrands' (1966 and 1976). The trot, however, was defined differently. The definition of trot utilized by these authors was actually analogous to the running trot defined by Hildebrand (1966 and 1976). The running trot is a symmetrical gait with a stance phase of less than 50% of the step cycle with the fore-and hind limb coupling pattern of a trot (Hildebrand, 1966 and 1976). The appearance of walking, trotting or galloping gaits is normally dependent on the speed of locomotion (Goslow et al., 1973).

Coss and coworkers (1978) examined the locomotion of normal adult cats moving overground in an unrestrained test situation. Walking gaits were observed at speeds as low as 1.0 m/sec, trotting at 1.0 m/sec to 2.5 m/sec and galloping

at speed greater than 2.5 m/sec. Similar results have been observed by other investigators examining cats locomoting overground (Goslow et al., 1973; Stuart et al., 1973) and on a motorized treadmill (Miller and Van der Burg,1973). The cat was observed to progress smoothly from one gait to the next as speed increased (Miller and Van der Burg, 1973).

Philippson in 1905 described the joint angle changes at the hip, knee and ankle for a dog as it progressed through the step cycle (cited from Grillner, 1975). The pattern of angle changes that he described has been called the Philippson step cycle (cited from Grillner, 1975). According to Philippson, the step cycle consists of swing and stance phases which were further subdivided into four component parts termed F (flexion), E1 (first extension), E2 (second extension) and E3 (third extension). The F conponent constituted the first part of the swing, and it was followed immediately by E1, which is also part of the swing. Stance was comprised of E2 initially which is then followed by E3 (cited from Grillner, 1975).

Goslow and coworkers (1973) investigated the joint angle changes in the hind limb step cycles of intact freely moving cats utilizing the Philippson step cycle classification scheme. The cats movements were examined during the walk (1.5 mph), trot (3.6 mph) and gallop (16.3 mph). These authors observed the presence of all four step cycles components containing the same pattern of joint angles changes described by Philippson in each of the three basic gaits. Goslow and coworkers', (1973) joint angle analysis

with a description of the Philippson step cycle is presented below (figure 1).

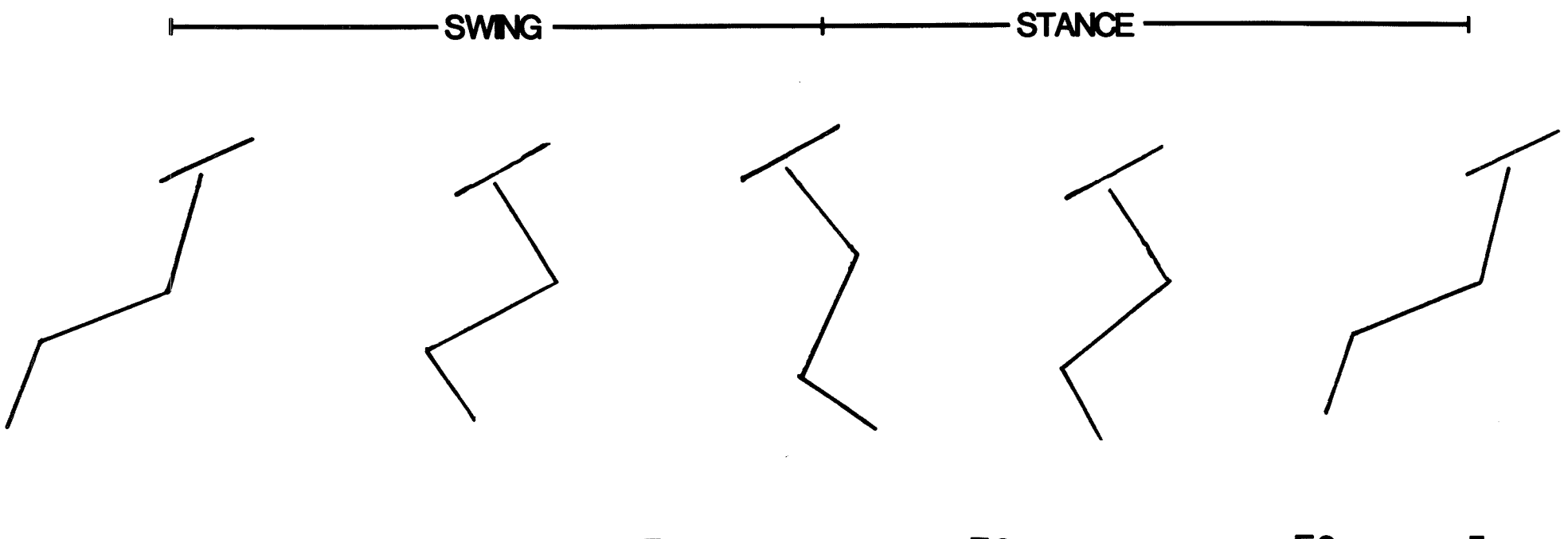
The F component occurs during the first part of the swing phase. During F simultaneous hip, knee and ankle flexion occur which functions to lift the foot from the ground and move the limb through the air (Goslow et al., 1973). The F component ends and the second component, E1, begins when the knee and ankle stop flexing. At the commencement of E1 the hip still flexes while the knee and ankle extend. Prior to the termination of E1 at the end of the swing phase, the hip also stops flexion and begins extension. The movements during E1 return the limb to the ground to begin the stance phase (Goslow et al., 1973).

The E2 component comprises the initial part of stance (Goslow et al., 1973). During this component the knee and ankle flex slightly or yield as the animal transfers its weight onto the stance limb. Concurrent to this, the hip extends. Because the knee and ankle generally seem to flex during the E2 period, this component has also been termed the yield (Grillner, 1975). The knee and ankle flexion are dependent on weight transfer for their occurrence (Grillner, 1975). In the latter part of stance after the yield has terminated all three joints extended simultaneously. This period, termed E3, functions to propel the animal forward (Goslow et al., 1973).

Other authors also have observed that the intact cat uses the pattern of movement described by the Philippson step

Figure 1. The Philippson Step Cycle
A schematic illustration of the Philippson step cycle redrawn from Goslow et al., 1973. The stick figures represent the positions of the hip, knee and ankle at the beginning of each component. Below each component are the main movements performed by each joint during the component.

THE PHILIPPSON STEP CYCLE



cycle during all gaits for both treadmill (Wetzel et al., 1975; Miller et al., 1975b) and overground (Engberg and Lundberg, 1969; Wetzel et al., 1975) locomotion. In addition, the components of the Philippson step cycle has been observed in the steps of a spinal kitten walking on a treadmill (Forssberg et al., 1980a).

Even though the step cycle components have been observed in gaits from all three gait categories (walk, trot and gallop), some variability has been described in the joint angle patterns of the components found in the different gaits. For example, Goslow and coworkers (1973) noted that the hip extension that occurs in the latter part of E1 during the trot was pronounced while E1 hip extension which occured during the walk and gallop was not. In contrast, Engberg and Lundberg (1969) found the hip extension during E1 to be more pronounced during the gallop than in the walk or trot. Other authors (Miller et al., 1975b) during a study of the gallop observed that hip extension during E1 was pronounced in the leading hind limb and not in the contralateral trailing hind limb. It should be noted that neither Goslow and coworkers (1973) nor Engberg and Lundberg (1969) mentioned whether the trailing or leading limb in the gallop was analyzed.

Another variant has been observed in the initial portion of the F component during the gallop. This variant was an initial hip extension at the beginning of the swing which drives the limb backwards when the limb first leaves the ground. This extension was attributed to the increased extensor thrust at the end of the stance phase during the

gallop (Goslow et al., 1973).

A third characteristic of the step cycle where variability is observed is the initiation of extension at the hip, knee and ankle during the swing phase. this characteristic was altered when different testing modalities or animal preparations were used. It did not appear to be effected by speed of locomotion (Wetzel et al., 1975). The order of appearance of extension at the joints during the swing phase in the hind limb of an intact cat locomoting overground is knee or ankle first followed fairly closely by the hip (Goslow et al., 1973; Wetzel et al., 1975). No specific trend for knee or ankle to lead the extension was observed (Goslow et al., 1973). For intact cats walking or trotting on a treadmill, however, there is a definite tendency for the knee to begin extension prior to the ankle during the swing (at the beginning of EI). The hip extends after the latter two joints (Wetzel et al., 1975). Forssberg and coworkers (1980a) in their report on the kinematics of the spinal kitten did not specifically mention the order of appearance of joint extension during hind limb swing. They did, however, present records the simultaneous joint angle changes for five step cycles produced by the spinal kitten. In these step cycles, knee and ankle extension occurred almost simultaneously followed slightly later by hip extension.

The total displacement or range of movement at a joint during a step cycle has been examined by several authors in both intact and spinal preparations. Goslow and coworkers

(1973) published range of movement data for intact cats locomoting freely overground at various speeds. At a walking speed (1.5 mph or 0.68 m/sec) the hip and knee joint displacements were both approximately 40° and the ankle joint displacement 55° . When the animals increased their speed to that of a trot (3.6 mph or 1.6 m/sec) the joint excursions at the hip and knee increased to approximately 45° and 50° respectively. The ankle joint displacement was less than that observed in the walk being 45° during the trot. At a speed where the cats galloped, the hip joint displacement actually decreased to 40° . This was thought to be due to the increased movement at the lower spine that occurs during the gallop. The extra spinal movement decreased the need for increased hip movement. The knee and ankle joint displacements were largest during the gallop being 50° and 80° respectively.

Forssberg and coworkers (1980a) also investigated articular range of movement during the step cycles of a spinal kitten. These authors observed that the three joints (hip, knee, ankle) were effected differently as the speed of the treadmill was increased from 0.1 to 0.8 m/sec (walking speeds). The hip joint displacement remained constant at 40° as the speed increased. It should be noted that the spinal kitten's hip displacement was similar to the hip excursion of freely moving intact cats. The knee and ankle joint displacements of the spinal kitten were affected by speed changes. These joints produced greater excursions as the speed increased. The articular displacements did stabilize

at speeds of 0.4 to 0.8 m/sec. At these speeds, the knee joint range was approximately 50° while the ankle range was 70° to 100°. These latter joint excursions for the knee and ankle of the spinal kitten were larger than those seen during walking in intact spinal cats (Goslow et al., 1973). The spinal kitten's knee and ankle joint excursions were actually close to those observed in intact cat during the gallop.

Goslow and coworkers (1973), investigated the duration of the step cycle as it relates to the speed of locomotion in cats moving freely overground. These authors noted that as the speed of locomotion increased the duration of the step cycle decreased. At speeds below 2 mph (0.89 m/sec) which is a walking speed the step cycle duration was greater than 500 msec (Goslow et al., 1973). At trotting speeds of 0.72 to 2.7 m/sec the step cycle duration ranged from 500 to 400 m/sec. In addition, at speeds where cats galloped (> 2.7 m/sec) the step cycle duration decreased to a range of 400 to 300 m/sec. These experimenters observed that the relationship between step cycle duration and speed fit a power curve (Goslow et al., 1973).

Miller and Van der Burg (1973) observed similar results to Goslow's group (Goslow et al., 1973) for cats locomoting on a motorized treadmill. Cats that walked at treadmill speeds below 1.4 m/sec produced step cycles with durations of 800 to 450 msec in duration (Miller and Van der Burg, 1973). At speeds of 1.4 to 6.0 m/sec the step cycle duration decreased to a range of 450 to 250 msec (Miller and Van der Burg, 1973;

Miller et al., 1975a). Wetzel and coworkers (1975) also demonstrated that step cycle deviations were similar for cats locomoting in overground and treadmill test situations at similar speeds. Decerebrate cats also decreased step cycle duration as the speed of the treadmill increased (Kulagin and Shik, 1970). These authors also observed the relationship between speed and step cycle duration in the decerebrate cat as fitting a power curve.

Spinal kittens (Forssberg et al., 1980a; Grillner, 1973) and cats transected in adulthood (Eidelberg et al., 1980) also were observed to decrease step cycle duration as the speed of the motorized treadmill on which they walked increased. One spinal kitten (Forssberg et al., 1980a) presented step cycle durations as long as 880 msec at a treadmill speed of 0.14 m/sec (Forssberg et al., 1980a). When the treadmill ran at 0.7 m/sec the kitten's step cycle duration was reduced to nearly 450 msec (Forssberg et al., 1980a). Eidelberg and coworkers (1980) demonstrated step cycle durations of 730 msec (SD ± 10 msec) and 603 msec (SD± 27 msec) for a spinal cat walking at treadmill speeds of 0.42 m/sec and 0.61 m/sec respectively. These durations were similar to the kitten described by Forssberg and coworkers (1980a) walking at a similar range of speed. Grillner (1973) presented a similarstep cycle versus speed relationship in a spinal kitten. He also demonstrated that this relationship was best described by a power curve.

The decrease in step cycle durations is primarily at the expense of the stance phase in intact (Miller and Van der

Burg, 1973; Goslow et al., 1973) decerebrate (Kulagin and Shik, 1970) and spinal cats (Grillner, 1973; Eidelberg et al., 1980; Forssberg et al., 1980a). The swing phase tends to remain at a fairly constant duration with changes in speed in all animal preparations in both treadmill and overground testing situations. (Miller and Van der Burg, 1973; Goslow et al., 1973; Forssberg et al., 1980a; Kulagin and Shik, 1970; Wetzel et al., 1975).

The decrease in stance duration with increased locomotion speed discussed above was noted as one of the major methods by which an animal could increase it's speed of locomotion (Miller and Van der Burg, 1973). The decrease in stance time and consequently the decrease in step cycle duration leads to an increased frequency of stepping. This increased stepping frequency could thus be utilized to increase an animal's speed (Miller and Van der Burg, 1973). The observation that decerebrate and spinal cats also decrease stance duration when treadmill speed is increased illustrates that this method of increasing the speed of locomotion was present in these preparations (Kulagin and Shik, 1970; Forssberg et al., 1980a). It is interesting to note that even though the duration of swing changes little as speed increases the percentage of the step cycle taken up by swing does increase with speed. This was observed for intact cats walking overground (Goslow et al., 1973) and on a treadmill (Miller and Van der Burg, 1973). This phenomenon was observed also for a spinal kitten (Forssberg et al.,

1980a) and decerebrate cats (Kulagin and Shik, 1970) walking on a treadmill. Correspondingly, these authors demonstrated that the percentage of the step cycle comprised by stance decreases as the speed at locomotion increases.

As discussed earlier, swing duration varies little as the speed of locomotion increases. In fact, as shown by Goslow and coworkers (1973) as cats locomoting overground increased their speed from 1 to 16 mph swing duration decreased by only 5%. These authors noted that of the two swing components, F and E1, most of the swing phase reduction occurred during E1 (Goslow et al., 1973). This means that the F component duration was extremely stable over changes in the speed of locomotion.

In the spinal kitten observed by Forssberg and coworkers (1980a), F and E1, followed a pattern similar to intact cats when the speed of locomotion was increased. The swing phase decreased slightly with increases in speed. Most of the reduction in swing duration that occurred was due to a shorter E1 component. In intact cats locomoting overground at a speed of 1 mph to 2 mph (walking speed) the F component composed slightly more than 1/2 of the swing phase (Goslow et al., 1973). Wetzel and coworkers (1975) found that the percentage of swing constituted by F differed in treadmill and overground locomotion. For cats walking (1.0 m/sec) or trotting (2.0 m/sec) overground the F component composed 46% of swing duration. When the same cats utilized similar speeds on the treadmill, the percentage of swing comprised by F increased to 67%. The percentage of the swing

phase constituted by the F component appeared to stay constant when the speed was changed, but only within a particular testing paradigm.

As mentioned in previous sections, the percentage of the step cycle comprised by the swing phase increases as speed of locomotion increases. The F component follows the same trend. It also constitutes a greater percentage of the step cycle as the speed of locomotion increases. This was demonstrated for intact cats locomoting overground (Goslow et al., 1973) and on a treadmill, (Wetzel et al., 1975). Forssberg and coworkers (1980a) observed a similar trend of the F component with increased treadmill speeds in a spinal kitten. As the treadmill speed was increased from 0.1 to 0.8, the percentage of step cycle constituted by F increased.

Since the components E2 and E3 make up the stance phase, it is not surprising to observe the marked effect changes in speed produced on the duration of these two components. Like the stance phase, the durations of E2 and E2 decrease when the speed of locomotion increased. This was observed in intact animals locomoting overground (Goslow et al., 1973). Even though a decrease occurred in both components as speed increased, the greatest effect was seen on the duration of E3, (Goslow et al., 1973). In addition, when E2 and E3 were calculated as percentages of step cycle duration, E2 was relatively unchanged with changes in speed. It composed approximately 15% of the step cycle at speeds ranging from 1 to 16 mph. The E3 component, however, illustrated a marked

decrease in the percentage of the step cycle it comprised as speed increased (Goslow et al., 1973).

Materials & Methods

1. Animal Preparation

Nineteen mongrel cats (2.3 kg - 3.5 kg) were utilized in this study. The majority of the cats were female since these are easier to care for after a spinal cord transection.

2. Surgical Techniques

Surgery was performed to transect the spinal cord of the animal, insert a cannula into the subarachnoid space, and insert wires into the iliac bone for suspending the cat. The following protocol was used. The cats were anesthetized with a mixture of N_2O, O_2 and halothane. Using sterile technique an incision was made in the skin and muscle covering the lower thoracic region from the level of thoracic vertebrae 8 to 12 (T_8 to T_{12}) and a laminectomy removing the T_{10} spinous process and lamina was performed. Following this the spinal cord was cooled for five minutes with frozen sterile Elliot's solution A (artificial CSF). The dura was opened, the spinal cord severed completely with scissors, and the rostral cord was lifted and examined to ensure a complete cut. A piece of sterile bone wax then was inserted between the cut ends of the cord and the area packed with Surgicel (Johnson & Johnson, New Jersey) and the wound closed. To insert the cannula, an incision was made at the lumbosacral junction and the lumbosacral ligament was cut. A small incision (approximately 1mm x 1mm) was made through the dura and arachnoid to gain access to the subarachnoid space. A

sterile cannula (PE-10 tubing) was inserted into the subarachnoid space through the incision until the distal end reached the level of the lumbosacral enlargement (approximately fourth lumbar vertibral level). The amount of tubing inserted into the subarachnoid space was measured beforehand by obtaining the distance from the lumbosacral junction to the fourth lumbar (L_4) vertebral spine. This distance was marked with thread at the insertion end of the cannula. The tubing was fixed to a plastic disc which was sutured to the skin overlying the lumbosacral junction, cut about four centimeters from its point of exit from the skin and capped. Whenever drugs were added to the subarachnoid space the cap was removed and a sterile 30 gauge needle with a 1.0 milliter (ml) syringe inserted. Immediately after surgery the cannula was kept free from blood clots by injecting 100 microliters (μl) of 20% (V/V) heparinized (1000 U.S.P. units/ml) Elliot's solution A. A similar amount of this solution was injected into the cannula three times daily for the first two days after surgery. In three of the cats (CID 28,29 and 41) the cannula was inserted into the paravertebral muscle or in the epidural space so that drugs did not enter the subarachnoid space. These animals were termed sham-operated controls or no drug controls in the study.

To insert the iliac wires for suspending the animals small incisions were made above the rostral ends of the iliac crests. A hole was drilled in the bone of each iliac crest

with a sterile dental drill bit. The stainless orthodontic steel wires (.56mm, Tru-chrome, Rocky Mountain, U.S.A.) were inserted and the wounds closed. These wires were utilized for support of the hind quarters during subsequent treadmill walking and weight bearing assessments.

After transection the cats were kept in padded cages. Their bladders were emptied once or twice daily. The animals were bathed daily and passive movements of the hind limbs were executed. Animals were given intramuscular (i.m.) injections of 500 mg of ampicillin sodium prophylactically on a daily basis. Urinary tract infections were treated with oral administrations of 100 mg. of nalidixic acid 2 to 3 times daily as necessary. Post-operative pain was treated for the first 2 post-operative days with 5.0 to 7.5 mg. of meperidine hydrochloride (i.m.). This was found to be an effective dose for reducing pain in the animals.

3. Drug Trials

After transection the cats were given 2 or 3 days for recuperation and return of spinal reflex activity. Following this a daily dose of 300 l of one of the test drugs was given intrathecally. The cannula was then flushed with 50µl of vehicle solution. The test drugs included norepinephrine bitartrate (NE) (Sigma, St.Louis) (0.1mM, 1.0mM and 10.0mM) dopamine hydrochloride (DA) (Sigma, St. Louis) (0.1mM; 1.0mM; 10.0mM) and serotonin creatine sulfate complex (5-HT) (Sigma, St.Louis) (10.0mM). All drugs were dissolved in Elliot's solution A (Abbott, Montreal) (artificial CSF). Control cats

were injected with 300μl of vehicle solution. No drug control cats (sham operated control cats) were injected with NE (0.1mM).

The cats' abilities to generate rhythmic walking movements and bear hindquarter weight were assessed prior to, and thirty minutes after intrathecal injection. Thirty minutes was determined by preliminary studies as the time required for NE drug injections to produce the greatest changes in motor ability. When motor changes occurred, they would increase steadily until they reached a plateau approximately 30 minutes after drug administration. These changes would last for about two hours and sometimes for longer periods after drug injection. In tests where no drug effect was observed after 30 minutes, there was also no effect observed before or after the test period 30 minutes after drug injection.

Drugs were administered immediately after preparation. Injections were performed by applying slow steady manual pressure on the piston of a 1.0ml syringe through a 30 gauge needle. The area of the spinal cord superfused by the cannula was checked during autopsy by injecting 350μl of 0.1% fast green into the cannula. The spinal cord was then examined to determine the extent of the drug superfusion.

4. Rhythmic Walking Movement Assessment

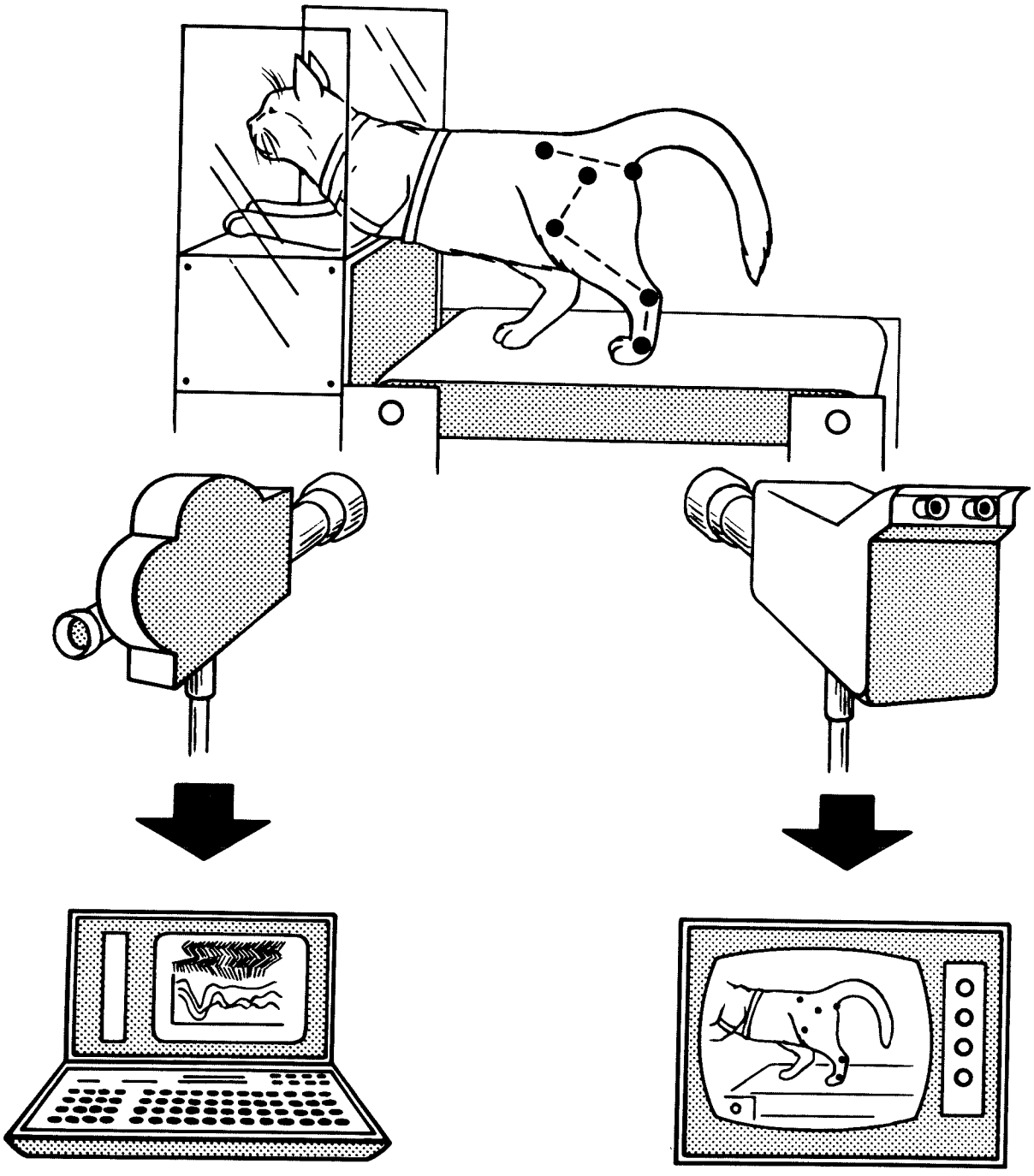
A. Independent Observer Rating of Rhythmic Movement

Rhythmic movement was assessed by two methods (see

figure 2.) In the first method the cats were videotaped prior to (predrug trial) and thirty minutes after (postdrug trial) intrathecal injections. The videotaped trials then were rated by two independent observers. During each pre-and postdrug trial videotaping was done of the animals walking both overground with a walker and on a treadmill. During overground walking the cats' forelimbs were free while each animal's hindquarters were suspended from an aluminum walker by an abdominal strap. Each cat's hindquarters touched the ground so that weight support was possible. The walker was mounted on casters allowing the animal to move freely in any direction. For the treadmill assessment each cat's hind-quarters were suspended by the iliac wires from a rod above the treadmill belt. Each animal's hind limbs touched the treadmill in such a way as to allow it to support it's own weight once sufficient muscle activity was produced. Each animal's forelimbs rested on a shelf at the front of the treadmill with it's thorax restrained in a fabric jacket which was tied securely to the shelf (figure 2). Food rewards were utilized. Trials were executed usually every second day for up to three weeks after spinal transection. Both the treadmill and overground (walker) tests were executed at each pre-and postdrug trial.

The videotaped trials were rerecorded in a random order and presented to two independent observers trained in the assessment of spinal animal walking. The observers had no previous knowledge of the treatments received by each animal or each others assessments. The cats were assessed for

Figure 2. Assessment of Rhythmic Walking Movement
The two methods of rhythmic walking movement assessment. On the left, presurgery, predrug and postdrug trials were filmed with a super 8 movie camera at 36 fps. The instantaneous joint angles on the cat's left hind limb were determined in a frame by frame computer analysis of the films and compared between presurgery and postsurgery trials. On the right the pre-and postdrug trials for each drug administration were videotaped, put in a random order, and shown to two independent observers who rated the movement on a scale from 1 to 5 (see text).



INDEPENDENT OBSERVERS

overground and treadmill walking according to the following rating scale:

1. Dragging both hind limbs. No evidence of alternative movements.
2. Alternating movements only with cutaneous stimulation or stimulation of the skin at the base of the tail.
3. Some spontaneous alternating movements of hindlimbs.
4. Well developed spontaneous movement of hind limbs.
5. Normal walking. This scale is based on the progression of accquisition of walking ability after spinal transection (see table 1)

5. Kinematic Analysis

The aim of the second method of assessment of rhythmic movement was to evaluate the quality of walking in the spinal cats after a drug injection. Kinematic techniques were utilized for this examination. The execution of this analysis involved filming the stepping generated by the cats prior to surgery, prior to drug administration, and after drug administration (figure 2). The film was analysed frame by frame for 6 consecutive step cycles. The instantaneous joint angles at the hip, knee, and ankle were determined from each frame of film. The instantaneous angle data was displayed graphically and analysed to determine the pattern of progressive changes in the joint angles during the step cycle. From the joint angle patterns the occurrence of the different components of the Phillipson step cycle were determined (Goslow et al.,1973). Comparisons were made between the presurgery, predrug, and postdrug trials utilizing the kinematic information.

The following method was used to produce the film data. Prior to surgery cats were enticed to walk on the treadmill

for food rewards. Each animals forelimbs and thorax was restrained as described above so that only its' hind limbs walked on the treadmill. Filming was done at 36 frames per second utilizing a super 8 movie camera. The same filming methodology was utilized to film cats during pre-and postdrug trials. The technique utilized for joint angle analysis was as follows: The animal's left hind limb was shaved and the bony points listed below were marked with red and white ink. These points were used to demarcate the joint angles for the frame by frame film analysis.

The bony points marked were (figure 3).

1. cranial dorsal iliac spine
2. superior aspect of the ischial tuberosity
3. greater trochanter (hip pivot point)
4. the lateral fibular malleolus (ankle pivot point)
5. the lateral aspect of the head of the fifth metatarsal.

These points were chosen since they are found easily and consistently on cats and skin slippage over these areas is minimal (Goslow et al., 1973; Miller et al, 1975a).

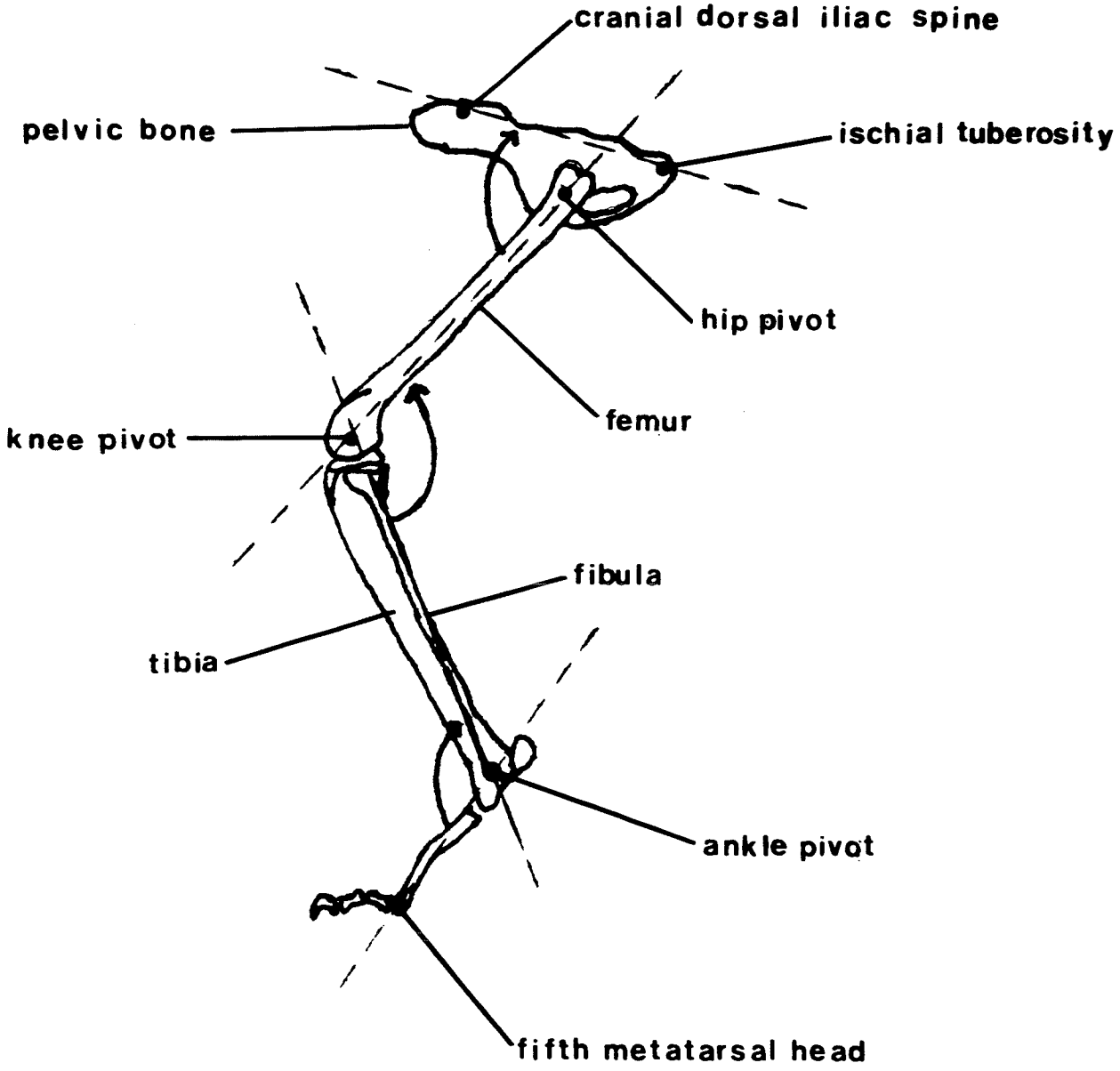
The angle measurement technique was a modification of Goslow and coworkers (1973).

It has been described previously by Wetzel and coworkers (1976).

1. Hip: This angle was determined by the intersection of two lines. One extended from the ischial tuberosity to the cranial dorsal iliac spine. The second line extended from the pivot point of the knee (midpoint between the insertion of extensor digitorum longus and the posterior fibular ligament) through the pivot point of the hip. This angle

Figure 3. Markings for Kinematic Analysis
Left hind limb skeleton redrawn from Goslow et al., (1973). The bony points marked on the skeleton were the ones marked on the cat's skin prior to filming. These points were utilized in calculations of the instantaneous joint angles (see text). The arrows denote the hip, knee, and ankle joint demarcations utilized in the kinematic analysis.

Lateral view left lower limb



actually lies above the pivot point of the hip, but accurately reflects changes in hip flexion and extension (figure 3).

2. Knee: This angle was demarcated by the intersection of the line extending through the pivot point of the hip to the pivot point of the knee, and a line from the pivot point of the ankle to the pivot point of the knee. The skin overlying the pivot point of the knee is loose and moves extensively during movement (Miller et al., 1975a). This point, therefore, was determined by triangulation from the greater trochanter and lateral malleolus. The distances between the greater trochanter and the knee pivot point (femur length) and the ankle pivot point and knee pivot point (lower leg length) were measured in each cat and utilized in a triangulation procedure (figure 3).

3. Ankle: This angle was denoted by the intersection of a line extending from the pivot point of the knee to the pivot point of the ankle with a line from the pivot point of the ankle through the long axis of the foot to the head of the fifth metatarsal (figure 3).

The film was projected frame by frame onto a HIPAD (Houston Instruments, Texas) digitizing tablet and the five bony points entered for computer analysis (4051 Tektronix computer, Tektronix, Inc., Oregon). Utilizing information on the bony positions and previously entered femur and lower leg lengths, the instantaneous angular measurements were calculated and graphs were drawn for the hip, knee and ankle.

6. Weight Bearing Assessment Procedure

Each animal's ability to bear it's hindquarter weight was assessed with the use of a force plate during every pre- and postdrug trial. The force plate assessment procedure consisted of lowering the animal by the iliac wires at a constant rate on to the platform of a scale with an analog output (force plate) (figure 4). The animal's weight was initially taken by the motor lowering it. As each cat was lowered, however, it's weight was transferred from the motor on to the force plate. The analog output from the force plate was recorded on a strip chart recorder (figure 5). The time axis of the analog record gives a measure of the distance the cat is lowered before pressure is exerted on the force plate. Each animal was started from a constant height above the force plate in every trial.

A cat that was unable to support it's hind quarter weight and had flaccid limbs would not exert pressure on the force plate until it had been lowered sufficiently for it's body to collapse on the force plate (figure 5A and B). However, an animal with hind limb extensor tonus would produce a force on the platform when lowered only a short distance - that is while still in a standing position (figure 5 C and D). The pressure on the force plate, therefore, is produced eariler in the lowering period. In figure 5C, the animal demonstrated increased extensor tonus, but was still unable to support it's hind quarter weight. In figure 5D the animal not only had increased extensor tone, but was also

Figure 4. Weight Bearing Assessment Apparatus
The cat was lowered by a constant speed motor onto a force plate. The analog output from the force plate was recorded on a strip chart recorder.

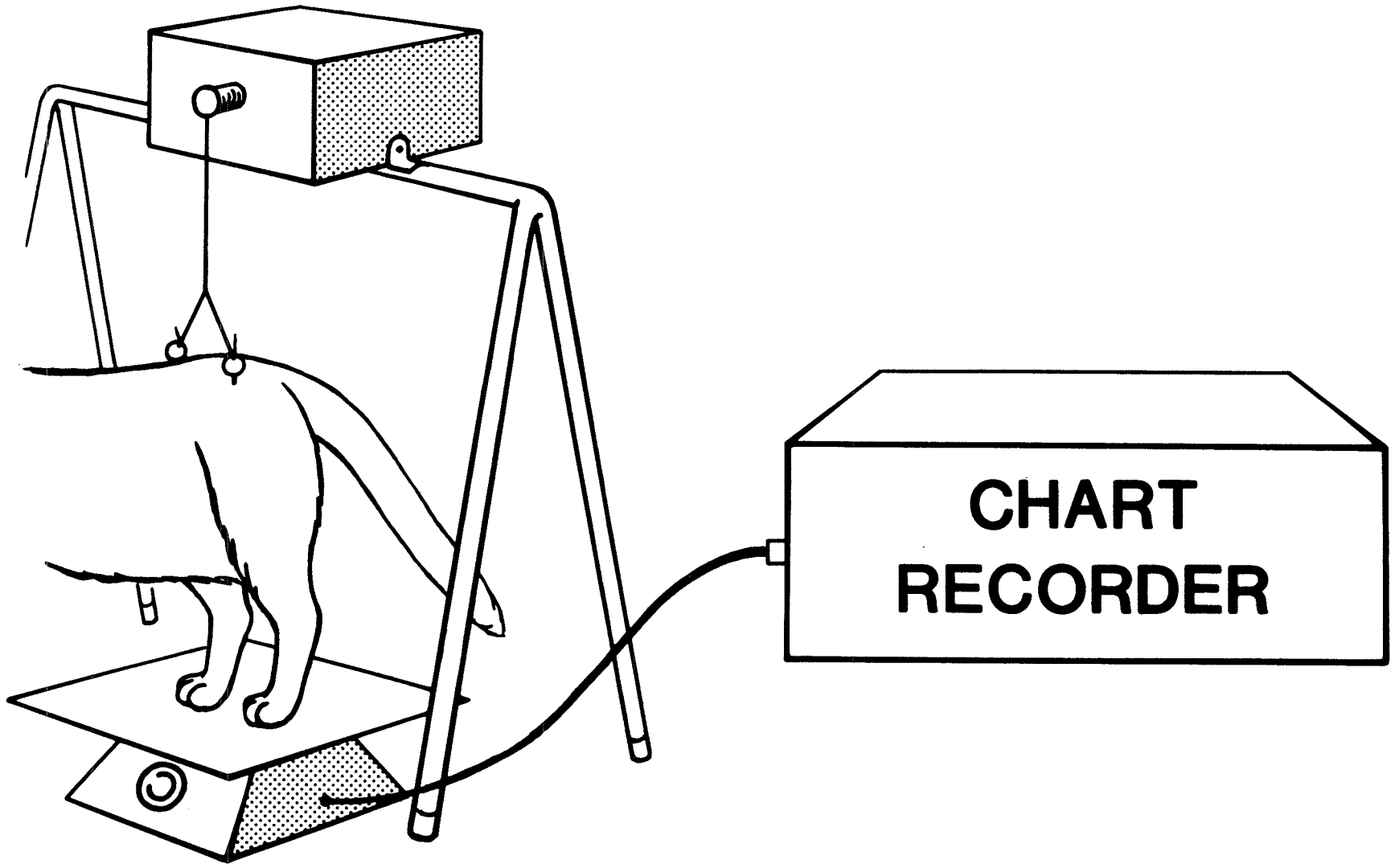


CHART
RECORDER

able to stand for 4 seconds. As an animal gains extensor tonus and the ability to bear weight there is a decrease in the time period for the cat to transfer it's weight on to the force plate. In addition, there is an increase in the amount of force produced by the animal at these earlier time periods. These two events are translated into an increase in the area encompassed by the analog weight bearing curves.

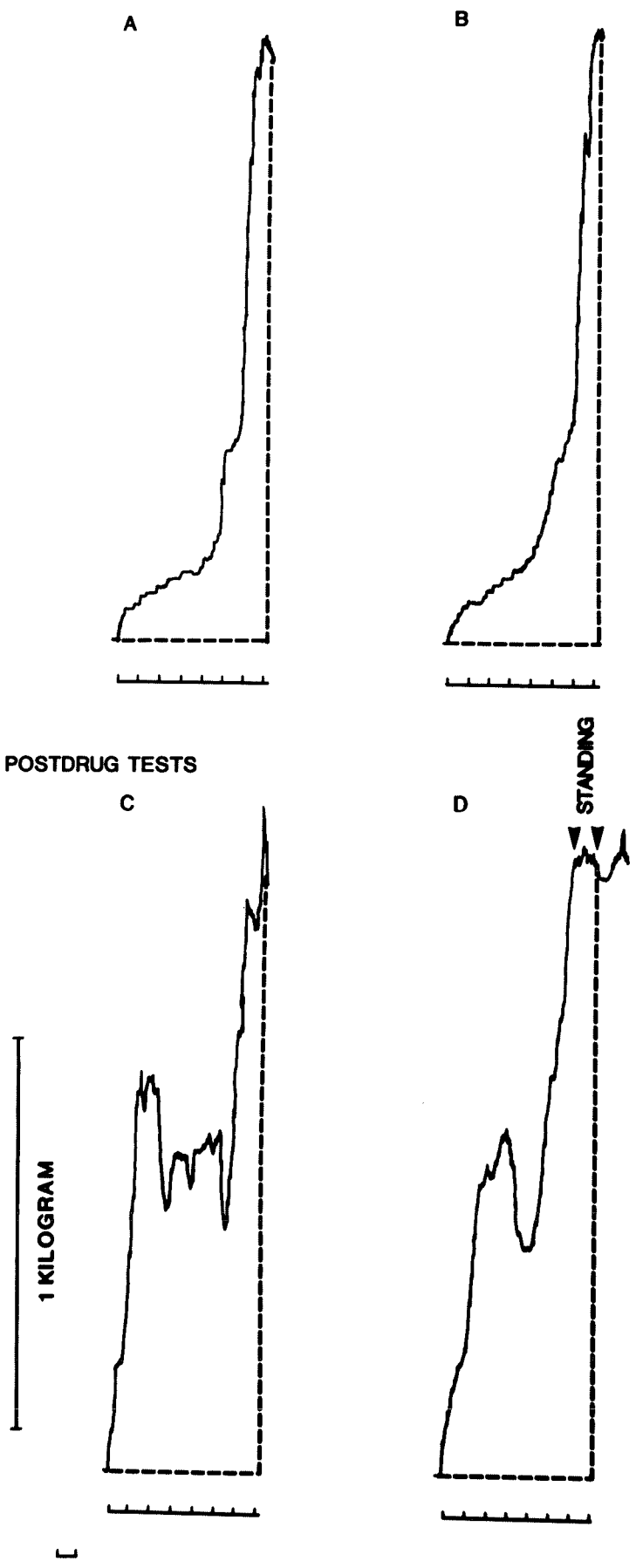
To assess changes in weight bearing the area underneath the curve was found by integrating the weight bearing analog record. The closed curve outline of each analog record was entered utilizing the HIPAD digitizing tablet and the area within the curve was calculated by a Hewlett-Packard 9836 computer (Hewlett-Packard, Colorado). The calculated area within the curve was termed the weight bearing index (WBI).

For records such as 5D where the animal was able to stand, the period of time for the animal to reach the force plate was increased. To standardize abscissa length only one time interval, the time period for the cat to reach the platform when it was unable to stand, was utilized when delimiting the closed curve which was subsequently entered into the computer.

Each animal was tested twice on the force plate for most pre-and postdrug trials. The two tests from each trial were each entered twice making a total of four determinations which were used to calculate the average WBI for each pre-or postdrug trial. The difference between the average WBI's of the pre-and postdrug trials was determined by subtracting the predrug WBI from the postdrug WBI. This difference was then

Figure 5. Weight Bearing Analog Records
Four analog recordings from one drug administration, A and B being the two predrug tests and C and D being the two postdrug tests. The dotted lines demarcate the left and lower boundaries of the closed curves utilized in the calculation of area. A common abscissa length was used (see text). Each curve was entered twice giving four determinations that were utilized to calculate the average predrug area of postdrug area. Units of the area are in kilogram seconds (kg sec). The areas encompassed by each curve are termed the weight bearing index (WBI). The average predrug WBI was subtracted from the average postdrug WBI and the difference was tested for significance with a paired t-test (see text).

WEIGHT BEARING ANALOG RECORDS



tested for significance using a paired t-test. An example of the calculations is illustrated in figure 5. The two determinations calculated figure 5A (predrug test 1) were 13.4 kg sec. and 13.5 kg sec. These two determinations plus those of the second predrug test illustrated in figure 5B (predrug test 2) (11.4 kg sec and 11.6 kg sec) were were utilized in the calculation of the average predrug WBI (WBI = 12.5 kg sec SD 1.1 kg sec). The two postdrug tests are shown in figure 5 C and D. The two determinations for figure 5C (26.2 kg sec and 25.9 kg sec) and 5D (24.3 kg sec and 24.1 kg sec) were utilized in the calculation of the postdrug WBI (WBI = 25.1 kg sec SD 1.1 kg sec). The pre-and postdrug trial difference was found to be significant on a paired t-test.

Results

I. Analysis of Rhythmic Movement with Rating by Independent Observers

The efficacy of a particular drug to modify the rhythmic movement status of an animal was assessed by analyzing the ratings given during each drug administration. Each drug administration consisted of a predrug and a postdrug trial. Any changes in rhythmic movement status were obtained by evaluating the difference between the pre-and postdrug ratings.

Only the drug administrations that fulfilled the following three criteria were utilized in the analysis. The criteria were: 1) both pre-and postdrug trials were videotaped. 2) the pre-and postdrug trials were rated by both observers. 3) any differences in ratings between observers for a particular pre-or postdrug trial were no more than one rating unit. Of the 137 drug administrations evaluated on the treadmill 135 (98.5%) fulfilled the criteria. For the 137 drug administrations evaluated in the overground paradigm 129 (94.2%) fulfilled these criteria.

Agreement between observers on the ratings of 270 pre-and postdrug trials obtained from the 135 drug administrations tested on the treadmill also was evaluated. The observers' ratings agreed in 89.3% of the trials. No significant difference was found in a comparison between the two observers utilizing a sign test (Sokol and Rohlf,1969)

Table 2. Agreement on Ratings Between Observers

	Treadmill (270 pre-and postdrug trials)		Overground (258 pre-and postdrug trials	
	number of trials	percentage of total trials	number of trials	percentage of total trials
Observers agree	241	89.3%	234	90.7%
Observers Disagree	29	10.7%	24	9.3%
P	not significant		not significant	

Table 3. Agreement on Changes in Rhythmic Movement Status Between Observers

	Treadmill (135 drug administrations)		Overground (129 drug administrations)	
	number of drug administrations	percentage of total drug administrations	number of drug administrations	percentage of total drug administrations
observers agree	116	85.9%	107	82.9%
observers disagree	19	14.1%	22	17.1%
P	not significant		not significant	

(Table 2). In addition the observers agreed on changes in rating in 85.9% of the 135 drug administrations. Again no significant difference was found between the two observers (sign test; Sokal and Rohlf, 1969) (table 3).

For the overground paradigm the observers' ratings agreed in 90.7% of the 258 pre-and post drug trials obtained from 129 drug administrations. Utilizing a sign test, no significant difference was found between the two observers (Sokol and Rohlf, 1969) (table 2). In addition the observers agreed on the occurrence of a change in the rating in 82.9% of the 129 drug administrations analysed. Again no significant difference was observed between the two observers (Sokal and Rohlf, 1969) (table 3).

The ratings given by the two observers were considered to be similar on the basis of the above comparisons (table 2 and 3). For this reason the ratings from two observers for each trial ratings were used to calculate an average rating for the trial. These averaged ratings were utilized as the predrug or postdrug trial scores in the subsequent analysis. Treadmill and overground tests were analysed separately.

Figures 6 (treadmill) and 8 (overground) illustrate the averaged pre-and postdrug ratings for control, NE 0.1mM, NE 1.0mM and NE 10.0mM drug groups. The CSF control and sham-operated control (no drug) groups were found not to be significantly different utilizing a one-way analysis of variance (tables 8 and 9) and multiple t-tests. For this reason the two control groups have been combined into one

Figure 6. Average Predrug and Postdrug Ratings (Treadmill) The averaged pre-and postdrug treadmill ratings for control, NE 0.1mM, NE 1.0mM and NE 10.1mM drug administrations. (0) predrug rating, (X) postdrug rating, (↓) postdrug ratings decreased, (=) postdrug ratings unchanged, (↑) postdrug ratings increase.

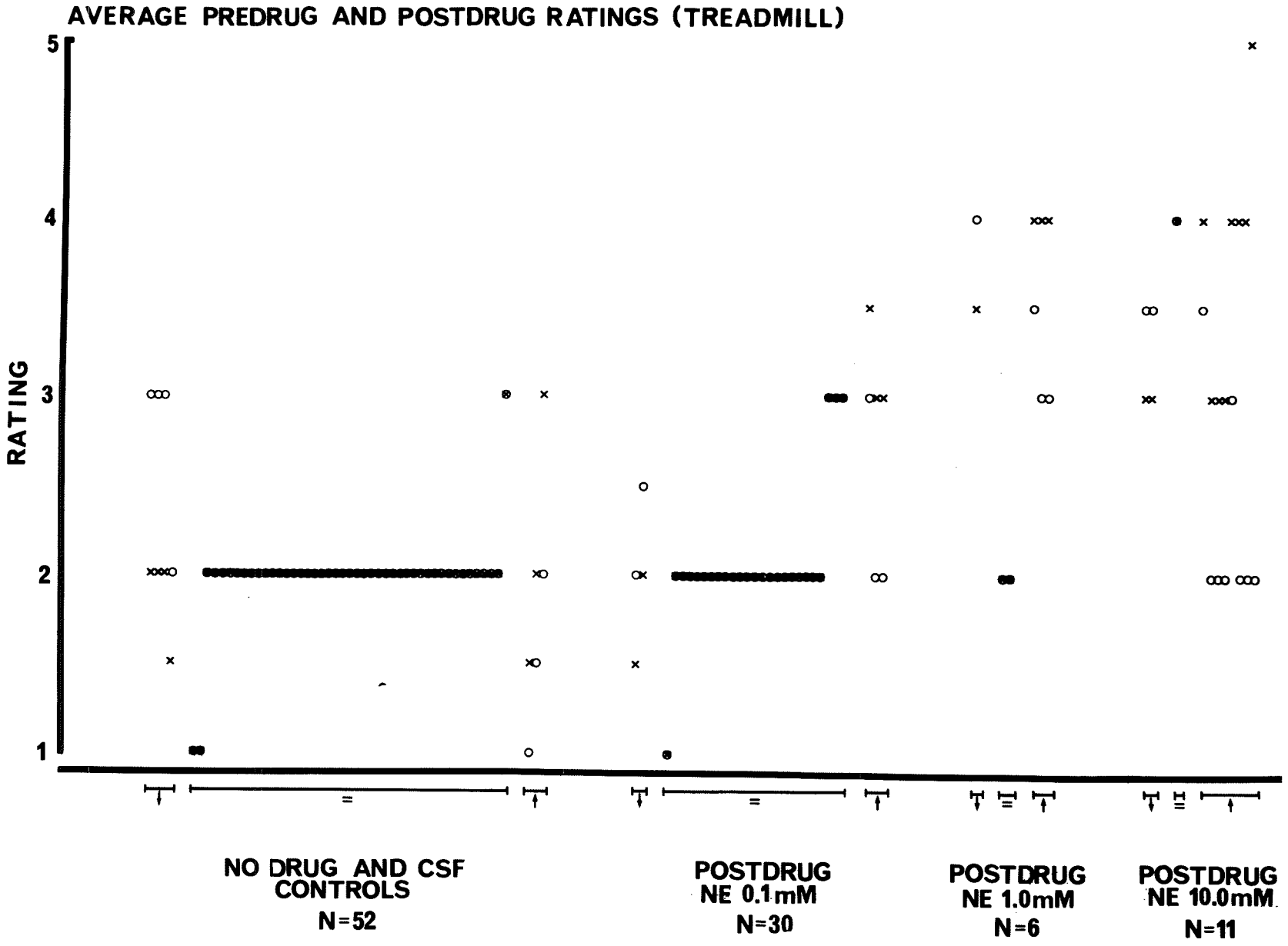

AVERAGE PREDRUG AND POSTDRUG RATINGS (TREADMILL)
RATING
5
4
3
2
1
NO DRUG AND CSF CONTROLS
N=52
POSTDRUG NE 0.1mM
N=30
POSTDRUG NE 1.0mM
N=6
POSTDRUG NE 10.0mM
N=11

Figure 7. Average Difference Between Predrug and Postdrug Ratings (Treadmill)
The averaged differences between pre-and postdrug ratings (postdrug rating - predrug rating) for treadmill results presented from a common baseline. (△) difference between ratings, (↓) differences negative, (=) no difference, (↑) differences positive.

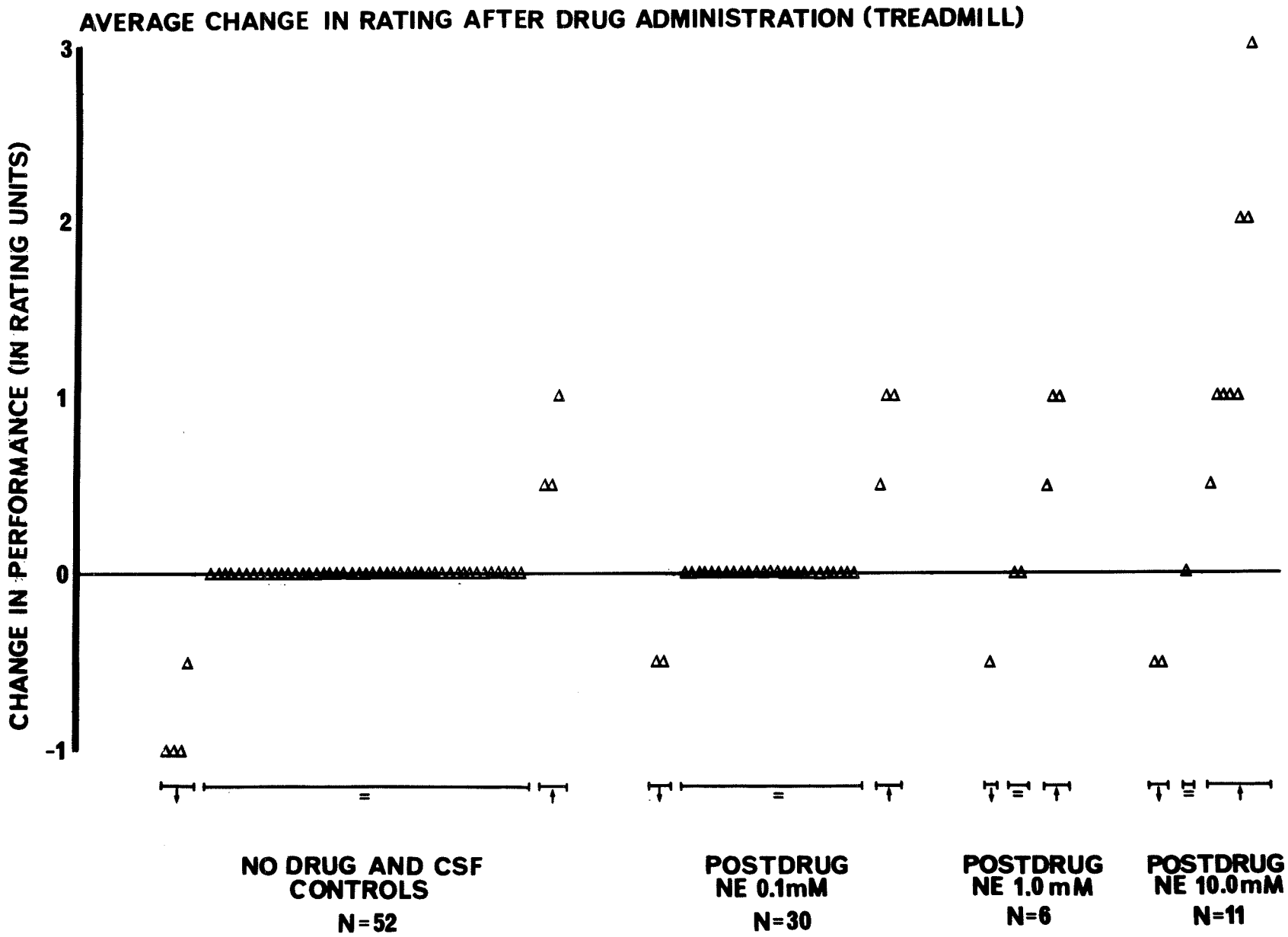

AVERAGE CHANGE IN RATING AFTER DRUG ADMINISTRATION (TREADMILL)
CHANGE IN PERFORMANCE (IN RATING UNITS)
3
2
1
0
-1
NO DRUG AND CSF CONTROLS
N=52
POSTDRUG NE 0.1mM
N=30
POSTDRUG NE 1.0 mM
N=6
POSTDRUG NE 10.0mM
N=11

Figure 8. Average Predrug and Postdrug Ratings (Overground) The averaged pre-and postdrug overground ratings for control, NE 0.1mM, NE 1.0mM and NE 10.0mM drug administrations. (O) predrug rating, (X) postdrug rating, (↓) postdrug ratings decreased, (=) postdrug ratings unchanged, (↑) postdrug ratings increased.

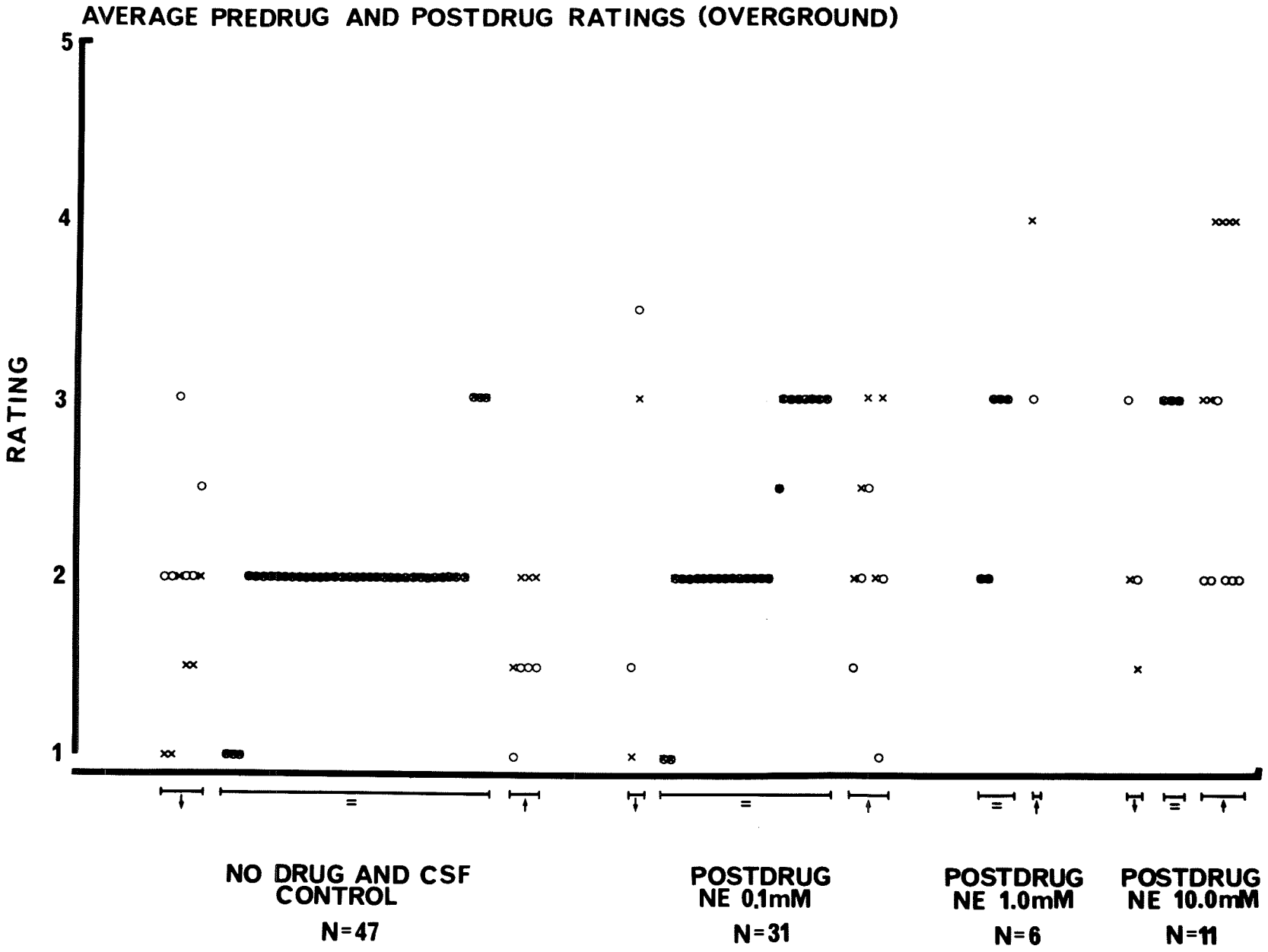
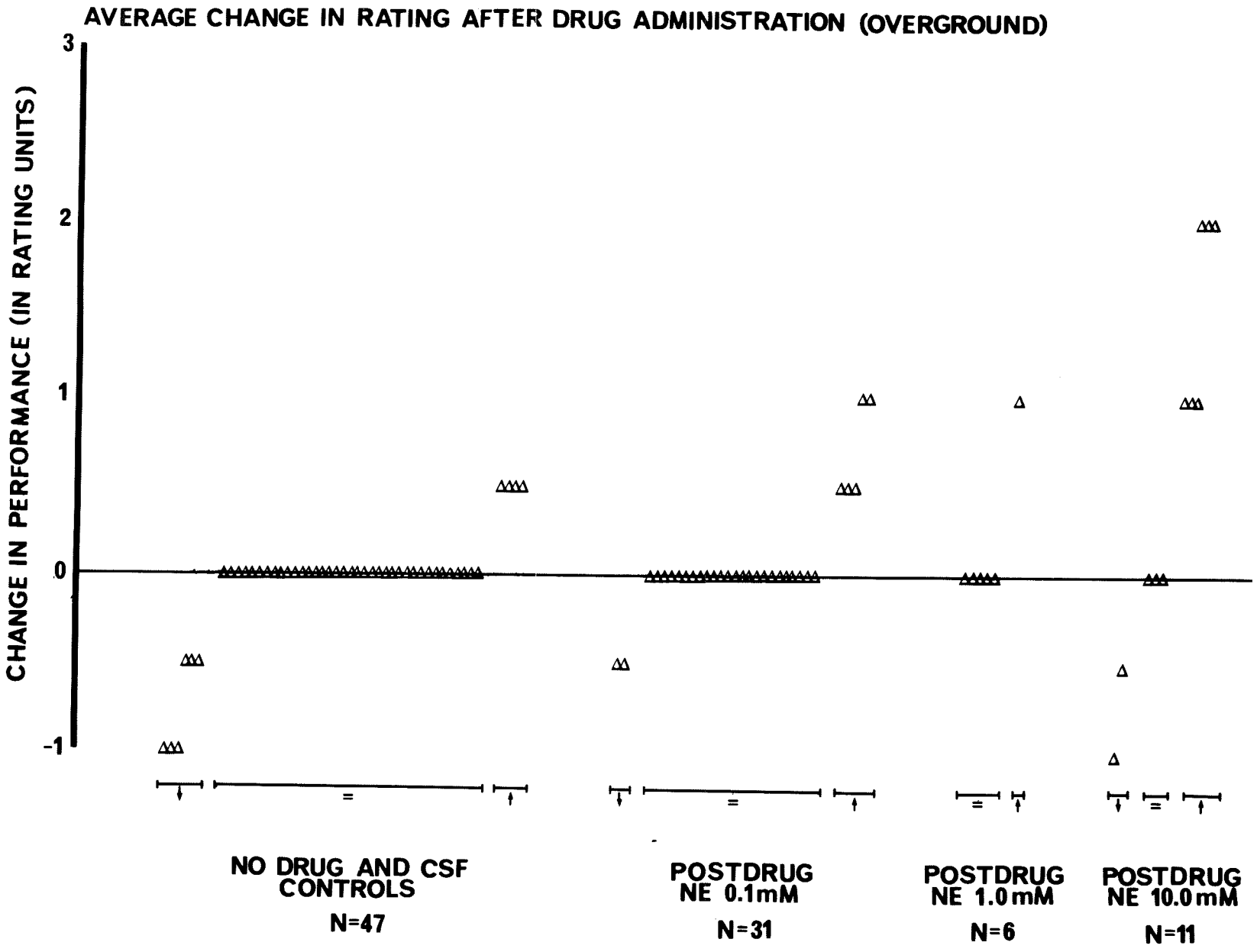

AVERAGE PREDRUG AND POSTDRUG RATINGS (OVERGROUND)
RATING
5
4
3
2
1
NO DRUG AND CSF CONTROL
N=47
POSTDRUG NE 0.1mM
N=31
POSTDRUG NE 1.0mM
N=6
POSTDRUG NE 10.0mM
N=11

Figure 9. Average Difference Between Predrug and Postdrug Ratings (Overground)
The averaged differences between pre-and postdrug ratings (postdrug rating-predrug rating) for overground results presented from a common baseline. (Δ) difference between ratings, ($\downarrow$) differences negative, (=) no difference, ($\uparrow$) differences positive.


AVERAGE CHANGE IN RATING AFTER DRUG ADMINISTRATION (OVERGROUND)
CHANGE IN PERFORMANCE (IN RATING UNITS)
3
2
1
0
-1
NO DRUG AND CSF CONTROLS
N=47
POSTDRUG NE 0.1mM
N=31
POSTDRUG NE 1.0mM
N=6
POSTDRUG NE 10.0mM
N=11

control group.

All four groups produced postdrug trials where ratings decreased, remained unchanged or increased from the predrug ratings were observed. Figures 7 (treadmill) and 9 (overground) showing the postdrug changes from a common baseline emphasize the differences. The majority of control (86.5% for treadmill and 78.7% for overground) and NE 0.1mM drug administrations (83.4% for treadmill and 77.5% for overground) did not produce a change in postdrug ratings (see tables 6 and 7). The NE 0.1mM group was found to be similar to the control groups utilizing a one-way analysis of variance (tables 8 and 9) and multiple t-tests.

Norepinephrine at 1.0mM and 10.0mM concentrations tended to produce an increase in the postdrug ratings (see tables 6 and 7). This effect was greatest with the NE 10.0mM concentration where postdrug rating increases were observed in 72.7% of treadmill and 54.5% of overground tested drug administrations. Also with this higher dose a cat could increase its performance by as much as two or three rating units. Increases observed after administration of lower concentrations of NE (0.1mM and 1.0mM) and in control animals were never greater than one rating unit. One rating unit was also the accepted error of measurement for the observers.

Tables 4 (treadmill) and 5 (overground) list the number of drug administrations where the animal's postdrug ratings decreased, remained unchanged or increased. A Kendall's tau (τ) coefficient was calculated for the treadmill and overground results (Sokol and Rohlf, 1969).

Table 4. Effect of Drug Concentration of Postdrug Performance (Treadmill)

Drug Concentration	Number of Drug Administrations	Postdrug ability decreased	Postdrug ability unchanged	Postdrug ability increased
combined controls	52	4	45	3
NE 0.1mM	30	2	25	3
NE 1.0mM	6	1	2	3
NE 10.0mM	11	2	1	8

N=99 τ =0.280 $P < 0.01$

Table 5. Effect of Drug Concentration on Postdrug Performance (Overground)

Drug Concentration	Number of Drug Administrations	Postdrug ability decreased	Postdrug ability unchanged	Postdrug ability increased
combined controls	47	6	37	4
NE 0.1mM	31	2	24	5
NE 1.0mM	6	0	5	1
NE 10.0mM	11	2	3	6

N=95 τ =0.225 $P < 0.05$

The tau statistic measures association between the two variables examined these being drug concentration and improvement in walking ability after the drug was given. The tau statistics calculated from tables 4 (τ =.280) and 5 (τ =.225) were greater than zero indicating a positive association between the two variables. When the statistic was tested against the null hypothesis that no association exists between drug concentration and improvement of walking ability it was significant at α=0.01 for the teadmill and α=0.05 for the overground results (Sokol and Rohlf, 1969).

Some preliminary studies utilizing DA (0.1mM, 1.0mM and 10.0mM) and 5-HT (10.0mM) were performed in addition. Tables 6 (treadmill) and 7 (overground) illustrate the results. Dopamine and serotonin were ineffective in producing consistent changes in the walking abilities of the cats treated. The changes in ratings that were produced were never more than one rating unit.

The proportion of drug administrations in which changes were produced utilizing the low doses of DA (0.1mM and 1.0mM) were tested against the proportion of drug administrations in which changes were produced in the CSF control, sham-operated control no drug, and NE 0.1mM drug groups. A one-way analysis of variance was utilized (tables 8 and 9). This analysis was weighted since the number of tests per animal varied. To stabilize the variance of the proportional data the Freeman-Tukey transformation was utilized (Mosteller and Youtz,1961)

Table 6 Percentage of Drug Administrations Where Postdrug Movement Changes Occurred (Treadmill)

Treatment Group	Number of Drug Administrations	Ability Decreased	Ability Unchanged	Ability Increased	Number of cats
combined controls	52	7.7%	86.5%	5.8%	9
NE 0.1mM	30	6.6%	83.4%	10.0%	9
NE 1.0mM	6	16.7%	33.3%	50.0%	6
NE 10.0mM	11	18.2%	9.1%	72.7%	10
DA 0.1 and 1.0mM	18	11.1%	72.2%	16.7%	3
DA 10.0mM	3	0	66.7%	33.4%	3
5-HT 10.1mM	5	20.0%	60.0%	20.0%	5

Table 7. Percentage of Drug Administrations Where Postdrug Movement Changes Occurred (Overground)

Treatment Group	Number of Drug Administrations	Ability Decreased	Ability Unchanged	Ability Increased	Number of cats
combined controls	47	12.8%	78.7%	8.5%	9
NE 1.0mM	31	6.4%	77.5%	16.1%	9
NE 1.0mM	6	0	83.3%	16.7%	6
NE 10.0mM	11	18.2%	27.3%	54.5%	10
DA 0.1 and 1.0mM	16	12.5%	62.5%	25.0%	3
DA 10.0mM	3	33.3%	33.3%	33.3%	3
5-HT 10.0mM	5	20.0%	60.0%	20.0%	5

Table 8. Comparison of Postdrug Effects Between Control Groups and Low Drug Concentration Groups (Treadmill)

Drug	Cat	Number of Drug Administrations	Proportion of Drug Administrations Where Postdrug Performance Improved	Proportion of Drug Administrations Where Postdrug Performance Worsened
Elliot's Solution A (CSF)	CID-33	9	0.11	0
	CID-35	9	0	0
	CID-36	10	0	0
	CID-45	2	0	0
	CID-53	6	0	0.5
No drug (sham-operated controls)	CID-28	4	0.25	0
	CID-29	8	0.125	0
	CID-41	2	0	0
NE 0.1mM	CID-38	8	0.125	0.125
	CID-39	8	0	0
	CID-40	7	0	0.14
	CID-44	2	0	0
DA 0.1and 1.0mM	CID-42	9	0.11	0.22
	CID-46	8	0.25	0
	CID-43	1	0	0
		P	0.05	not significant

Table 9. Comparison of Postdrug Effects Between Control Groups and Low Drug Concentration Groups (Overground)

Drug	Cat	Number of Drug Administrations	Proportion of Drug Administrations where postdrug performance improved	Proportion of Drug Administrations where postdrug performance worsened
Elliot's SolutionA (CSF)	CID-33	7	0.14	0.14
	CID-35	8	0	0
	CID-36	10	0	0.3
	CID-45	2	0.5	0
	CID-53	6	0	0.17
No drug (sham-operated controls)	CID-28	3	0.33	0
	CID-29	7	0	0.14
	CID-41	2	0	0
NE 0.1mM	CID-38	9	0.22	0.11
	CID-39	8	0	0
	CID-40	7	0.28	0.14
	CID-44	2	0	0
DA 0.1 and 1.0mM	CID-42	7	0.28	0
	CID-46	8	0.25	0.125
	CID-43	1	0	1.0
		P	not significant	not significant

The analysis of variance for the treadmill results (table 8) demonstrated a significant difference between the treatment groups when the comparison was made of the proportion of tests where cats showed improvement. Multiple t-tests which were employed to compare the proportion of drug administrations that showed improvement between treatment groups demonstrated differences between the following groups ($\alpha = 0.05$): a) Elliot's Solution A (CSF) and sham-operated groups b) NE and DA groups c) Elliot's Solution A and DA groups. However, since simultaneous t-tests were utilized, an adjustment had to be made such that the overall α-error protection is equal to $\alpha = 0.05$. To achieve this the Bonferroni technique was used (Miller, 1981). When this was done none of the t-tests produced a significant result.

The analysis of variance of the overground results did not produce any significant differences between treatment groups.

II Kinematic Analysis of Noradrenergic-Induced Stepping

Trials from five cats were filmed and analysed in detail to obtain information on the characteristics of the gaits utilized by the cats (see table 10). After transection stepping was induced in three of the cats with NE 10.0mM (C10-26,38, and 40); in one cat stepping was induced with NE 0.1mM (CID-7); in the fifth cat stepping was evoked with NE 0.1mM and NE 10.0mM in two separate trials (CID-42). All five cats produced continuous alternating movement at a 4 rating level. However, in the four cats treated with NE

Table 10. Trials Utilized in the Kinematic Analysis

cat	presurgery control	predrug control	postdrug NE 0.1mM	postdrug NE 10.0mM
CID-7	trial:1	trial:10	trial:11	*
CID-26	trial:1	trial:4	*	trial:5
CID-38	trial:1	*	*	trial:23
CID-40	trial:1	trial:26	*	trial:27
CID-42	trial:1	*	trial:24	trial:28

* - no trial available

10.0mM (CID-26,38,40 and 42) stepping occured even when the treadmill was at a standstill. In the two cats in which stepping was induced with NE 0.1mM (CID-7 and 42), continuous stepping was only observed while the treadmill ran. No other stimulation was necessary to evoke the locomotion, however.

A scissors-type gait often was produced by the NE-treated animals. This meant that a cat would adduct a hind limb during the swing phase (swing the limb towards the midline) causing the swinging limb to knock or trip over the contralateral hind limb during its stance phase. When filming, a piece of plexiglass was held between the two hind limbs to prevent interference during stepping.

The trials analysed from each cat are shown in Table 10. A presurgery and a posttransection-postdrug trial was obtained from each of the 5 cats. In additon, stepping induced prior to drug administration with tail stimulation (predrug control trials) was filmed in three cats (CID-7,26,40). Six typical consecutive step cycles were chosen from each filmed trial except in CID-42 trial: 1 where only 5 step cycles had been filmed. All trials were executed on a treadmill moving at a speed of 0.4 to 0.6 m/sec.

1. Presence of Step Cycle Components

The joint angles that occurred at the hip,knee and ankle during the step cycle were obtained from a frame by frame analysis of each of the six step cycles in every trial (see methods). Six joint angle curves, one from each step cycle,

were obtained for each joint. These six joint angle curves were overlaid to obtain average hip, knee and ankle joint curves for each trial (see figure 10). These average curves were examined to ascertain the presence of the step cycle components F,E1,E2,and E3.

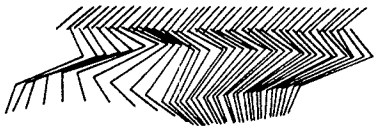
The four step cycle components were present in the presurgery control trials of each of the 5 cats. The hip joint angles in the E1 component displayed a pattern similar to intact cats walking overground (Goslow et al, 1973) where hip flexion ended fairly soon after E1 began. The hip remained in a stable position throughout the rest of E1 close to the same joint angle reached at the end of hip flexion (see figure 10B).

The stepping observed in the three cats (CID-7,26 and 40) filmed prior to drug administration was poor. In each case the stepping was induced only with tail stimulation. In addition, in none of the predrug control trials were the cats capable of supporting their own weight with their hind limbs. In these trials the animals were dependent on the sacral ring suspension system to maintain an upright position. The F and E3 components were present in all three predrug control trials. The E1 component if present at all was brief and occurred immediately prior to the stance phase (figure 10 E to H). The E2 or yield component was not observed in any of the animals. This component, however, is dependent on weight transfer for its occurrence (Grillner, 1975). It was possible that the sacral suspension necessary to keep the animals upright hindered the transfer of weight which was

Figure 10. Overlays of Step Cycles
Overlays of the step cycles from CID-40. Trial:1 presurgery control, Trial: 26 predrug control, Trial: 27 postdrug NE 10.0mM. The stick figures found above each graph represent one of the six step cycles entered. This step cycle is typical of the six that were entered. The stick figures represent the movement of the left hind limb of the cat as one watches from the right side of the cat.

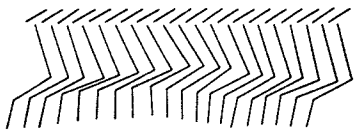
A

CID 40 TRIAL 1 PRESURGERY CONTROL



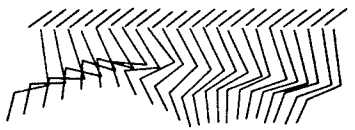
E

CID 40 TRIAL 26 PREDRUG CONTROL

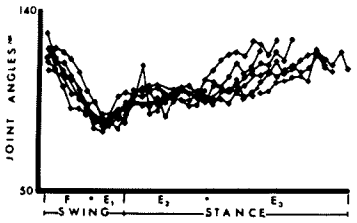


I

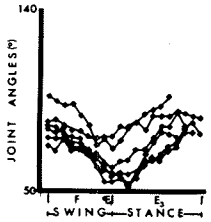
CID 40 TRIAL 27 POSTDRUG (NE 10 mM)



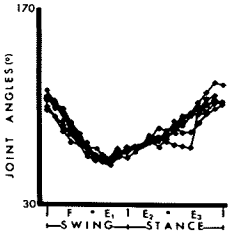
B CID 40 TRIAL:1 HIP JOINT ANGLES



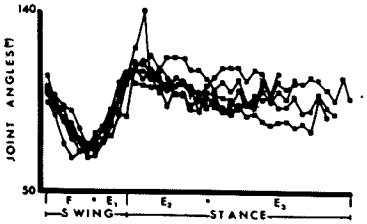
F CID 40 TRIAL:26 HIP JOINT ANGLES



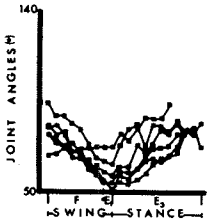
J CID 40 TRIAL:27 HIP JOINT ANGLES



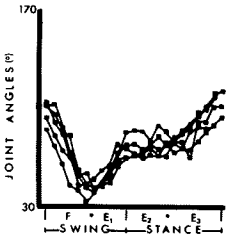
C CID 40 TRIAL:1 KNEE JOINT ANGLES



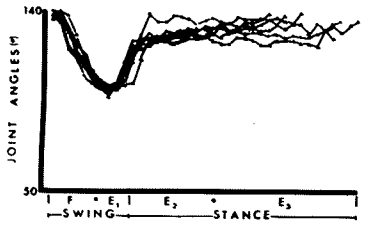
G CID 40 TRIAL:26 KNEE JOINT ANGLES



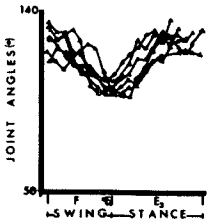
K CID 40 TRIAL:27 KNEE JOINT ANGLES



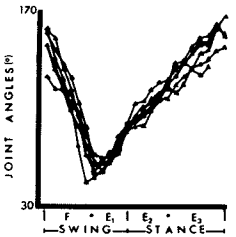
D CID 40 TRIAL:1 ANKLE JOINT ANGLES



H CID 40 TRIAL:26 ANKLE JOINT ANGLES



L CID 40 TRIAL:27 ANKLE JOINT ANGLES



requisite in bringing about the yield component.

The two cats (CID-7,42) that walked after administration of NE 0.1mM also produced stepping in which the F and E3 components were conserved. The E1 component was present but poor; that is extension at the knee and ankle was not pronounced and hip extension occurred just prior to the beginning of the stance phase. The E1 or yield component was present in one cat (CID-42) but not in the other (CID-7). Both animals were capable of supporting the weight of their hindquarters with their hind limbs however. In the E2 component that was produced during CID-42's steps the yield was observed at the knee, but not at the ankle.

The animals in the final group received NE 10.0mM (CID-26,38,40,42). All of these animals produced steps in which all 4 step cycle components were present. The components differed in some characteristics from those seen in the presurgery controls trials, however. For example, pronounced hip extension was observed during the E1 component in 3 of the cats (CID-26,38,42). As much as 10 to 30 of hip extension was produced by these animals. The hip extension during E1 in the fourth cat (CID-40) was not as pronounced, being only 5 to 6 (figure 10J). Another component that illustrated differences from the presurgery controls was E2 (yield component). In two of the cats, CID-40 and CID-26, slight flexion was observed at the knee but the ankle was held quite rigid (figure 10 K,L). The other two animals (CID-38 and 42) produced a yield component

in which slight flexion was noted at both knee and ankle. All four animals appeared to walk without extra support, utilizing the ring suspension system to compensate for a lack of balance.

As noted earlier the three articulations, the hip, knee and ankle begin extension during the swing phase. For intact cats walking on a treadmill investigators have noted the order of joint extension to be knee first followed closely by the ankle and then the hip (Wetzel et al., 1975). In addition all three joints are seen to begin extension within short time intervals of each other during the swing (Goslow et al., 1973). The 5 presurgery control animals all displayed a similar pattern to the one described as above by Wetzel and coworkers (1975). The knee extended first followed by the ankle and then the hip. All three articulations began extension within a 74 msec (SEM±5.4 msec) time interval. This orderly appearance of joint extension was altered in the predrug control and the postdrug NE 0.1mM and NE 10.0mM groups. Neither the knee or ankle predominated in leading the extension. In fact, occasionally the hip would extend first or all three joints would begin extension simultaneously. The three joints began to extend within time intervals of 48.3 m sec (SEM±18.3 msec) for the predrug control group, 61.7 m sec (SEM±6.9 msec) and 67.2 m sec (SEM± 10.3 msec) for the post drug NE 0.1mM and NE 10.0mM groups respectively. No significant differences were found in the size of the time interval between the 4 groups (presurgery, predrug and postdrug NE 0.1mM and NE 10.0mM) when multiple

t-tests with the Bonferroni techique for simultaneous statistical inference (Miller,1981) were used.

2. Articular Range of Movement

The average range of movement or joint displacement during a step cycle was calculated for each trial. The displacements obtained from the postdrug trials were compared with their corresponding presurgery trials.

Figure 11 presents the articular displacements from presurgery and corresponding postdrug NE 0.1mM trials of cats CID-7 and CID-42. After NE 0.1mM the cats were capable of producing steps with joint displacements that were not significantly different (paired t-test $\alpha=0.05$) from the presurgery control trials at most joints.

In figure 12 the joint displacements from trials where cats received NE 10.0mM (CID-26,38,40,42) were compared with the corresponding presurgery controls. The joint displacements generated after NE 10.0mM were either not significantly different or greater (paired t-test$\alpha=0.05$) than those seen in the presurgery control trials. In none of the NE 10.0mM trials did the cats produce joint displacements that were less than the displacements produced by the presurgery controls.

The displacements calculated from the three predrug control trials (CID-7,26,40) were compared with the displacements produced after drug administration (figure 13). The cat that received NE 0.1mM (CID-7) produced steps with significantly larger hip joint range ($p<0.01$) but the

Figure 11. Range of Movement of Presurgery Controls and Postdrug NE 0.1mM Cats
Histogram comparing the ranges of movement from presurgery control trials and corresponding post-transection postdrug NE 0.1mM trials. Standard deviations calculated from 6 step cycles are marked.

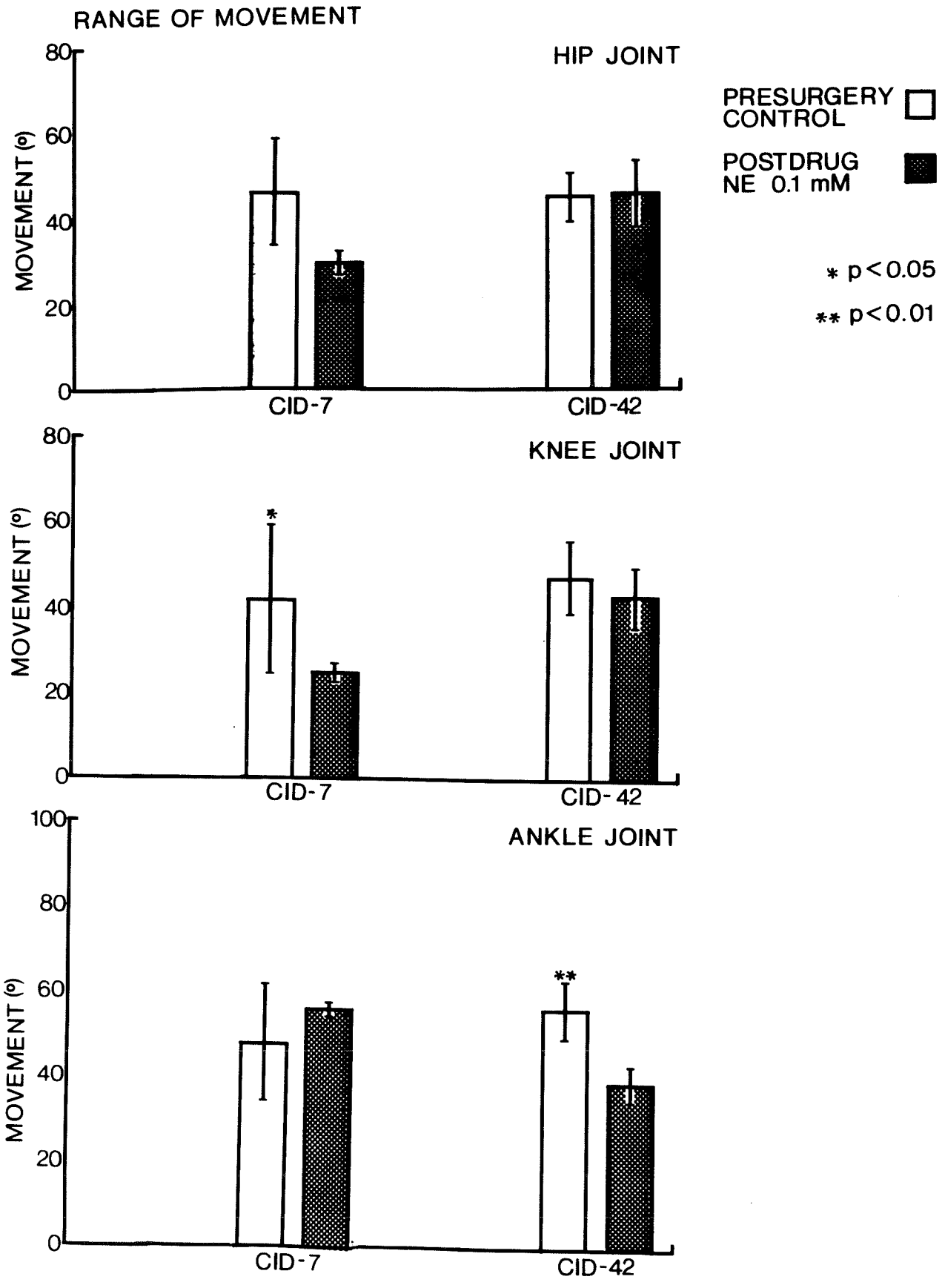

RANGE OF MOVEMENT
HIP JOINT
KNEE JOINT
ANKLE JOINT
MOVEMENT (°)
80
60
40
20
0
100
CID-7
CID-42
PRESURGERY CONTROL
POSTDRUG NE 0.1 mM
* p<0.05
** p<0.01
*
**

Figure 12. Range of Movement of Presurgery Control and postdrug NE 10.0mM Cats
Histogram comparing the ranges of movement from presurgery control trials and corresponding post-transection postdrug NE 10.0mM trials. Standard deviations calculated from 6 step cycles are marked (CID-42 presurgery control trial only 5 step cycles were used).

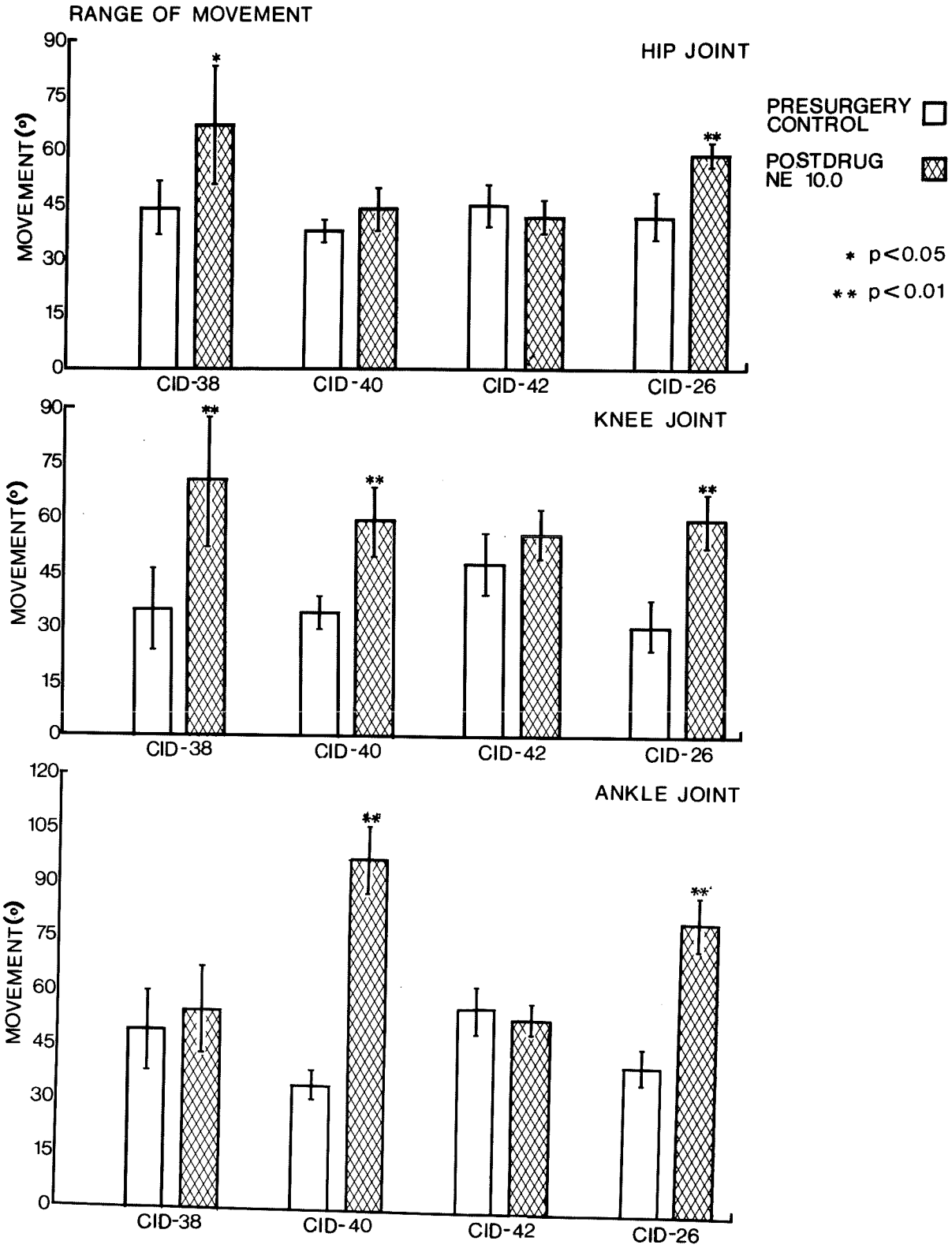
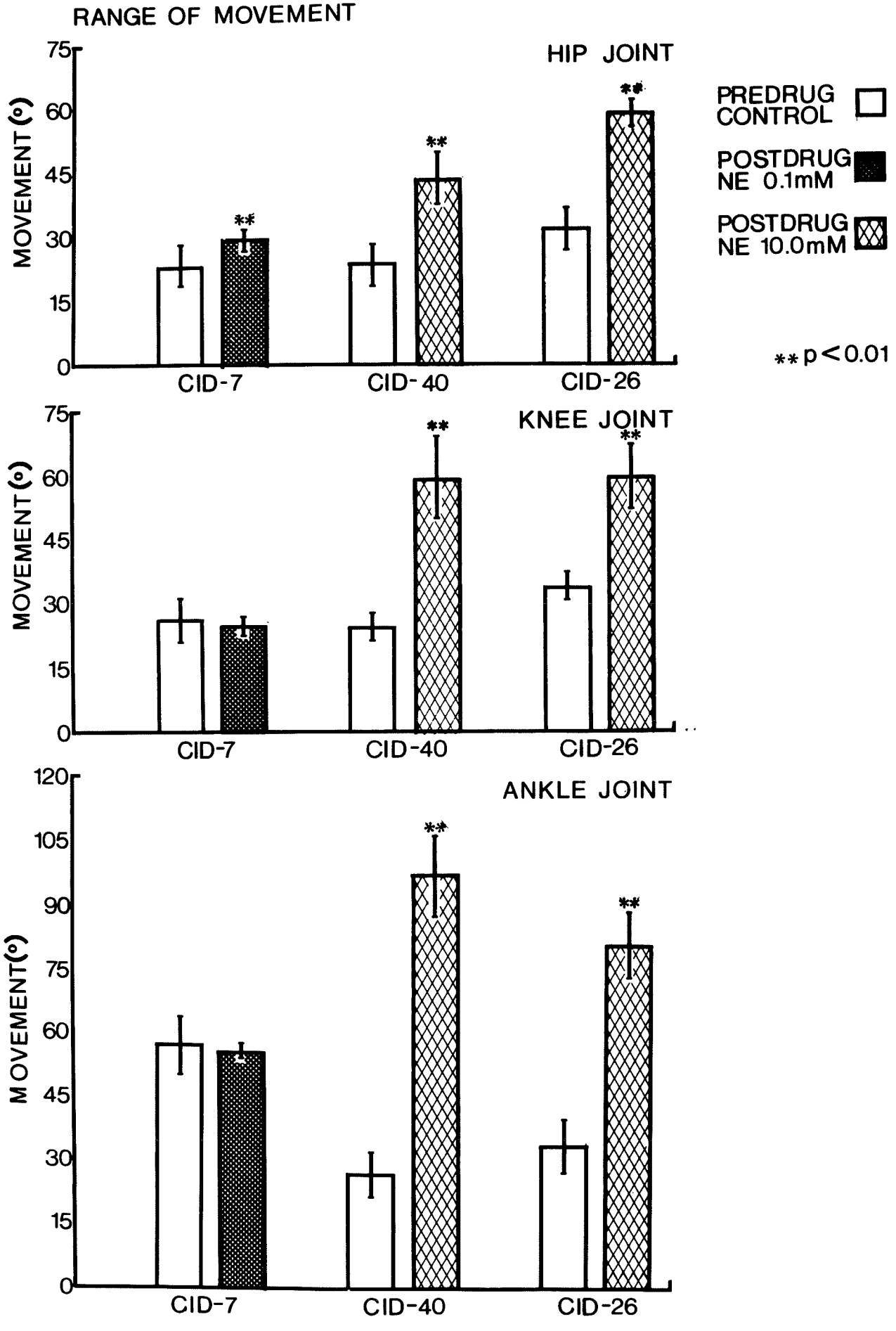

RANGE OF MOVEMENT
HIP JOINT
MOVEMENT(°)
90
75
60
45
30
15
0
CID-38
CID-40
CID-42
CID-26
PRESURGERY CONTROL
POSTDRUG NE 10.0
* p<0.05
** p<0.01
KNEE JOINT
ANKLE JOINT
120
105

Figure 13. Range of Movement of Predrug Control and Postdrug Cats
Histogram comparing ranges of movement from post-transection pre-and postdrug trials. Standard deviations calculated from 6 step cycles are marked.


RANGE OF MOVEMENT
HIP JOINT
KNEE JOINT
ANKLE JOINT
MOVEMENT (°)
75
60
45
30
15
0
120
105
90
75
CID-7
CID-40
CID-26
**
PREDRUG CONTROL
POSTDRUG NE 0.1mM
POSTDRUG NE 10.0mM
** p < 0.01

displacement at the knee and ankle were not significantly different from predrug control. The two cats receiving NE 10.0mM (CID-26 and 40) generated steps with significantly larger ($p < 0.01$) displacements at all three joints. Paired t-tests were utilized in the statistical comparisons.

3. Classification of Gaits

The films from the five cats were examined to determinethe types of gaits utilized by the animals. The duration of the step cycle and its swing and stance phases were determined for each trial for both the left and right hind limbs during 4 to 6 consecutive step cycles. The swing phase was defined as beginning at the first frame where the foot was observed in the air and ending at the frame where the foot first made contact with the ground. Stance was defined as beginning at the frame immediately after that where the foot first touched the ground and ending at the last frame where the foot touched the ground. Step cycle duration was defined as the sum of the swing and stance durations. These data were utilized to construct gait diagrams of the hind limb stepping.

The gait diagrams were constructed by utilizing modifications of Hildebrand's technique (Hildebrand, 1966). This involved two sets of calculations. In the first set of calculations the percentage of the step cycle taken up by the stance phase was determined for each step cycle. The average stance duration for each trial (expressed as a percentage of

the step cycle) was found by obtaining the average of the percent stance durations for the series of step cycles analysed in each trial. Seperate determinations were made for the right and left hind limb stance phases.

The second set of calculations consisted of determining the average interval from initial left hind limb foot contact to initial right hind limb foot contact in addition to the opposite interval from initial right hind limb foot contact to initial left hind limb contact. Prior to the averaging process these two sets of intervals were expressed as a percent duration of the step cycle. The gait diagrams were constructed utilizing the results from the two sets of calculations (see figure 14).

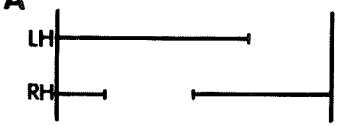
Classification of gaits as symmetrical or asymmetrical was not done since data on the consistency of the treadmill speed were not available. Gaits, however, could be classified on the basis of the percentage of the step cycle occupied by the stance phase. The gaits were classified into two broad categories. The first category, the walk, included gaits where the duration of the stance phase was greater than 50% of the step cycle (Hildebrand, 1966 and 1976). The second category was termed the fast gaits. These gaits were defined as having a stance phase of less than 50% of the step cycle. Normally this category would include such gaits as runs (Hildebrand, 1966 and 1976) and asymmetrical gaits (Muybridge, 1899; Stuart et al, 1973; Goslow et al, 1973; Miller and Vander Burg, 1973: Miller et al, 1975a). All trials were performed on a motorized treadmill at a speed of

Figure 14. Gait Diagrams and Gait Classification.
Vertical bars denotes the step cycle and horizontal bars the proportionate length the stance phase. LH- left hind limb, RH - right himd limb.

GAIT DIAGRAMS

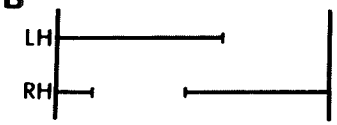
CID-7

A



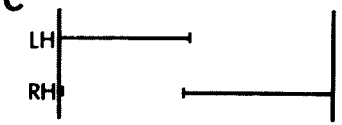
PRESURGERY : WALK
CONTROL

B



PREDRUG : WALK
CONTROL

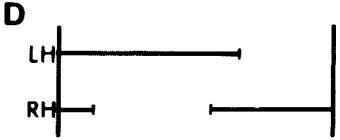
C



POSTDRUG : WALK
NE 0.1 mM

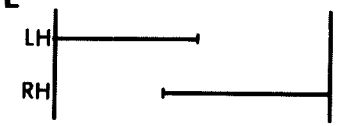
CID-42

D



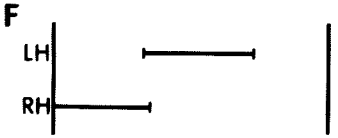
PRESURGERY : WALK
CONTROL

E



POSTDRUG : WALK
NE 0.1 mM

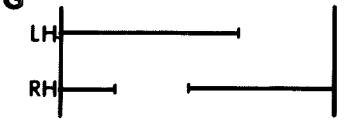
F



POSTDRUG : FAST GAIT
NE 10.0 mM

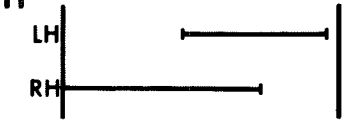
CID-26

G



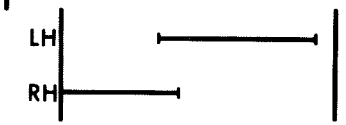
PRESURGERY : WALK
CONTROL

H



PREDRUG : WALK
CONTROL

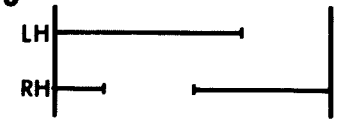
I



POSTDRUG : WALK
NE 10.0 mM

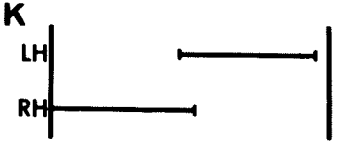
CID-38

J



PRESURGERY : WALK
CONTROL

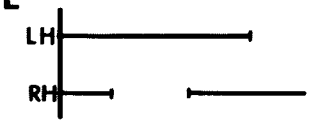
K



POSTDRUG : WALK
NE 10.0 mM

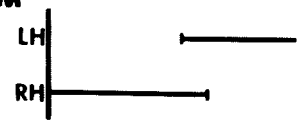
CID-40

L



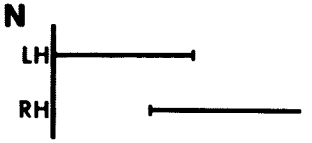
PRESURGERY : WALK
CONTROL

M



PREDRUG : WALK
CONTROL

N



POSTDRUG : WALK
NE 10.0mM

0.4 to 0.6 m/sec. For both intact (Miller and VanderBurg, 1973) and spinal animals (Eidelberg et al,1980; Grillner, 1973; Forssberg et al, 1980a) these treadmill speeds produce walking gaits.

Figure 14 illustrates the result of the analysis. During the presurgery control trials all cats utilized walking gaits (figure 14,A,D,G,J,L). This gait was observed also in the predrug control (figure 14, B,H,M), and postdrug NE 0.1mM trials (figure 14 C,E), and most of the postdrug NE 10.0mM trials (figure 14,I,K,N). The one exception was CID-42 (figure 14F) after the administration of NE 10.0mM. This animal utilized a fast gait.

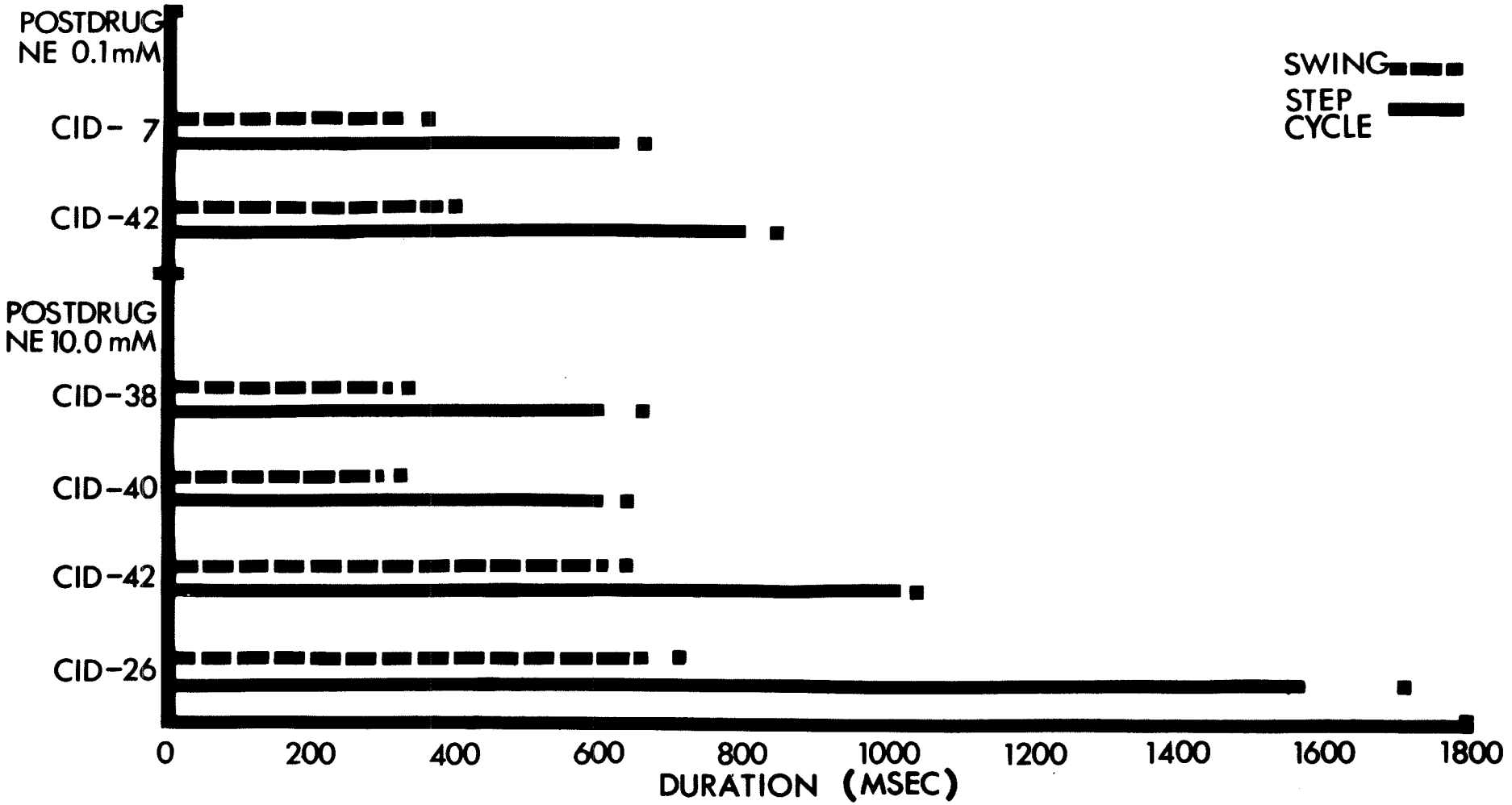
4. Duration of the Step Cycle

The duration of the step cycle for the left hind limb was calculated from the films of the postdrug trials of the 5 cats. The step cycle duration was obtained by counting the number of frames encompassed by each step cycle within a trial as described in the section on classification of gaits. The average duration of the step cycle for each trial was calculated from 5 to 6 consecutive step cycles. Each calculated average was multiplied by 27.8 msec (1/36 of a second) to obtain the duration in milliseconds. The cats were filmed on a treadmill moving at an approximate speed of 0.4 to 0.6 m/sec.

The results are presented in figure 15. For all of the cats except CID-26 the step cycle durations are similar to thoseobserved of spinal cats and kittens walking at a

Figure 15. Duration of Swing and Step Cycle after NE Administration
The average step cycle and corresponding swing durations are illustrated. Cats were treated with NE prior to filming. ■ Standard deviation.

DURATION OF SWING AND STEP CYCLE AFTER NE ADMINISTRATION



comparable treadmill speed (Grillner, 1973; Forssberg et al., 1980a; Eidelberg et al., 1980) and intact cats locomoting at similar treadmill speeds (Miller and Van der Burg, 1973).

An unusually long duration step cycle was produced by CID-26 (figure 15). Once this animal's stance limb reached the limit of extension, the stance foot would drag on the treadmill in this extended position while the contralateral hind limb finished its swing. The stance limb would then begin to swing. The animal was therefore walking at a rate which was slower than the actual treadmill speed.

5. Swing Duration

The swing durations for the left hind limbs of the six cats were calculated in addition. The average duration (in frames) of the swing phase for 5 to 6 step cycles was obtained for each postdrug trial. This average was again multiplied by 27.8 msec to obtain the duration in milliseconds. The cats walked on a moving teadmill at speed of 0.4 to 0.6 m/sec.

The results of this analysis are found with their corresponding step cycle durations in figure 15. The swing durations of most of the NE treated spinal cats were approximately 300 msec. This duration is similar to those observed in spinal kittens by Forsberg and coworkers (1980a) and Grillner (1973). These kittens were walking at similar treadmill speeds to the NE treated cats.

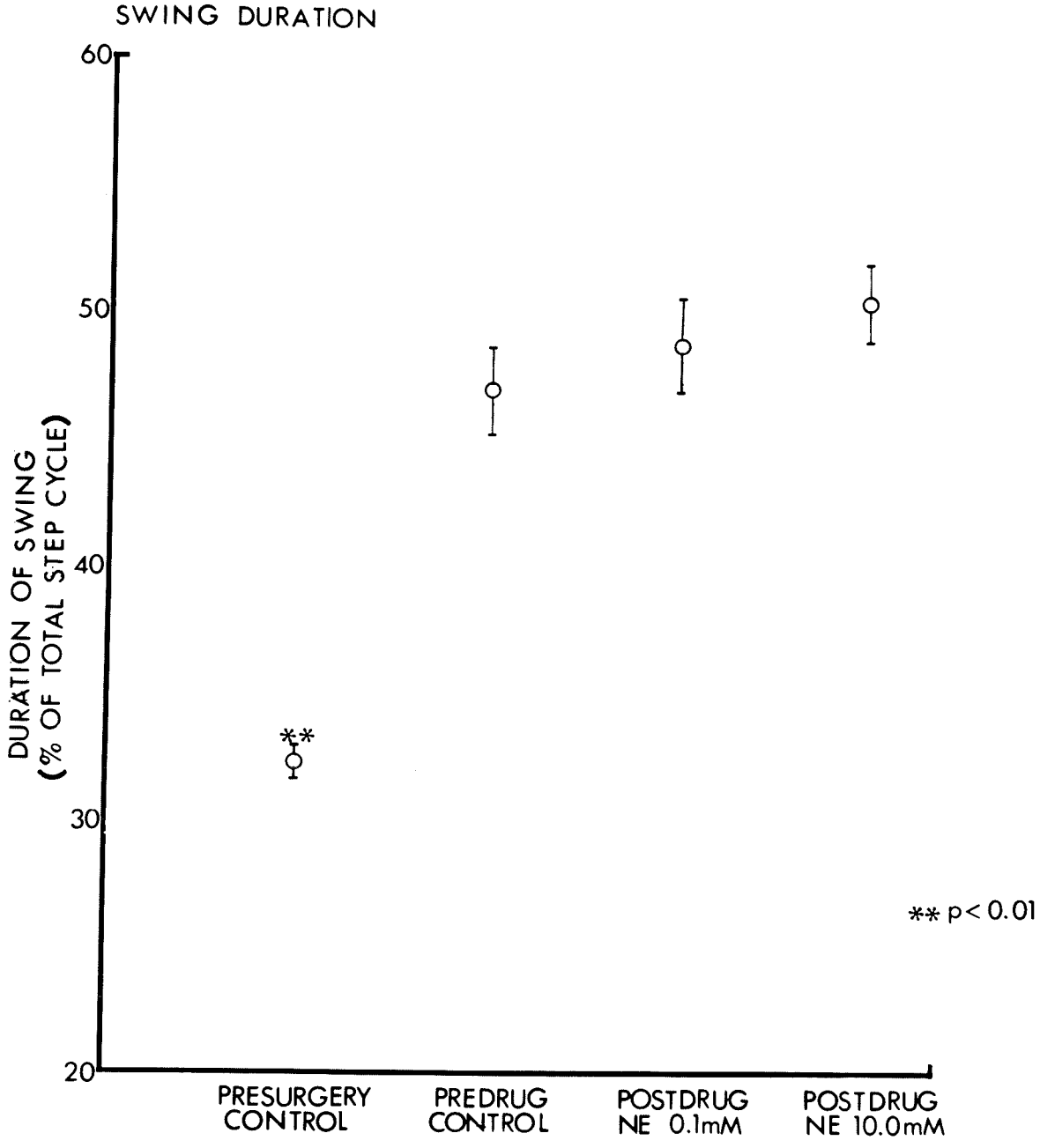
Two of the NE treated cats CID-42 and CID-26 had

particularly long swing durations that were greater than 600 msec (figure 15). The spinal kitten described by Forssberg and coworkers (1980a) did not produce swing durations of that magnitude even at treadmill speeds as low as 0.15 m/sec. The long duration swing phases produced by CID-42 and 26 were unlikely to be a function of treadmill speed.

The average swing duration expressed as a percentage of the step cycle was calculated for each of the four treatment groups. Data from 5 to 6 step cycles was obtained from each trial. The four treatment groups included presurgery controls (N=29 step cycles from CID-7,26,38,40,42), predrug controls (N = 18 step cycles from CID-7,26,40), postdrug NE 0.1mM (N = 12 step cycles from CID-7,42) and postdrug NE 10.0mM (N = 24 step cycles from CID-26,38,40,42). The results are illustrated in figure 16.

The swing constituted approximately 50% of the spinal cat's step cycles. This was observed for all three groups of spinal cats (predrug controls, postdrug NE 0.1 and NE 10.0mM groups). This percentage was similar to that described for a spinal kitten walking at treadmill speeds of 0.4 to 0.6 m/sec (Forssberg et al, 1980a). This percentage of the step cycle constituted by the swing phase in the presurgery control group was approximately one-third (32.0% SEM $\pm$ 0.57). This was similar to literature values for intact cats walking at a treadmill speed of 0.5 m/sec (Miller and Van der Burg, 1973). The presurgery control value was significantly different ($p<0.01$) than that calculated for the

Figure 16. Swing Duration
Average swing durations of the four treatment groups expressed as a percentage of the total step cycle with SEM's marked are illustrated. The presurgery control group was significantly different from all 3 spinal groups ($p \leq 0.01$) when the Bonferroni technique was utilized (Miller,1981). (Presurgery control N=29 step cycles, predrug control N=18 step cycles, post drug NE 0.1mM N=12 step cycles. Post drug NE 10.0mM N=24 step cycles).


SWING DURATION
60
50
40
30
20
DURATION OF SWING
(% OF TOTAL STEP CYCLE)
**
** p< 0.01
PRESURGERY CONTROL
PREDRUG CONTROL
POSTDRUG NE 0.1mM
POSTDRUG NE 10.0mM

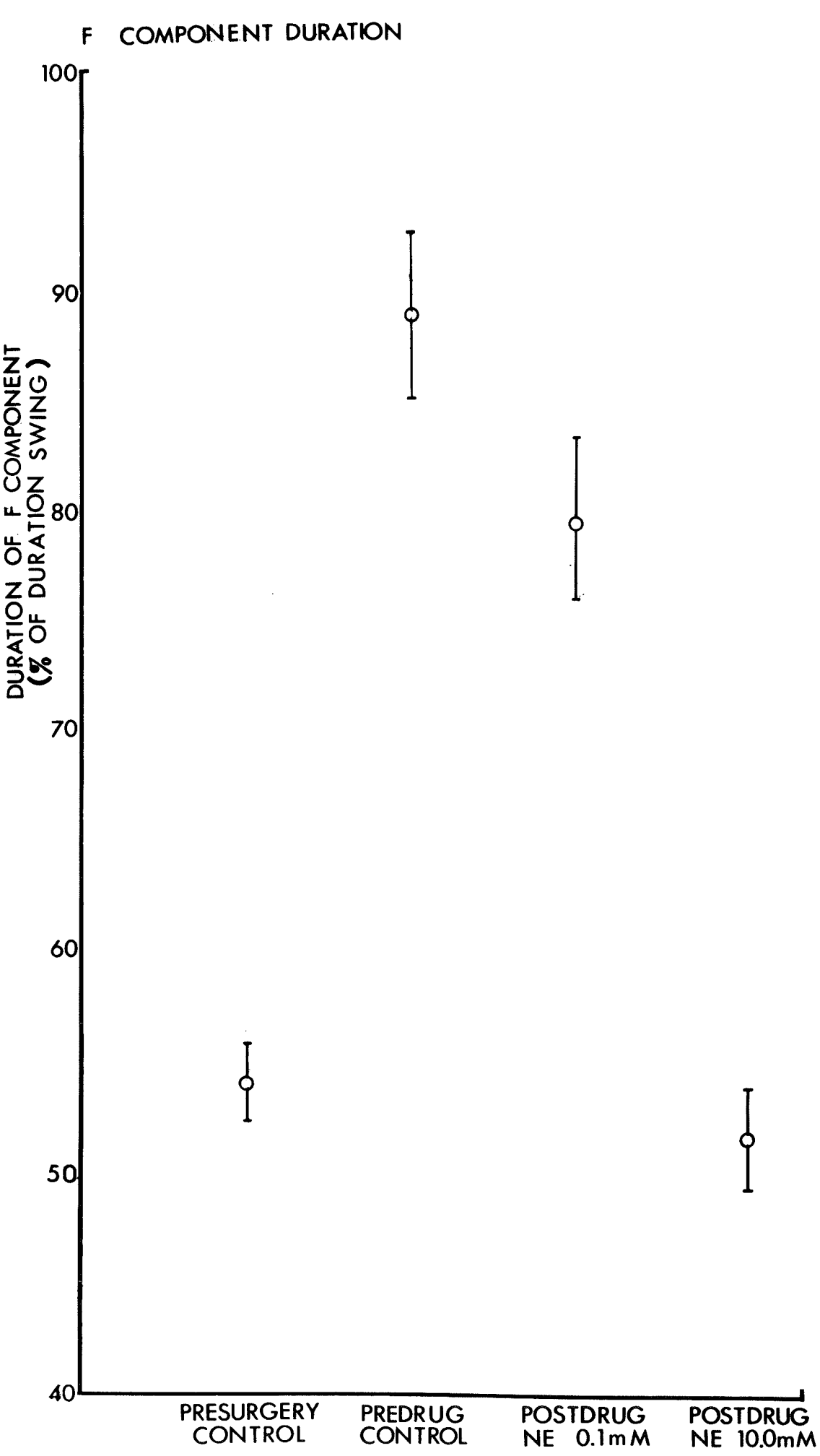
spinal cats. Statistical testing utilized multiple t-tests and the Bonferroni technique for simultaneous statistical inference was utilized in the statistical comparison (Miller,1981).

6. Duration of the F Component and Hip Flexion

The F component of the step cycle as described earlier occurs during the initial portion of the swing phase. During this period all three joints, the hip, knee and ankle, are observed to flex. This component occurs in all the trials of the five cats examined. In intact cats locomoting on a treadmill the percentage of the swing phase comprised by the F component remains constant at walking and trotting speeds (Wetzel et al., 1975). Its value was calculated to be 67% of the swing phase. In this section the effect of treatment on the percentage of swing constituted by the F component will be described.

The average duration of the F component expressed as a percentage of the swing phase was calculated for each treatment group (figure 17). For the presurgery control group this percentage was 54.1% (SEM ± 2.34 N = 29 step cycles). This presurgery control group value was lower than the one calculated by Wetzel and coworkers (1975) for intact cats locomoting on a treadmill. In the predrug control group, F comprised 89.0% (SEM ± 3.73 N = 18 step cycles) of the swing phase. This value was significantly different from the presurgery control group ($p<0.01$). The percentage of the swing comprised by F in the postdrug NE 0.1mM group lay

Figure 17. F Component Duration
Illustrated are the average F component durations expressed as a percentage control and NE 10.0mM groups were not significantly different, but both groups were significantly different from both the predrug control and NE 0.1mM groups ($p<0.01$). The predrug control and NE 0.1mM groups were not significantly different. The Bonferroni technique was utilized in the statistical tests (Miller,1981). (Presurgery control N=29 step cycles; predrug control N=18 step cycles; postdrug NE 0.1mM = 12 step cycles; postdrug NE 10.0mM N=24 step cycles).


F COMPONENT DURATION
DURATION OF F COMPONENT (% OF DURATION SWING)
100
90
80
70
60
50
40
PRESURGERY CONTROL
PREDRUG CONTROL
POSTDRUG NE 0.1mM
POSTDRUG NE 10.0mM

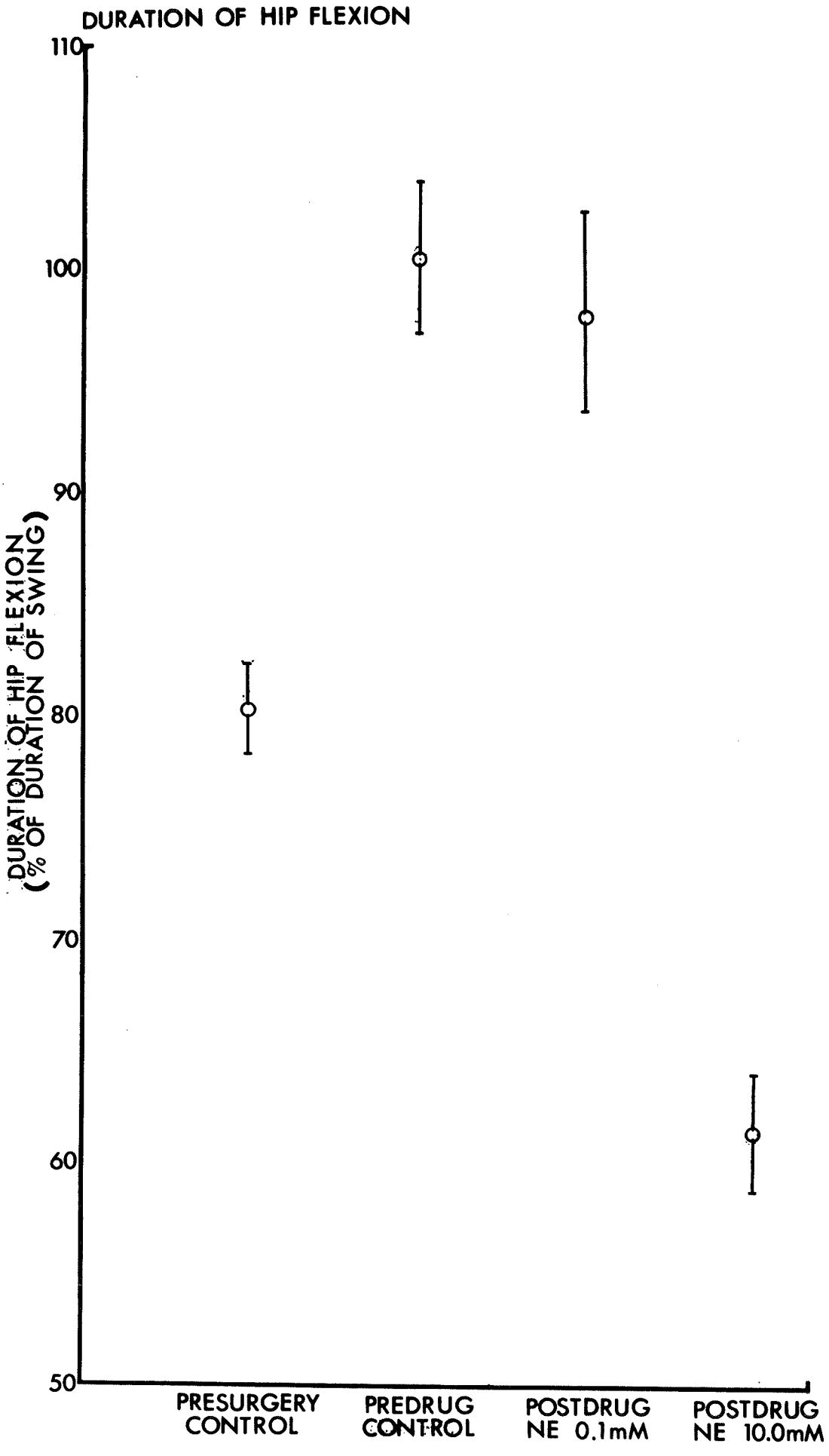
between the two former groups at a value of 79.5% (SEM ± 3.40 N = 12 step cycles). This percentage was significantly different from the presurgery control group ($p<0.01$) but not the predrug control group. In the NE 10.0mM group the calculated value for the F duration was 51.6% (SEM ± 2.27 N = 24 step cycles). This value was not significantly different from the presurgery control group, but it was highly significantly different from both the predrug control and NE 0.1mM groups ($p< 0.01$). The statistical tests utilized were multiple t-tests and the Bonferroni technique for simultaneous statistical inference (Miller, 1981).

In addition to the investigation of the F component duration another characteristic of the swing phase, hip flexion duration, was examined. As described earlier, the termination of hip flexion is closely related in time to the termination of the F component and the beginning of E1. The hip flexion interval was defined as the period from the beginning of swing to the last moment where hip flexion is observed.

The duration of hip flexion was expressed as a percentage of the swing phase, and the average duration of hip flexion was calculated for each treatment group. (Figure 18).

The hip flexion duration followed a pattern similar to that of the F component duration within the treatment groups (see figures 17 and 18). This was not surprising since the termination of hip flexion closely followed the end of the F

Figure 18. Duration of Hip Flexion
Illustrated are the average hip flexion durations expressed as a percentage of the swing phase with SEM's marked. The presurgery control group was significantly different from the other three groups ($p \leq 0.01$) as was the postdrug NE 10.0mM ($p < 0.01$). The postdrug NE 0.1mM and predrug control values were not significantly different. The Bonferroni technique was utilized in multiple t-tests utilized tests (Miller,1981) (presurgery control N=29 step cycles; predrug control N=18 step cycles; postdrug NE 0.1mM N=12 cycles; postdrug NE 10.0mM = 24 step cycles).


DURATION OF HIP FLEXION
110
100
90
80
70
60
50
DURATION OF HIP FLEXION
(% OF DURATION OF SWING)
PRESURGERY CONTROL
PREDRUG CONTROL
POSTDRUG NE 0.1mM
POSTDRUG NE 10.0mM

component. In presurgery control group hip flexion occurred during 80.3% (SEM ± 2.05 N = 29 step cycles) of the swing phase. This was highly significantly different ($p < 0.01$) from the predrug control (hip flexion = 100.2% of swing phase SEM ± 3.38 N = 18 step cycles) and the postdrug NE 0.1mM (hip flexion = 97.5% of swing phase SEM ± 4.17; N=12 step cycle) group values. In both these groups hip flexion occurred throughout the swing phase, and the values of the hip flexion duration were not significantly different from each other. An interesting effect was observed in the NE 10.0mM group. The cats receiving the higher NE concentration utilized hip flexion for only 61.2% (SEM ± 2.6 N = 24 step cycles) of the swing. This was significantly different from the other three groups ($p<0.01$)(Figure 18). The statistical tests utilized were multiple t-tests and Bonferroni technique for simultaneous statistical inference (Miller,1981).

III Weight Bearing Analysis

Concurrent with the videotaping of the pre-and postdrug trials each cat's hind limb extensor tone was measured using the force plate. The weight bearing analog records were screened by superimposing the predrug and postdrug records for each drug administration. The drug was not considered to produce a change if both sets of pre-and postdrug records were the same. The pre-and postdrug trials that demonstrated a difference during screening were further processed by calculating the Weight Bearing Index (WBI). The average difference between the pre-and postdrug WBI's for each trial

Table 11. Postdrug Weight Bearing Effects Expressed as Percentages of the Number of Drug Administrations

Treatment Group	Number of Drug Administrations	Weight Bearing decreased	Weight Bearing unchanged	Weight Bearing increased	Number of cats
Elliot's Solution A (CSF)	34	8.8%	88.2%	2.9%	6
No drug (sham-operated controls)	14	0	100%	0	3
NE 0.1mM	31	3.2%	83.9%	12.9%	9
NE 1.0mM	6	0	50%	50%	6
NE 10.0mM	6	33.3%	33.3%	33.3%	6*
DA 0.1 and 1.0mM	16	0	100%	0	3
DA 10.0mM	3	0	100%	0	3
5-HT 10.0mM	5	60%	20%	20%	5

*In 5 drug administrations from 4 cats weight bearing during the post drug trial could not be assessed. These cats walked continuously and were incapable of keeping both feet on the force plate.

was found (postdrug WBI-predrug WBI) and tested for significance using a paired t-test.

Table 11 illustrates the effect of the test drugs on the weight bearing. For the DA 0.1 and 1.0mM, DA 10.0mM, NE 0.1mM, CSF and no drug control groups, weight bearing was largely unaffected by treatment. When higher concentrations of NE were utilized, effects were observed. Norepinephrine at a concentration of 1.0mM produced postdrug increases in the weight bearing of 3 (50%) of the 6 cats tested. The highest concentration of NE utilized (10.0mM) was given in 11 drug administrations. However, in 5 of these drugs administrations the postdrug trials could not be assessed. In these trials continuous stepping was produced in the cats, and the animals were unable to maintain both feet on the force plate simultaneously. Of the 6 NE 10.0mM drug administrations that were assessed, weight bearing increases were observed in 2 (33.3%), no change observed in 2 (33.3%), and a decrease observed in the last two. Of the cats treated with 5-HT (10.0mM) 3 (60%) demonstrated a decrease in weight bearing, one was unchanged and one demonstrated an increase.

IV Importance of Cannula Placement

In one animal (CID-50) the cannula was placed in the subarachnoid space so that the tip was above the level of the lumbosacral enlargement. Three doses of NE (10.0mM) were administered on separate days. Analysis of the assessment of

the pre-and postdrug trials by the observers demonstrated no change in rating of rhythmic movement when the cat was evaluated in the treadmill paradigm. When evaluated in the overground paradigm, the animal did demonstrate an increase in rating (from a 1 rating level to a 2 rating level) in two of the three drug administrations. The increased movement that was observed was more noticable in the right hind limb. In addition the cat did not demonstrate any changes in weight bearing after administration of the norepinephrine.

When a 350 µl injection of 0.1% fast green was administered through CID-50's cannula at autopsy, the dye predominated in the subarachnoid space of the thoracic region. A small amount of dye also was observed on the upper right portion of the lumbo-sacral enlargement.

Discussion

Norepinephrine given intrathecally at concentrations of 1.0mM and 10.0mM is an effective means of producing locomotor movements in chronic spinal cats. The production of locomotion by NE is consistent with the production of locomotion by clonidine (Forssberg and Grillner, 1973) and L-DOPA (Budakova, 1973; Jankowska et al., 1967). The steps produced by the cats after administration of NE 10.0mM were well developed as revealed by kinematic analysis and displayed characteristics which were similar to the presurgery (intact) control cats. These similar characteristics included:

1. All four step cycle components were present in both groups.
2. The percentage of the swing phase comprised by the F component was similar in both groups (Figure 17).

The stepping of the NE 10.0mM cats also demonstrated some characteristics that are usually observed when intact cats gallop. These characteristics included:

1. a large range of motion at the ankle (see Figure 12) (Goslow et al., 1973).
2. pronounced hip extension at the end of the E1 component (Miller et al., 1975b).

Norepinephrine also produced increases in the WBI (increased extensor tonus). This phenomenon was observed in some of the postdrug trials after administration of NE at all three of the concentrations utilized (see Table 11).

The increased weight bearing ability is consistent. The effects of the α-agonist clonidine in acute and chronic

spinal cats and the effects of L-DOPA in acute spinal cats and rabbits. Clonidine increased hind limb extensor tonus in acute spinal cats (Forssberg and Grillner, 1973) and chronic spinal cats (Barbeau and Rossignol, 1982). In acute spinal cats L-DOPA increase limb extensor tonus (Miller and Van der Meche, 1976). In acute spinal rabbits L-DOPA induced rhythmic locomotor discharges which were predominant in the extensor motor nerves (Viala and Buser, 1971).

The lowest concentration of NE (0.1mM) utilized was not effective in producing increases in walking ability. Several investigators (Viala et al., 1974; Anden et al., 1966b), however, have noted that the effects of L-DOPA are potentiated by the use of MAO inhibitors. It is possible that administration of MAO inhibitors could potentiate the effects of NE. Preliminary studies with intrathecal administration of the MAO inhibitor, tranylcypromine, 30 minutes prior to administration of NE 0.1mM, support this hypothesis.

Dopamine at low concentrations (DA 0.1mM and DA 1.0mM) was not effective in producing improvement of locomotor abilities of cats (see tables 6 and 7). The short term effects of low concentrations of DA were not significanlty different from cats treated with NE 0.1mM or the control cats (see Tables 8 and 9). Three injections of the highest concentration of DA utilized (DA 10.0mM) were administered once to three separate animals. Minimal improvement in walking ability was observed in only one of these animals,

and no improvement was observed in the other two animals.

If DA is the mediator of the L-DOPA effects on locomotion, the administration of DA at high concentrations should be as effective or more effective than NE at inducing locomotion. Since marked improvement in locomotor abilities after administration of DA was not observed this would suggest that DA does not mediate the effect of L-DOPA on locomotion.

It should be noted that DA did not appear to increase the cats' abilities to support hind limb weight. It should be noted that the administration of the dopaminergic agonist, apomorphine, in chronic spinal cats produced periods of sustained flexion which disrupted locomotor ability in these cats (Barbeau and Rossignol, 1982). An increase in flexor tonus would cause a decrease in the weight bearing ability of the cats. Although decreases in hind limb WBI were noted on occasion in cats after treatment with DA, these changes were not significantly different from the predrug control trials.

The effectiveness of NE and not DA for the induction of spinal stepping and weight support is consistent with the hypothesis that NE is the mediator of L-DOPA's motor effects. This is also evidence against Commissiong's theory (Commissiong, 1981b) that DA is the mediator of L-DOPA's physiological effects. However, high doses of DA (DA 10.0mM) had some minimal effects on locomotion in one animal. It is possible that these effects were mediated through NE. Anden and coworkers (1966b) noted that the short latency FRA reflex effects continued to be depressed by L-DOPA in cats 2 to 4

weeks after spinal transection. In addition, Baker and coworkers (1982) observed that L-DOPA was capable of inducing locomotor-like discharges in the motor nerves of chronic spinal cats. It is thought that the effects of L-DOPA on FRA reflexes are mediated through DA or NE since adminstration of the dopa decarboxylase inhibitor, NSD 1015, depresses L-DOPA effects in acute spinal cats (Anden et al., 1966b). It is likely that the effects of L-DOPA in the chronic spinal cats also would be mediated through NE or DA. Anden and coworkers (1966b) found low concentrations of NE and DA to be present in the spinal cord below the level of transection after administration of L-DOPA. It is possible that a small amount of L-DOPA was converted to NE and DA in these chronic animals. Anden and coworkers (1966b) suggested that possible denervation supersensitivity below the level of spinal transection would increase the effectiveness of low concentrations of catecholamines. Recent studies by Jones and Alcantara (1982) support Anden and coworkers' (1966b) hypothesis of denervation supersentivity. These authors noted an increase in NE-stimulated cyclic AMP accumulation and an approximate doubling of beta receptors below the level of transection in the spinal cord of the rat (Jones and Alcantara, 1982). It is possible, therefore, for DA to be converted into NE and the resulting NE mediate the changes in locomotor ability.

Another explanation for the lack of effectiveness of DA is that DA was degraded in the spinal cord before producing

it's effects. Pretreatment of the cats with a MAO inhibitor could potentiate the effects of DA. However, in light of the possibility of conversion of DA to NE in chronic spinal animals, the use of an inhibitor of dopamine-beta-hydroxylase is also necessary to properly answer the question of DA's effectiveness.

An increase in walking ability was observed in only one of the animals treated with 5-HT (see Tables 6 and 7). This indicates that serotonin administration is not an effective method of producing increases in walking ability. These results concur with the effects of 5-HTP observed by Griller and Shik (1973) in acute spinal cats. These authors also noted little sign of alternating activation of flexors and extensors after 5-HTP administration.

The effects of 5-HT on weight bearing appear to be more complex. The WBI was observed to decrease in 3 of the 5 spinal cats and increase in one cat. The animals were tested 30 minutes after 5-HT administration. In addition, during preliminary studies, 5-HT caused an initial increase in extensor tonus followed by a decrease in extensor tonus. Grillner and Shik (1973) investigated the effect of 5-HTP on hind limb tonus in acute spinal cats. These investigators observed excessive extensor tonus which sometimes changed to prolonged flexor tonus. It should be noted, however, that the 5-HT agonist quipazine was found to increase extensor tonus only when given chronic spinal cats chronic spinal cats (Barbeau and Rossignol, 1982). In addition, 5-HTP increased the extensor EMG output in chronic spinal rats (Bedard et

al., 1979; Barbeau et al., 1981). In contrast to these extensor effects, 5-HTP administration in acute spinal rabbits produced rhythmic discharges in the motor nerves which were more predominant in the flexor motor nerves (Viala and Buser, 1971).

One hypothesis to explain 5-HT's apparent dual effects on muscle tonus is that 5-HT may convey both flexor and extensor tonus changes through different receptors. Peroutka and coworkers (1981) have found two distinct central serotonergic receptors in rat brain. These receptors are termed serotonin 1 and serotonin 2 respectively. The presence of these receptors in the spinal cord has yet to be determined. It should be noted, however, that 5-HT has both depressive and facilitatory effects on spinal neurons when applied by iontophoretic techniques (Salmoiraghi and Stefanis, 1965; Engberg and Ryall, 1965. The serotonin 1 receptors are thought to mediate inhibitory synaptic serotonin effects in rat brain while serotonin 2 receptors mediate excitatory synaptic serotonin effects in rat brain, (Peroutka et al., 1981). Iontophoretic application of quipazine mimiced the excitatory effect of 5-HT on rat spinal motorneurons (Neuman and White, 1982). It is possible that quipazine excites serotonin 2 receptors which through some mechanism increases extensor tonus. The serotonin 1 receptor might be involved in producing the increased flexor tonus effects. Two distinct 5-HT receptors might, therefore, mediate the different effects of the iontophoretically

applied 5-HT and the dual effects of 5-HT on hind limb tonus. However, to substantiate this hypothesis more extensive investigation should be performed.

The production of locomotion after NE administration supports the hypothesis that NE is a putative neurotransmitter for the release of the spinal locomotor CPG. Norepinephrine does fulfill some of the 5 classical criteria for the identification of a neurotransmitter.

The first criterion for transmitter identification is that the putative transmitter and appropriate synthetic machinery must be present in the presynaptic terminal. The fulfillment of this criterion by NE is supported by a large amount of evidence. Not only has NE been found in the spinal cord, it also appears to have a close anatomical relationship with the brain stem locomotor regions. Studies by several investigators have identified NE in the ventral horns, central region and dorsal horns of the rat, rabbit and cat (Anderson and Holgerson, 1966; Zivin et al., 1965; Fleetwood-Walker and Coote, 1981). The major source of NE for the spinal cord appears to be the brainstem (Dahlstrom and Fuxe, 1964; Stevens et al., 1981), and the CA fibers from the brainstem appear to descend from areas located near the brainstem locomotor regions (Steeves et al., 1975; Garcia-Rille et al., 1983; Stevens et al., 1982). In addition,noradrenergic fibers descend in the ventralateral funculi in the cat (Fleetwood-Walker and Coote, 1981). This is the same region of the cat spinal cord where the locomotor pathways descend (Steeves and Jordan, 1980). Besides to the studies on the presence of NE in the spinal cord,

antibodies to the NE synthesizing enzyme DBH have been utilized to demonstrate the presence of this enzyme in the spinal grey matter of the rat (Westlund et al., 1983). Anden (1965) also has found decarboxylase activity in the rabbit and cat spinal cords. It appears, therefore, that the presence of NE and its synthesizing enzymes has been established in the spinal cord.

A second criterion for the identification of NE as a neurotransmitter involved in release of the spinal locomotor generator is that the substance be released upon presynaptic depolarization. Norepinephrine has been shown to be released into the CSF surrounding the spinal cord in the cat during stimulation of the sciatic nerve (Yaksh and Tyce, 1981) and during intrathecal perfusion of a potassium solution in the subarachnoid region of the spinal cord (Yaksh and Tyce, 1980). However, the release of NE into the CSF surrounding the spinal cord during stimulation of the locomotor regions in the brainstem has yet to be shown.

The third criterion met by NE in the spinal cord is that mechanisms for removal of the transmitter be present in the spinal cord. Kessler and coworkers (1976) have found the principle metabolite for NE in the CSF surrounding the monkey spinal cord. This would indicate that degradative enzymes for NE are present in the spinal cord. Uptake mechanisms for NE have also been noted in the spinal cord of the rat (Jones and Alcantara, 1983).

A fourth criterion for the identification of NE as a

putative neurotransmitter in spinal locomotion is that the injection of the putative neurotransmitter into the synaptic cleft produces the same response postsynaptically as does depolarization of the presynaptic membrane. The fact that both and receptors have been noted in the rat spinal cord supports this criterion (Jones et al., 1982). However, the exact location of the synapse at which NE would act to release the spinal locomotor CPG is unknown. Knowledge of the exact location of this synapse would be required to be able to fulfill this criterion. It should be noted that Jordan et al., (1977) utilizing cats revealed that NE-containing neurons rarely formed intimate contact with motorneurons. However, the NE terminals did appear to make contact with interneurons found near the motorneurons (Jordan et al., 1977). The effects of NE applied by iontophoresis to interneurons appears to be mainly depressive (Briscoe et al., 1966; Salmoiraghi and Weight, 1968; Jordan et al., 1977). However, besides these depressive effects occasional excitatory effects of NE on interneurons have been noted (Salmoiraghi and Weight, 1968; Jordan et al., 1977).

If the excitatory effects of NE on interneurons are involved in release of the spinal locomotor CPG, it is possible that these excitatory effects simply would activate the CPG. However, the major effect of NE on interneurons appears to be inhibitory. An increase in motorneuron excitability can occur also if inhibitory effects on motorneurons are themselves inhibited. Norepinephrine might inhibit inhibitory effects on motorneurons. This mechanism

could also be involved in release of the spinal locomotor CPG. The actual mechanism of CPG activation by NE, however, is unknown. The results of the present experiments do not elucidate the mechanism by which NE effects the CPG.

The fifth criterion for the identification of NE as a putative spinal locomotor neurotransmitter is that the pharmacology of the putative transmitter matches that for natural stimulation. The effects of NE noted in this study support this criterion. However, further study utilizing noradrenergic blockers are necessary to substantiate that NE activates the spinal locomotor CPG. Other pharmacological evidence supports the criterion that the pharmacology of NE matches that for natural stimulation. This evidence includes the fact that the depression of the short latency FRA reflex induced by L-DOPA has been reversed by the blocker phenoxybenzamine (Anden et al., 1966b). In addition locomotion has been induced in spinal cats with the agonist clonidine (Forssberg and Grillner, 1973) and the NE percursor L-DOPA (Jankowska et al., 1967). However, there is also evidence that does not support NE's fulfillment of this criterion. Steeves and coworkers (1980) were unable to antagonize the locomotor effects of the MLR utilizing phenoxybenzamine. In addition, substancial depletion of NE from the cat spinal cord does not inhibit walking in the intact cat or prevent the occurrence of locomotion with MLR stimulation (Steeves et al., 1980). These negative findings do not necessarily preclude NE as a transmitter involved in release of the locomotor CPG.

Norepinephrine could be one of several neurotransmitters involved in release of the spinal locomotor CPG. Antagonism of one of these transmitters may not prevent the effects of other neurotransmitters.

The possibility that other neurotransmitters may be involved in releasing the locomotor CPG is supported by anatomical evidence. The magnocellular and gigantocellular tegmental fields do not contain catecholamines (Poitras and Parent, 1978). These areas do receive projections from the MLR (Garcia-Rill et al., 1983, Steeves and Jordan, in preparation). In addition, these areas send projections to the spinal cord (Tohyama et al., 1979a). These descending reticutospinal projections are located in the same region of the spinal cord where locomotor pathways are located in the ventrolateral funiculi (Tohyama et al., 1979a; Steeves and Jordan, 1980)

Preliminary studies utilizing low concentrations (0.1mM) of somatostatin, substance P and glutamate have been performed to test these drugs as possible locomotor neurotransmitters. Glutamate produced an increase in rhythmic movement rating of one unit (from rating 1 to rating 2) during one administration. However, no effect was observed after administration of the same concentration of glutamate in another cat. The other substances were ineffective in producing changes in rhythmic movement abilities at these low concentrations. The effects of these substances at higher concentrations have yet to be investigated.

Intrathecal administration of NE appears to be an effective method of induction of locomotion in chronic spinal cats. The possibility that NE may evoke stepping in chronic spinal monkeys or in humans with spinal cord injuries has yet to be investigated. If NE did induce stepping in spinal human beings, this drug or its agonists might prove therapeutically useful in attempts at rehabilitation.

Intrathecal injection may be a useful method of drug administration for studies in monkeys and humans. It could possibly have theraputic application under certain circumstances This method has several advantages. First of all, intrathecal injection offers a convenient method of crossing the blood brain barrier. Secondly, the drug is concentrated at the proposed site of action by circumventing the diluting effect of the systemic circulation. A third advantage of intrathecal administration is that drugs can be administered directly to the site of action in the CNS without affecting other areas of the CNS. This third advantage is supported by the fact that fast green applied to the region of intrathecal injection appeared to be limited to the subarachnoid space of the spinal cord.

Summary and Conclusions

The major findings from this study are as follows:

1. Norepinephrine can induce locomotion and increase weight support in chronic spinal cats. These findings are evidence which supports NE as a putative neurotrasmitter involved in the release of the spinal locomotor CPG. The possibility of NE's use as a therapeutic agent in spinal cord injury was discussed.

2. Both 5-HT and DA were much less effective at inducing locomotion. The minimal locomotor effects produced by DA may be mediated by NE. It is possible that 5-HT may be involved in the regulation of hindlimb tonus. However, the effects of 5-HT on hindlimb extensor tonus are complex. These effects may be mediated by more than one 5-HT receptor.

3. Intrathecal injection is a convenient and advantageous method of drug administration. This method can be utilized in the testing of the effects of many different drugs on locomotion or as a method of therapeutic drug administration.

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