THE UNIVERSITY OF CALGARY

Sensorimotor deficits after pyridoxine intoxication. Kinematic and electrophysiological observations in a new animal model of deafferentation.

by

Gregory Mark Bishop

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF NEUROSCIENCE

CALGARY, ALBERTA

JULY, 1998

Abstract

The role of sensory feedback in the control of movement can be assessed by examining motor deficits after deafferentation. To overcome limitations of surgical deafferentation, a new animal model of non-invasive, chemical deafferentation by pyridoxine was developed in the cat. Within 3-4 days after pyridoxine treatment began, the animals developed severe motor deficits as animals A) showed abnormal magnitude and variability of trunk and relative paw motion trajectories during walking, B) lost ability to compensate for external disturbances, C) walked with a crouched posture and plantigrade strategy, and D) showed disrupted forelimb-hindlimb phase coordination. Motor strength and motoneurone function were unaffected. In contrast, muscle and cutaneous sensory nerves were strongly affected as compond potentials, vibration and neurogram responses were abolished. Single medial gastrocnemius afferent unit recordings revealed a dramatic loss of group I, and group II afferents while group III afferents including mechano-sensitive units were readily isolated.

Acknowledgements

Throughout the past three years during my M.Sc. studies I have received limitless support and assistance from those I wish to thank below. I must thank Dr. M. Hulliger for providing an intellectually challenging and stimulating work environment. I truly appreciate his never ending guidance. A large thank you goes out to Alberta Heritage Foundation for believing in my research and my abilities enough to support my studies. I am very grateful for the efforts and support of my supervisory committee Drs. R. G. Lee, G. Melvil-Jones, R. Zernicke and R. B. Hawkes. In addition, I would like to thank Dr. J. Ronsky for participating in my thesis defence. A large note of gratitude is forwarded to Dr. A. Bulloch for considerable encouragement and direction. Equal in appreciation are my thoughts regarding four very special people: Ania Zanussi, the proprietor of a smile and hug unequalled; Scott Day, for his bottomless heart; Mats Djupsjobacka, a noble man who is passionate about life; and Jonathon Foweraker, a truely excellent scientist and Canadian. Also, a warm thank you is extended to Brendan Rauw and Leigh Davis for their substantial contributions and friendship. Above all, I must once again praise, thank and bless my Mother and Father for their endless encouragement and devoted love for which I am deeply indebted.

The compilation of my Masters Thesis is dedicated in whole to two groups of very important beings.

My family

To my Mother, one who has blessed me with her love and understanding.

To my Father, from whom I have undying support and golden direction.

To my Brother, here with me every step of the way.

My cats

Their lives will not be forgotten.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

CEP	compound evoked potential
MG	medial gastrocnemius
SP	superficial peroneus
CP	common peroneus
DR	dorsal root
VR	ventral root
CNS	central nervous system
PNS	peripheral nervous system
NMR	nuclear magnetic resonance
EMG	electromyogram
GTO	Golgi tendon organ
PLP	pyridoxal phosphate
CPG	central pattern generator
i.p	intraperitoneal
i.v.	intravenous
RMS	root mean squared
IPSP	inhibitory post synaptic potential
EPSP	excitatory post synaptic potential

CHAPTER ONE: INTRODUCTION

1.1 General

Researchers in the field of motor control have been investigating the role of proprioceptive information in the control of locomotion and goal directed movements for over a century (Mott and Sherrington, 1895; Munk, 1909). One way to determine the role of proprioceptive input is to abolish it selectively (e.g. by transecting the sensory nerves entering the central nervous system), and then to look for deficits in motor performance. Selectively abolishing proprioceptive information has been carried out in numerous different ways in animals ranging from insects to humans. In nearly all cases, purposeful, or rhythmic, motor action persisted after deafferentation, but the accuracy and adaptability of the actions were diminished.

1.2 Proprioception

Proprioception refers to the body's sensation of its own movements. Proprioceptive information is signalled by the proprioceptors throughout the body. This information is then sent to the spinal cord for local processing and/or is sent along the spinal cord to higher brain centres for more complex processing. At each stage this information can be used to regulate movements.

1.2.1 Spinal cord proprioceptors and exteroreceptors

Body movement is sensed internally by muscle spindles, Golgi tendon organs (GTO), joint receptors and skin receptors. Muscle spindles with primary (Ia) or secondary (II) endings are arranged in parallel with the muscle fibres. They signal information to the

spinal cord about muscle length (Ia and II) and rate of change of muscle length (Ia) (Matthews, 1972). Spindle primary endings have larger axonal diameters than the secondary endings. The larger axonal diameter of the Ia afferents produces larger action potentials and faster conduction velocities. These two attributes were the reason why Ia afferent information was, and remains to be, considered the more influencial muscle spindle afferent. Each muscle spindle is innervated by 10-12 gamma (δ) motor neurons and often by a beta (β) motor neuron for control of muscle spindle sensitivity. Golgi tendon organs (GTO) are arranged in series with muscle fibres and are located at musculotendinous junctions (Matthews, 1972; Horcholle-Bossavit, Jami, Petit, Vejsada and Zytnicki, 1990). Their axons (Ib) signal information to the spinal cord about active muscle force exerted by a few motor units. Joint mechanoreceptors are located in encapsulated joints such as the knee. Their axons are much smaller with slower conduction velocities and are far outnumbered by the skin and muscle afferents. Joint receptors signal to the spinal cord information about the position of the joint (see later in Introduction 1.2.1). Skin or cutaneous receptors are located throughout the body and respond to the stretching of the skin and movement. Cutaneous receptor axons are large and classified as group I afferents with fast conduction velocities.

In 1900, Sherrington wrote his own explanation of muscle sense. It states: "The perceptions of muscular sense may be grouped into: (i) those of posture, (ii) of passive movement, (iii) of active movement, and (iv) those of resistance to movement. Changes in consciousness accompany all movements of the body which occur at speeds above a certain liminal (amount), and over distances of beyond a liminal extent." Therefore, Sherrington believed that different types of muscle sense come from various sensory

mechanisms that are integrated to produce a single internal representation of the body. He postulated that muscle sense originated from the muscle receptors, including muscle spindles, GTO's, and joint receptors but not cutaneous receptors.

This view was held for half a century, until the 1960's, when it was demonstrated that there was no projection between the primary muscle spindles and the cerebral cortex. At this time, Rose and Mountcastle concluded that, "The sense of position and of movements of the joints depends solely on the appropriate receptors in the joints themselves. There is no need to invoke a mysterious 'muscle' sense to explain kinaesthetic sensations..." (1959). This notion was adopted by many including Jones (1972) who introduced the term kinaesthesia. Jones thought that kinaesthesia was mediated by joint mechanoreceptors and not by muscle receptors.

At the same time, Goodwin, McCloskey and Matthews (1972) studied the effects of vibration on kinaesthetic sense. They showed that by vibrating human tendon and exciting spindle Ia afferents predominantly, if not selectively, a range of movement illusions were produced. In addition, muscle afferent responses during illusory muscle pulling induced the perception of joint movement (Matthews and Simmonds, 1974). Goodwin et al. (1972) and Matthews and Simmonds (1974) concluded that muscle spindles, predominantly the Ia afferents, provide information that is used for the perception and control of movement.

Recent data on Golgi tendon organ inputs during locomotion showed that GTO's excite extensor motor neurones during the stance phase and can prevent the initiation of a new

flexor burst (Pearson and Collins, 1993; Pearson, Ramirez and Jiang, 1992). The reduction of Ib input near the end of the stance phase appears to signal the time for a new step to occur, indicating a role for GTO's in regulating movements.

Whether joint receptors contribute to kinaesthesia has become the question of many experiments. Joint receptors, recorded individually, have been shown to be active at various points in a limb's working range, but to be silent in the middle of that range (Grigg, 1975; Hulliger, Nordh, Thelin and Valbo, 1979; Gandevia and McCloskey, 1976). Since joint receptors are not active throughout a joints' entire range of motion, they can not be exclusively responsible for kinaesthesia. The question then became whether joint receptors contribute to kinaesthesia at all. After deafferentation of the joint receptors by intracapsular injections of local anaesthetic (Clark, Horch, Bach and Larson, 1979), and as a consequence of hip replacement surgeries (Cross and McCloskey, 1973; Grigg, Finerman and Riley, 1973), position sense seemed little affected. In contrast, a deterioration of walking was reported for the cat after acute anaesthesia of the knee joint (Ferrel, Baxendale, Carnachan and Hart, 1985). Joint receptors may complement the kinaesthetic and sensorimotor control of other proprioceptors and may have a special role in inhibiting muscles when joints are damaged (Iles, Stokes and Young, 1990).

Even though Sherrington (1900) hypothesized that cutaneous receptors were active during muscular contraction and limb movement, he felt that they were not used in the development of muscle sense. In 1979, Hulliger et al. re-iterated the fact that cutaneous receptors were active during voluntary finger movements, and when the hand was held in a constant position. In addition, Clark et al. (1979) showed that local anaesthesia of

the hand or fingers produced impairments of position sense. Therefore, cutaneous receptors must also play a role in position sense.

It has been suggested that controlled movements are simply the outcome of CNS modulation of the reflex transmission of afferent activity (Merton, 1953; Feldman, 1974; Houk, 1979). Therefore, afferent feedback drives locomotor reflexes. Recent investigations of proprioceptors in the walking systems of cats, locusts and crayfish have identified reflex pathways that regulate the timing of the transition from stance to swing, and control the magnitude of ongoing motor neuronal activity (Pearson, 1995). Dietz, Schmidtbleicher and Noth (1979) investigated the latency of force production of ankle extensors during walking after sudden increments in electrical stimulation of the tibial nerve. They concluded that spinal stretch reflexes play an important role in human running and sprinting as the increase in EMG activity shortly after ground contact is evoked by proprioceptors via a spinal pathway within a time consistent with monosynaptic transmission.

1.2.2 Spinal central pattern generators

One of the most complex movement patterns for any organism to coordinate is locomotion. It requires the accurate orchestrated activation of numerous muscles and, in vertebrates, is produced by neural networks that are referred to as central pattern generators (CPG) (Grillner, 1975). These neuronal assemblies are located in the central nervous system (CNS; i.e. spinal cord) and are capable of generating the basic patterns of motor output for rhythmic activities such as walking and swimming (Rossignol and Dubuc, 1994). In addition, the CPG's are capable of producing these rhythmic activities

in the absence of sensory feedback (Rossignol and Dubuc, 1994). This was originally conceived when Brown (1911) observed coordinated activation of flexor and extensor muscles after the cord had been isolated from descending supraspinal input and from all afferent input. Later, Grillner (1975) suggested that in locomotion, sensory feedback dependent reflexes are prepared to operate but are without any effect so long as the movement proceeds according to the set central program. It was hypothesized that CPG's can operate independently of sensory feedback because they themselves send information up to the brainstem and the reticular formation about the spinal CPG activity. The initiation of CPG activity is, however, dependent upon descending inputs from the reticular formation and other supraspinal centres, while ongoing alternating activity may be controlled intrinsically or via sensory feedback from stretch receptors (Grillner, Deliagina, Ekeberg, el Manira, Hill, Lanser, Orlovsky and Wallen, 1995; Grillner, Wallen and Brodin, 1991).

However, when an animal producing fictive locomotion or air stepping movements is lowered to the ground, the locomotor rhythm ceases or is slowed (Bassler, 1983; Bassler, 1993; Duysens and Pearson, 1980; Giuliani and Smith, 1985). In addition, hindlimbs of spinal cats can adjust to locomotion of different speeds when walking on a split-belt treadmill (Forssberg, Grillner, Halbertsma and Rossignol, 1980). Obstruction of hip movement (Andersson and Grillner, 1983; Grillner and Rossignol, 1978) or loading the extensor muscles at the end of stance phase (Conway, Hultborn and Kiehn, 1987; Duysens and Pearson, 1980) can suppress the rhythmicity of the effected leg during periods of walking in the other three limbs of a cat. Stretch of the cat flexor muscles during the stance phase resets locomotor rhythm to generate flexion ipsilaterally and

extension contralaterally (Hiebert, Whelan, Procazka and Pearson, 1996). Further, GTO's have been shown to produce IPSPs before locomotion, but during locomotion they produce EPSPs in motor neurones (Pearson and Collins, 1993; Gossard, Brownstone, Barajon and Hultborn, 1994). In the absence of sensory feedback, obstacles or inclines are not compensated for, and if the animal is not supported it falls (Giuliani and Smith, 1987; Goldberger, 1977). A necessary condition for swing phase initiation in locomotion is the unloading of extensor muscles (Pearson, Ramirez and Jiang, 1992; Conway, Hultborn and Kiehn, 1987). During locomotion, the silence of group Ib (GTO) afferents innervating extensor muscles is interpreted as extensor muscle unloading. Whelan, Hiebert and Pearson (1995) have shown that if these afferents are electrically stimulated near the end of the stance phase in spontaneously walking decerebrate cats, the onset of swing phase is delayed. In addition, muscle spindles of hip flexor muscles sensitive to hip extension trigger the transition of stance to swing phases (Grillner and Rossignol, 1978). Taking all of these observations together, sensory input must be an integral and necessary part of locomotor pattern generation.

1.2.3 Supraspinal motor control

Supraspinal control of locomotion refers to the control or regulation of movement exerted by central nervous system centres rostral to the spinal cord. Supraspinal control of locomotion has largely been implicated in the initiation, regulation and coordination of locomotion. Stimulation of the mesencephalic locomotor region has been shown to elicit a complete quadrupedal locomotor pattern in the presence (Shik, Severin and Orlovsky, 1966) and absence (Jordan, Pratt and Menzies, 1979) of peripheral feedback. Baev, Beresovdkii, Kebkalo and Savoskina (1988) have convincingly shown that stimulation of

the subthalamic locomotor region also triggers locomotion, and can do so even when most of the midbrain has been destroyed.

Additional supraspinal coordination and regulation of locomotion is provided by the motor cortex, vestibular system and cerebellum. Russel and Zajac (1979) have shown that the vestibulospinal system regulates cycle timing and structure in cat fictive locomotor rhythm. That motor cortex is important in regulating step cycle components has been emphasized by Liddle and Phillips (1944) and Eidelberg and Yu (1981). In addition, Armstrong and Drew (1984a, 1984b) have shown that 80% of motor cortical cells are rhythmically active in relation to stance or swing phases during walking. Yet, it is not clear whether Armstrong and Drew were able to sample all cell types and sizes with equal frequency.

The cerebellum is also likely to play a role in the control of movements considering its input and output arborizations. However, the cerebellum's precise role in coordination and regulation of limb movements is still controversial. Holmes' (1922) investigations of gun shot victims of World War I have clearly illustrated that cerebellar lesions are associated with abnormalities in the rate and regularity of locomotor movements (see review by Ito, 1978). In addition, work with experimental animal models of various cerebellar lesions has shown that the cerebellum is indeed involved in controlling interlimb coordination during walking (Shimamura and Kogure, 1983; Shik and Orlovsky, 1976), and regulates the ongoing muscle activity in locomotion (Ito, 1978 and 1979). It has been hypothesized that the cerebellum acts as a comparator as it assesses the difference between the efferent copy for intended movement with the reafferent inputs

of the actualized movements (Von Holst, 1954; Gandevia and McCloskey, 1977; Cole and Sedgwick, 1992). As a result, the cerebellum produces an error signal which is thought to be used by the motor cortex to organize subsequent movements.

1.2.4 Summary

It has been demonstrated that in the absence of afferent feedback the spinal cord is capable of generating the rhythmic alternation of flexor and extensor activity that forms the basis of locomotion. However, locomotor output must be modulated by afferent feedback regarding the state of the moving limbs, as locomotion adapts to suit the external environment. Neural control of locomotion has three different tasks: 1) generate the stereotypic locomotor movements that result in the propulsion typical for a given species, 2) to adapt these movements to the environment and the goals of the animal, and 3) to maintain equilibrium of the animal during the different body positions encountered during locomotion (Forssberg, Grillner and Halbertsma, 1980). Taken together, the spinal pattern generators alone are capable of producing basic rhythmic output. The addition of supraspinal initiation and coordination, and of proprioceptive information renders the rhythmic output successful in the ever changing environment.

1.3 Deafferentation

For more than a century, deafferentation studies have demonstrated the components of reflex, postural and locomotor movements that are independent of afferent input from the limb (Mott and Sherrington, 1895; Hering, 1897; Munk, 1909; Lassek, 1953; Twitchell, 1954; Goldberger and Murray, 1980; Grillner and Zangger, 1984; Giuliani and Smith, 1987; Goldberger, 1988a, 1988b; Taub, 1976). The bulk of the earlier deafferentation

observations were similar in that basic forms of voluntary movement were either preserved, or observed to recover rapidly, but that motor performance was generally abnormal. Yet, the role of sensory feedback in motor control remains enigmatic.

1.3.1.1 Deafferentation - animal studies

The earliest study of deafferentation was that by Mott and Sherrington (1895). After transecting the posterior roots of the forelimb or hindlimb in monkeys, they observed that movements of the hand and foot were practically abolished. Therefore, they concluded that movement initiation requires the support of afferent information and proposed that coordinated movement results from the concatenation of reflex responses. With the same animal model, Munk (1909) also observed that the deafferented limbs were not used by the animal immediately following deafferentation. However, if the unaffected limb was bound, the animal eventually managed to use its deafferented limb. Munk then concluded, in contrast to Mott and Sherrington, that use of a limb can be achieved without afferent impulses from the limb. Nearly half a century later, Lassek (1953) supported Sherrington's findings as he observed an almost complete paralysis in deafferented limbs of the monkey. The deafferented limb was not used "in supporting the body weight in the sitting position, for progress, for grasping the wire meshes of the cages, for climbing or for eating." Twitchell (1954) also observed severe motor deficits after deafferenting one arm in monkeys. However, Twitchell also observed that, within one week, considerable recovery had occurred, and Twitchell believed that deafferented limb use was the result of adapting the flexion and extension of the neck reflexes. After deafferenting the neck muscles, Twitchell observed that both neck reflexes and purposive movements disappeared. A more recent account of monkey deafferentation has shown

that not only can postoperative disuse of a deafferented limb be overcome with proper reinforcement procedures, but also that bilaterally deafferented animals may spontaneously use their forelimbs for a variety of skilled manoeuvres (Taub, 1976). This indicated that sensory input from the limbs is not strictly required, neither to initiate movement nor to perform complex motor acts, as originally believed by Mott and Sherrington.

Mott and Sherrington (1895), Lassek (1953), and Twitchell (1954) claimed that the monkey is almost paralysed after deafferentation. In contrast, Munk (1909), Hering (1897), Taub (1976), and Polit and Bizzi (1979) claimed that monkeys are far from paralysed after deafferentation, and that in fact they can perform many tasks with the deafferented limb. Therefore, the views of motor deficits after deafferentation of monkeys are conflicting. Not only did the monkeys of the various researchers show different deficits, but often they were of opposite character. In addition, what's more disturbing is that monkeys observed by one worker apparently showed different behaviour when viewed by another (cf. Mott and Sherrington, 1895 vs. Hering, 1897). The main difference between results of deafferented monkeys seems to be the extent to which the animals were forced to use the deafferented limb as they were prevented from using their normal limbs (Taub, 1976).

In more recent years, surgical deafferentation has been carried out in the cat (Goldberger, 1988a, 1988b; Grillner and Zangger, 1984; Giuliani and Smith, 1987). Goldberger (1988a) observed that during locomotion, cats with unilateral surgical hindlimb deafferentation often placed the affected foot off the runway and were unable to correct the erroneous limb placement. With learning, the animals walked along the opposite side

of the runway so that the affected limb would always make contact, but the deafferented hindlimb continued to qualitatively demonstrate abnormal limb trajectories, and the time required to cross a narrow walkway exceeded that for control animals. In an accompanying set of experiments, Goldberger (1988b) investigated the difference between spared root and complete deafferentation preparations in hemisectioned ($lumbar_1$) cats. Surprisingly, there was a dramatic difference. Animals with the $lumbar_6$ dorsal root spared were capable of walking whereas those animals with a complete hindlimb deafferentation could not. Within one week, the complete lumbosacral deafferented animals recovered walking ability. However, the kinematic profile of locomotion remained abnormal whereas the kinematic profile for the spared root animals was identical to those of normal animals. In addition, Goldberger showed that if deafferentation preceded hemisectioning by 6 months, there was no recovery of locomotion in animals with complete deafferentation while those animals with a spared root were able to recover locomotion to the same state it was before hemisectioning. Therefore, Goldberger concluded that the sensory feedback provided by the spared dorsal root is used and demonstrates greater control over locomotion than descending commands.

The effects of unilateral hindlimb deafferentation in the spinal cat on air-stepping (Giuliani and Smith, 1987) and paw-shake (Koshland and Smith, 1989) behaviours have also been examined. The main finding for the air-stepping task was a disruption of interlimb (ipsi- and contralateral hindlimbs) coordination, as a consistent phase relationship in soleus EMG between the normal and deafferented limb was no longer present (Giuliani and Smith, 1987). When investigating the paw-shake response,

Koshland and Smith (1989) observed that the hindlimb responses were decreased in duration and that cycle periods increased in magnitude and variability after deafferentation. Together these results indicate that the sensory feedback from a hindlimb is used to regulate the step-cycle duration and provides the necessary information for bilateral coordination. However, the underlying pattern of activity in the single limb seems to be driven by the central pattern generators, irrespective of that limb's sensory feedback. Grillner and Zangger (1984) showed that after unilateral dorsal root transection of the hindlimb, mesencephalic cats retain nearly normal motor patterns in the flexor and extensor muscles acting across the hip, knee, ankle and toe joints.

1.3.1.2 Evaluation of methods - animal deafferentation

The results of deafferentation studies have been varied and confusing. In hindsight, much of the inconsistencies and controversies can be attributed to the following factors. First, the classical method of deafferentation by dorsal rhizotomy is non-selective, in that it abolishes sensory feedback from all modalities. Cutting fibres from chemoreceptors and nocioceptors may produce side effects that potentially affect motor control. Second, surgical deafferentation is usually restricted to a few spinal segments because of the time required and the amount of trauma it produces. This leaves large portions of sensory inputs (i.e. contralateral dorsal roots) unaffected and able to exert reasonable control, especially for simple movements. Thirdly, this form of deafferentation is traumatic and results in shock requiring a period of recovery. Within this recovery period, motor deficits are likely to be masked by compensation as the animals learn to compensate for the loss of limb sensory feedback (through other feedback mechanisms, e.g. vision or knowledge of results; Ghez, Gordon and Ghilardi, 1995). Fourthly, the degree of motor

deficit is relative to the complexity of the movement studied.

1.3.2.1 Deafferentation - human studies

In addition to the results of animal deafferentation, patients with apparently selective large-fibre sensory neuropathies show often striking motor dysfunction (Rothwell, Traub, Day, Obeso, Thomas and Marsden, 1982; Schaumburg, Kaplan, Windebank, Vick, Rasmus, Pleasure and Brown, 1983; Sanes, Mauritz, Dalakas and Evarts, 1985). The etiology of these neuropathies was mostly unknown (Lajoie, Teasedale, Cole, Burnett, Bard, Fleury, Forget, Paillard and Lamarre, 1996), and the associated motor deficits spanned a wide range. After deafferentation, patients have a wide-based gait, difficulty in making repeated finger movements without vision, and difficulty in maintaining posture, especially when vision is excluded (Sanes et al., 1985; Rothwell et al., 1982; Sainburg, Poizner and Ghez, 1993; Sainburg, Ghilardi, Poizner and Ghez, 1995; Gordon, Ghilardi and Ghez, 1995). Introduction of an unexpected viscous load or external interference at the onset of, or during, a movement resulted in abnormal and highly erroneous movements (Rothwell et al., 1982; Sanes et al., 1985). In deafferented patients, abnormally high degrees of co-contraction are often seen, but for rapid movements, normally characterized by a biphasic or triphasic muscle activation, the EMG patterns of agonist and antagonist muscles appeared unchanged (Forget and Lamarre, 1987; Sanes et al., 1985; Rothwell et al., 1982). Deafferented patients are also less able to make accurate multijoint goal directed movements with the upper limb (Ghez, Gordon, Ghilardi, Christakos and Cooper, 1990). In one case, an even more remarkable inability to learn new motor skills was described (Rothwell et al., 1982). Yet, motor tasks requiring movement of the larger muscles and joints seem to be relatively unaffected

(Rothwell et al., 1982). However, the interpretation of these findings was again controversial as Gordon et al. (1995) and Sainburg et al. (1993) have shown that the programming of multijoint movements is critically dependent on proprioceptive information from the limb.

1.3.2.2 Evaluation of methods - human deafferentation

Even though motor performance following deafferentation has been investigated for well over a century, controversy remains as to the deficits produced by deafferentation. This may be due to the following factors. First, spontaneously occurring deafferentation is variable in the extent to which motor and sensory nerves are affected. Secondly, it is often impossible to rule out additional primary deficits in the central nervous system. Thirdly, the time between initial sensory loss and clinical recording is variable and may permit compensation for the loss of sensory feedback through other feedback mechanisms. Fourthly, performance is dependent upon the task selected for testing.

1.3.3 Summary

The following conclusions about deafferentation, regardless of the many technical troubles and inconsistencies of these experiments, are well recognized and thought to be valid. First, basic ability to produce voluntary force and move the limbs is preserved after deafferentation. However, movements are generally uncoordinated and inaccurate, especially when visual guidance is absent. Secondly, coordination of different aspects of a limb in precision tasks are impaired. Thirdly, gait is possible after deafferentation, but it is irregular and uncoordinated, and in humans, it requires continuous conscious attention. Fourthly, tasks involving simultaneous changes in several variables (i.e.

fastening shirt buttons and locomotion) and adaptation to external perturbations in movement (i.e. increased load upon arm during goal-directed movement) are not easily corrected for.

1.4 Pyridoxine-induced deafferentation

High-dose pyridoxine treatment is currently being used for various medical problems. These include conditions as diverse as Down's syndrome, migraine headache, alcoholism withdrawal seizures, carpel tunnel syndrome, diabetic peripheral neuropathy, preeclampsia edema, premenstrual syndrome, homocystinuria, hyperoxaluria, asthma, sickle cell anaemia, and, of course, vitamin B_6 deficiency (Dalton and Dalton, 1987; Bassler, 1988; Bendich and Cohen, 1990). However, overdoses of pyridoxine (vitamin B_6) cause a large-fibre peripheral sensory neuropathy, which is associated with major motor impairment (Hoover and Carlton, 1981a; Schaumburg et al., 1983; Windebank, Low, Blexrud, Schmelzer and Schaumburg, 1985).

1.4.1 Metabolic action of pyridoxine

Pyridoxine is a water soluble complex consisting of three different chemically active forms, pyridoxine, pyridoxamine, and pyridoxal (Snell, 1990), which are summarily referred to as B_6 vitamers. These compounds are metabolized in the liver into the active metabolite, pyridoxal phosphate (PLP), and a dead end catabolite, pyridoxic acid (Merrill and Henderson, 1990), which is taken up by the kidney and excreted in the urine. Pyridoxal phosphate acts as a coenzyme in over 100 PLP dependent enzymes (Dakshinamurti, Paulose, Visvanathan, Siow and Sharma, 1990). It plays an important role in biochemical transformation of numerous amino acids and in nitrogen-related

metabolism, in the form of decarboxylation, transamination, deamination, racemization, and desulfhydration (Dakshinamurti, 1982). Many putative neurotransmitters are synthesized and/or metabolized with the aid of PLP dependent enzymatic reactions. These include dopamine, norepinephrine, serotonin, tyramine, taurine, histamine, gammaaminobutyric acid, and acetylcholine (Dakshinamurti, 1977; Ebadi, 1981). Transamination of enzymes is the principal means by which defective enzyme molecules are removed and PLP is redistributed. While these multiple functions readily account for the CNS dysfunction associated with pyridoxine deficiency (Dakshinamurti, 1982), it is not immediately evident why pyridoxine neurotoxicity should be selective for peripheral sensory neurones.

1.4.2 History of pyridoxine studies

The pyridoxine neuropathy was first observed in the dog (Antopol and Tarlov, 1942; Schaeppi and Krinke, 1985; Hoover and Carlton, 1981a), and was subsequently studied in the rat (Krinke, Naylor and Skropil, 1985; Windebank et al., 1985; Xu, Sladky and Brown, 1989) and eventually described in man, either as a megavitamin syndrome (Schaumburg et al., 1983) or resulting from medical treatment (Albin, Albers, Greenberg, Townsend, Lynn, Burke and Alessi, 1987). Further, in laboratory animals, pyridoxine causes selective degeneration of large sensory axons, while sparing motor axons (Schaumburg et al., 1983; Hoover and Carlton, 1981b; Windebank et al., 1985). Clinically, it is associated with loss of proprioception and the sense of light touch, while the sensation of pain and temperature remain normal. The most striking observation resulting from pyridoxine-induced deafferentation is the dramatic motor deficits in locomotion. These deficits have been characterised as ataxic gait without loss of strength

(Schaumburg et al., 1983; Krinke et al., 1985; Xu et al., 1989). Electrophysiological testing of the resulting neuropathy in rats demonstrated a 60% reduction in single axon potential amplitude and a 17% reduction in its conduction velocity of the caudal nerve (Windebank et al., 1985). The significant reduction in potential amplitude was thought to be indicative of axonal degeneration and not demyelination. Nerve conduction studies in humans with pyridoxine neuropathy revealed that sensory action potentials were absent in the median and ulnar nerves with normal motor conduction (Schaumburg and Berger, 1988; McClachlan and Brown, 1995) and that there were quantitatively abnormal sensory thresholds and vibration response (Berger, Schaumburg, Schroeder, Apfel and Reynolds, 1992). The maximum nerve conduction velocity and the action potential amplitude observed in sensory nerves in dogs were also dramatically diminished (Schaeppi and Krinke, 1985). The selectivity of the experimental B_6 neuropathy was further confirmed in histopathological studies. In dogs and rats, neurone degeneration in the CNS was shown to be restricted to the projection regions of large-diameter first-order afferent fibres (spinal cord and dorsal column nuclei; e.g. Hoover and Carlton, 1981b; Schaeppi and Krinke, 1985). Even though pyridoxine has repeatedly been shown to selectively impair sensory, but not motor, neurones, the mechanism of pyridoxine's neurotoxic effects remain unknown (Bendich and Cohen, 1990; see Discussion 4.2.2).

1.4.3 Summary

Pyridoxine induced deafferentation is non-invasive, rapid, and avoids complications from surgical trauma associated with dorsal rhizotomy. However, the nature and extent of the motor impairment resulting from pyridoxine intoxication have never been examined in any detail. In addition, the selectivity of the deafferentation procedure for large fibre

afferents or specific sensory receptor modalities remained to be demonstrated in recordings from single afferent units. Further, the pyridoxine syndrome has not been previously demonstrated in the cat, as all experimental animal studies have so far been carried out in rats or dogs.

1.5 Objectives

1) to establish a model of non-invasive chronic large-fibre deafferentation in the cat.

2) to examine the electrophysiological deficits elicited by selective large-fibre deafferentation.

3) to examine the short-term motor deficits elicited by selective large-fibre deafferentation.

1.6 Hypotheses

1) pyridoxine elicits a selective large-fibre sensory neuropathy in the cat.

2) acute electrophysiology reveals loss of large fibre sensory function but normal motor neurone function.

3) pyridoxine-induced deafferentation results in locomotor deficits (i.e. general ataxia).

4) reflex responses to rapid stretch and behavioral responses to sudden disturbances (e.g. falls) are abolished.

Note: At the last supervisory committee meeting it was suggested that the order of presentation in the Methods, Results and Discussion should be electrophysiological material before kinematic material. The motivation for presenting electrophysiological before kinematic material was to discuss the cause (extent of sensory deprivation) before

the effects (motor deficits). In retrospect, this sequence is not necessarily the best order for two main reasons. First, the extent of sensory deprivation studied during the terminal electrophysiological experiment is not necessarily the same as the extent of sensory deprivation at the height of the motor syndrome (1 to 2 weeks earlier). Secondly, this proposed order is opposite to the chronological order of the data collection (kinematic data before electrophysiological data). Therefore, the sequence of presentation in the Methods, Results and Discussion is kinematic material before electrophysiological material.

CHAPTER TWO: METHODS

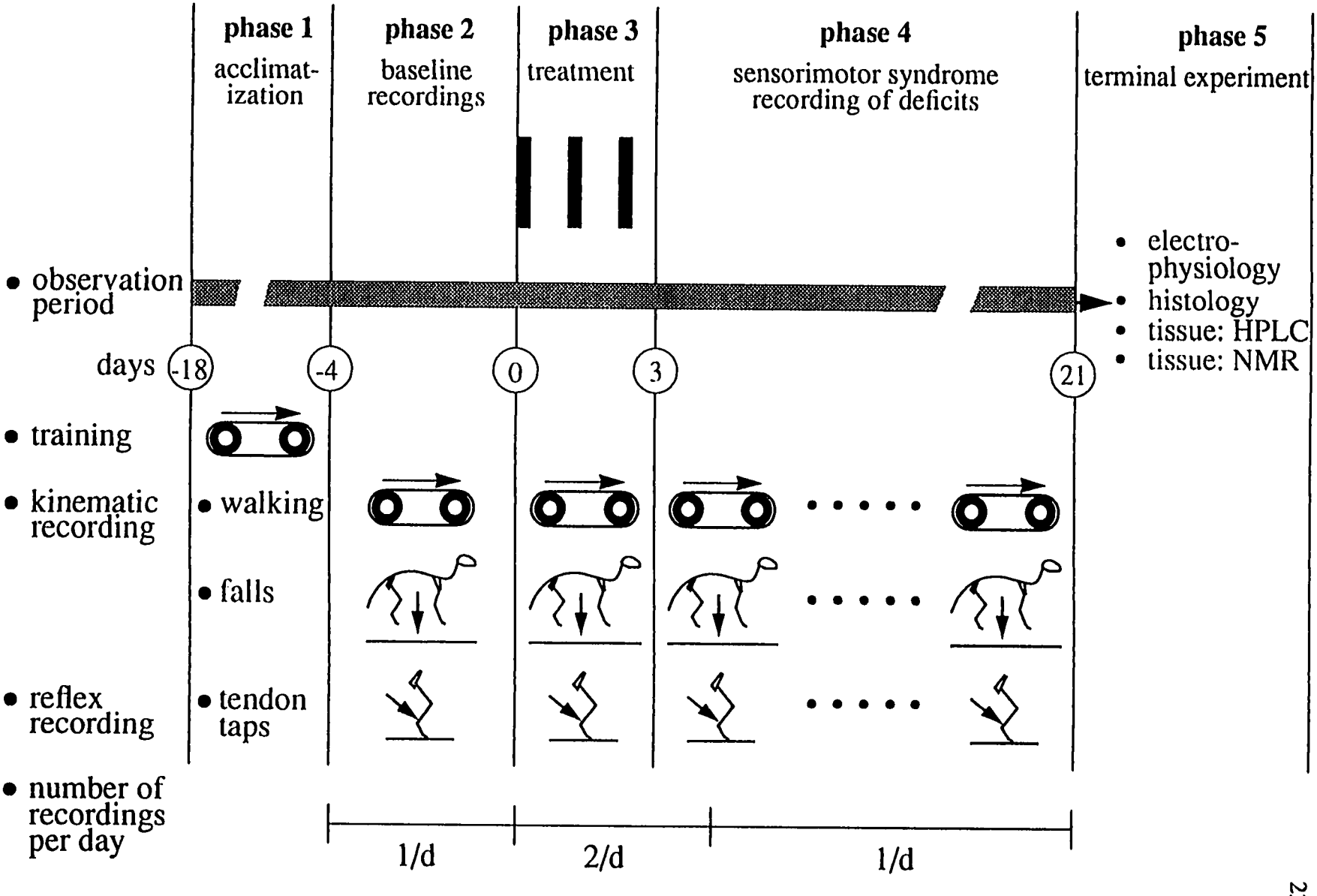
2.1 Overview of experimental studies

The general goal was to establish a model of non-invasive, large-fibre selective, sensory deafferentation in the cat. This deafferentation was induced chemically with high-dose pyridoxine (vitamin B_6) treatment rather than the conventional surgical deafferentation. The loss of sensory feedback produced dramatic motor deficits. The selectivity for large fibres of the chemically induced deafferentation was evaluated in a terminal acute electrophysiological experiment and during chronic recordings of tendon tap reflex responses. The electrophysiological experiment consisted of a battery of tests including measurements of tetanic force, and recordings of compound evoked potentials, compound vibration responses, muscle nerve neurogram responses to trapezoidal stretch, and single afferent unit characteristics. Motor deficits were studied with chronic kinematic recordings of locomotion and landing from falls.

2.1.1 Overview of experimental protocol

The experimental protocol encompassed pre-treatment, treatment, and post-treatment periods and a terminal acute electrophysiological experiment (Table 1). During the pre-treatment period, animals underwent acclimatization to the recording paradigms and were trained to walk on a treadmill. Animals were than injected with 350 mg/kg for 3 to 4 days during the treatment period and allowed to recover from the motor deficits during the post-treatment period. Throughout the pre-treatment, treatment and post-treatment periods, kinematic and reflex recordings were made. One week (cats 5,6 and 7) or 2 weeks (cats 8-16) after the height of the motor syndrome (typically day 4) a terminal

Table 1. Treatment and recording protocol. The entire protocol is divided into 5 different phases as illustrated at the top. Throughout phases 2-4, kinematic recordings of treadmill locomotion and landing from falls, and electromyographical recordings of tendon tap reflex responses are made once daily, or twice daily during the critical period as illustrated at the bottom. For 3/8 animals, phase 4 lasted from day 3 to day 10. For the remaining 5/8 animals, phase 4 lasted from day 3 to day 20 as shown here.


phase 1
acclimat-
ization
phase 2
baseline
recordings
phase 3
treatment
phase 4
sensorimotor syndrome
recording of deficits
phase 5
terminal experiment
• observation period
• electro-physiology
• histology
• tissue: HPLC
• tissue: NMR
days
-18
-4
0
3
21
• training
• kinematic recording
• walking
• falls
• reflex recording
• tendon taps
• number of recordings per day
1/d
2/d
1/d

acute electrophysiology experiment was performed (Table 2).

2.2 Animal preparation

2.2.1 Animals

The study was performed on 17 male cats, weighing between 2.5 and 5.0 kg (mean 3.38 +/- 0.62 kg; Table 2). Six out of the 17 animals served as controls and were only studied in the terminal acute electrophysiological experiment. Eleven out of the 17 animals were treated with pyridoxine. Of the 11 treated animals, 8 underwent extensive behavioral training and subsequent kinematic and reflex recordings. The other 3 treated animals only had limited kinematic and reflex recordings made to document quantitatively that they developed the same sensorimotor syndrome as the animals that were studied in greater detail. All animals, control and treated, underwent an acute electrophysiology experiment. However, in the terminal experiment for the three treated animals with limited chronic recordings, tetanic muscle force was the only electrophysiological variable measured, while nervous system tissue samples were collected. These tissue samples were collected for NMR spectroscopy analysis of alterations in nervous tissue metabolism induced by pyridoxine intoxication. All protocols were approved by the University of Calgary Animal Care Committee.

2.2.2 Animal training

The 8 pyridoxine treated animals with the extensive kinematic and reflex recordings were trained to walk at three speeds (0.2, 0.4, and 0.8 m/s) on a Plexiglas enclosed treadmill with food reward and verbal encouragement. The treadmill speeds were selected as the comfortable speed of normal overground walking (0.4 m/s), the fastest speed of normal

Table 2. Summary of animals used and their testing schedules. Also illustrated is the initial (in brackets) and final (at terminal acute experiment) masses, the number of pyridoxine injections administered to each animal, and the duration in days of the recordings. Note that cats 5-13 provided data for both the kinematic and electrophysiological analyses, while in cats 14-16, only tetanic force recordings were taken during the terminal acute experiment.

Pyridoxine-treated animals

Cat #	Mass final (initial)	# Injections (350 mg/kg)	Dates begin-end	# of Days begin - end
5	3.9 (4.6)	3	Dec 11/96 - Dec 20/96	9
6	2.95 (3.7)	3	Jan 11/97 - Jan 21/97	10
7	3.1 (3.75)	4	Jan 18/97 - Jan 28/97	10
8	3.5 (3.7)	4	Jul 8/97 - Jul 25/97	17
9	3.0 (3.4)	4	Jul 16/97 - Aug 5/97	20
11	3.0 (3.25)	4	Jul 31/97 - Aub 18/97	18
12	2.75 (3.0)	4	Aug 4/97 - Aug 25/97	21
13	3.6 (3.8)	4	Aug 11/97 - Aug 28/97	17
14	3.4 (3.55)	3	Dec 16/97 - Jan 5/98	20
15	3.95 (4.4)	3	Dec 16/97 - Jan 6/98	21
16	3.2 (3.25)	3	Dec 17/97 - Jan 8/98	22

Control animals

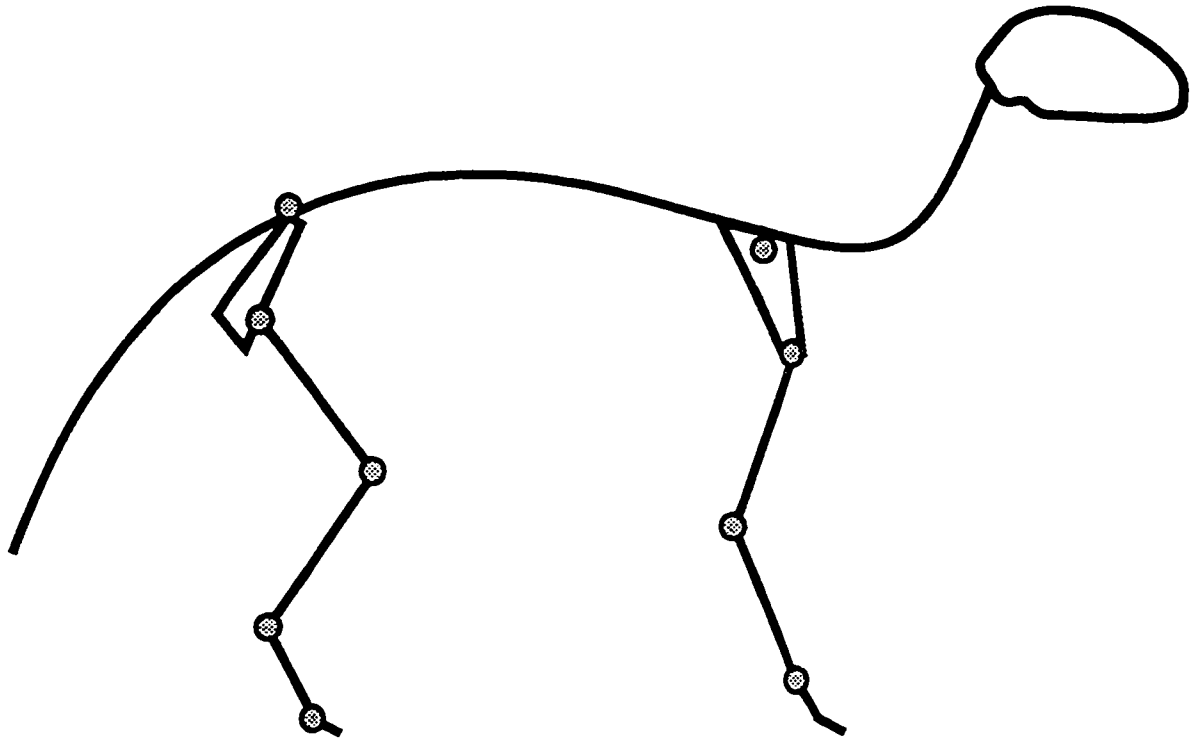
e351	3.75	0	Nov 4/96	0
e361	4.1	0	May 20/97	0
e362	5.0	0	May 28/97	0
e373	2.75	0	Dec 18/97	0
e374	3.0	0	Jan 2/98	0
e378	2.5	0	Jan 21/98	0

walking before changing to trotting (0.8 m/s) and the fastest speed of locomotion for cats at the height of the motor syndrome (0.2 m/s). These treadmill speeds were determined in pilot studies. Training sessions occurred over the period of 2-to-3 weeks before pyridoxine treatment began (Table 1). Each session lasted 30 minutes, matching the expected duration of subsequent recording sessions. Cats were trained to walk on the treadmill at three fixed speeds using food reward and verbal encouragement. This positive reinforcement was used to encourage walking at a constant speed and while positioned in the middle of the treadmill so that drifting or fluctuating walking speeds could be diminished. Upon completion of training, all animals were able to walk at the three specified speeds and were comfortable with walking for the period required for a full kinematic recording session.

2.2.3 Animal treatment and care

The entire treatment and recording period spanned either 2 weeks (3 animals; cats 5, 6 and 7) or three weeks (8 animals; cats 8-16; Table 2). Before recording sessions began, all animals received a short-lasting Halothane anaesthesia and were shaved. The joint centers of the right hindlimb and forelimb were determined by palpating bone motion during imposed movement. These joint axes and prominent bony locations (Table 3) were marked by lightly tattooing the overlying skin. The tattooed skin markers ensured reproducible marker placement between sessions. During the treatment period, animals received 3 or 4 daily intraperitoneal (i.p.) injections of pyridoxine hydrochloride (350 mg/kg; dissolved in deionized water; Sigma Chemicals) on consecutive days until motor deficits were manifest. This concentration of the pyridoxine injections yielded a pH of 2.7. At such a low pH value, animals initially showed signs of abdominal irritation upon

Table 3. Schematic illustration of kinematic marker placement. List and illustration of the bony landmarks used for kinematic marker placement during treadmill locomotion and landing from falls recordings. Note that for the hindlimb, both the ankle and 5th metatarsal locations were recorded, whereas the forelimb wrist and not 5th metacarpal location was recorded because of spatial resolution. These marker placements were kept constant between recordings for an animal by lightly tattooing the skin at these landmarks before recordings began (see Methods 2.2.3).



Forelimb medial border of spine of scapula

greater tubercle of humerus (shoulder)

lateral epicondyle of humerus (elbow)

radial styloid process (wrist)

Hindlimb spinous process of lumbar 7 vertebra (lumbar spine)

greater trochanter of femur (hip)

lateral epicondyle of femur (knee)

lateral malleolus of tibia (ankle)

head of 5th metatarsal (toe)

pyridoxine injection. The acidic irritations were then decreased with preceding local anaesthetic (Lidocaine 2%; Austin Chemicals) injections. However, even with the preceding Lidocaine injections, the pyridoxine continued to produce stomach irritations. In a recent pilot project, the pyridoxine solution was successfully neutralized using a buffer solution (Sutherland, Hulliger, Tyson and Bishop, unpublished).

Most animals developed an incapacitating motor syndrome within 3-4 days of the first administration. This motor syndrome was so severe it impaired adequate food and liquid intake over a period of about 5 days. During this time, the animals typically lost between 0.1 and 0.4 kg of body mass. Therefore, animals were carefully fed by one of the experimenters. In some animals weight loss was very rapid (within 24 h). This suggested that animals were experiencing dehydration rather than tissue loss. This was confirmed by skin palpation and observation of reduced turgor. As an anti-dehydration measure, these animals received sub-cutaneous injections of Dextrose saline solution (300 ml, once or twice per day, if necessary over a period of up to 14 days). With this treatment, all animals maintained satisfactory health and tolerated the pyridoxine treatment, and most regained pre-treatment body weight.

2.3 Kinematic recordings

Kinematic recordings of treadmill locomotion and landing from short falls were made once daily before, during and after pyridoxine treatment. Three sessions of pre-treatment treadmill walking and landing from short falls were collected as control recordings. During the critical period up to the full development of the syndrome, two daily sessions were held 12 hours apart to capture the different stages of the development of the motor

syndrome. Three dimensional motions of the body segments were recorded using a two-camera high-speed video digitizing system (Motion Analysis Corp., Santa Rosa, California) which captured the images of spherical reflective markers placed on the animal (see Table 3). The two cameras were placed at each of two corners of an isosceles triangle with 2 m sides with the third corner being the centre of the treadmill. The camera separation angle of 60° allowed for the greatest separation while maintaining high resolution of markers from both cameras. The images from both cameras were synchronized and digitized at a frame rate of 60 Hz. The laboratory coordinate system was defined as a right handed coordinate system having the x axis (longitudinal) in the direction of walking, the z axis (vertical) pointing vertically, and the y axis (transverse) orthogonal to the x and z axes, using a custom built calibration frame with 20 isotropically distributed markers. Self-adhesive spherical reflective markers were attached to specified (tattooed) anatomical locations (Table 3) of the right hindlimb and forelimb. All kinematic recordings were taken with the animals situated in the enclosed plexiglass treadmill casing and were supplemented by video recordings of the entire acquisition session for further qualitative assessments.

2.3.1 Treadmill locomotion

Treadmill locomotion recordings for each animal were taken during walking at three treadmill speeds (0.2, 0.4, and 0.8 m/s), with 10-15 trials at each speed. Individual trials lasted 3-4 seconds and were refered to as step sequences. At the slowest speed (0.2 m/s), a step sequence of 4 s would encompass 1-2 consecutive steps, while at the fastest speed (0.8 m/s), up to 6-8 consecutive steps could be recorded. Particular attention was paid to collect data only during episodes of steady-state walking at the target speed, when

the animals remained at a constant position in front of the cameras. This could only be achieved with frequent food awards and continual verbal encouragement. Even so, appreciable numbers of walking trials had to be discarded, when the criteria of walking at a constant position on the treadmill and at the target speed was not met. When locomotor ability was reduced, recordings were typically limited to the lowest speed. Under those conditions, the speed requirements were sometimes relaxed to allow animals to walk at a speed slightly below the target speed to obtain a minimum set of recordings at the lowest speed. Otherwise no recordings of pathological locomotion would have been available for a given session. However, even under relaxed speed conditions, a steady speed and constant location in front of the cameras were still required. At the height of the motor syndrome, most animals were unable to walk, and no locomotion recordings could be collected.

2.3.2 Landing from falls

The animals' landing responses from short falls (15 cm) were recorded after each locomotion recording while the animals were still wearing the reflective markers. The animals were lifted by an experimenter until their backs were touching the ceiling of the treadmill casing (approximately 15 cm) and then suddenly released. Recordings were taken for a 4 s episode, with the drops timed to occur approximately at the middle of the recording.

2.4 Electrophysiological recordings

2.4.1 Tendon tap reflexes

Tendon tap reflex responses were tested once daily before, during and after pyridoxine

treatment. Three recordings of pre-treatment tendon tap reflex responses were collected as control recordings. During the critical period up to the full development of the syndrome, two daily sessions were held 12 hours apart to capture the different stages of the development of the motor syndrome.

Tendon tap reflex responses were elicited by light finger taps on a hand-held, custom-built transistor switch mounted on a light plastic beam with a notch matching the curvature of the Achilles tendon. Reflex responses were recorded electromyographically using self-adhesive surface electrodes attached longitudinally over the lateral gastrocnemius muscle of the left hindlimb. The EMG signal was amplified using optimized gain settings (typically a gain of 3,000), band-pass filtered (10 Hz - 1 kHz), and sampled with a digital oscilloscope (80 kHz) for a 50 ms period following a trigger signal generated by the mechanical switch. Tendon tap responses were initially recorded when the animals were maximally relaxed, resting on the lap of the experimenter. The recordings were then repeated during standing, when the animals supported most of their body weight by their hindlimbs as the fore-trunk was raised by the experimenter. The addition of the standing tendon tap reflex monitoring was used to eliminate the possibility that reflexes could be absent because of a lack of background motoneurone pool activity. Each recording session consisted of at least 20 mechanical stimuli and recorded reflex responses for each paradigm, relaxed and standing, throughout the recording period.

2.4.2 Acute terminal experiment

The purpose of the terminal acute electrophysiological experiment was to examine the function of the peripheral nervous system both at single unit and ensemble levels. The

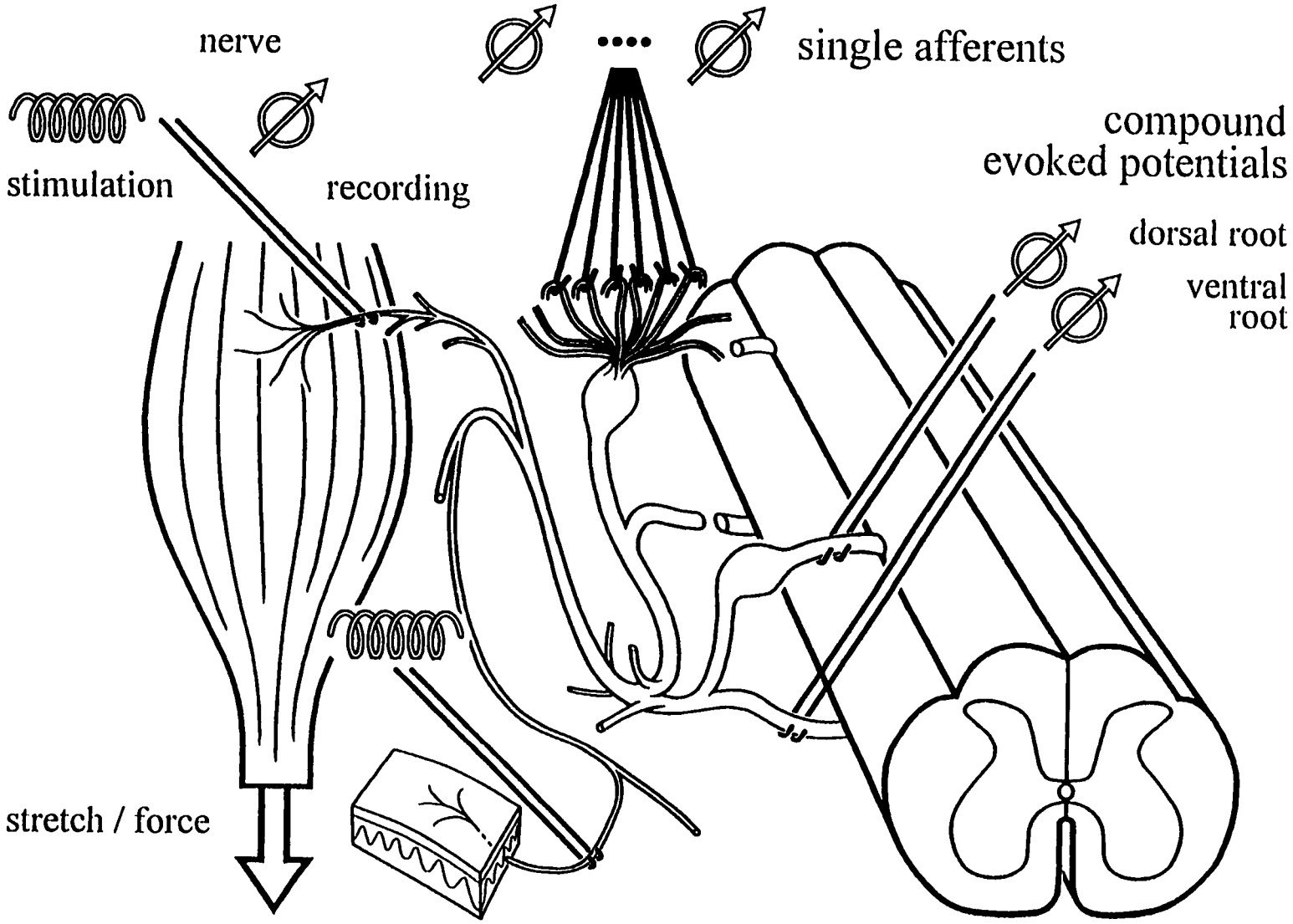
purpose of recording ensemble activity (compound evoked potentials and muscle nerve neurogram responses) was to allow the assessment of the functional status of populations of sensory receptor afferents and motor neurones. Further, recordings of single afferent unit responses demonstrated which different receptor categories and sensory modalities may or may not be affected by pyridoxine neurotoxicity.

2.4.2.1 Cat preparation

The terminal acute electrophysiological experiment in the cat was studied using a medial gastrocnemius nerve-muscle preparation, as shown in Figure 1 (Matthews, 1972). All procedures were carried out under general pentobarbitone anaesthesia that was induced (40 mg/kg i.p.) and maintained (variable intermittent doses intravenously (i.v.)) using the barbiturate pentobarbitone. Fluid balance was maintained with a low-rate Dextrose infusion (i.v.). Animal body temperature was continuously monitored and maintained at 38° C using a thermistor in series with a servo-controlled heating pad. The left carotid artery was cannulated and linked to a commercial pressure gauge with a custom designed electronics interface, allowing visual and auditory monitoring of mean arterial blood pressure. Any significant decline in mean arterial blood pressure was counteracted with small dose injections of dextran (Macrodex, Pharmacia; i.v.). In the event of marked, and sustained, blood pressure drops, even after administration of dextran, adrenergic drugs were utilized. Care was taken to record all measurements up to, and including, the neurogram responses to trapezoidal stretch before using adrenergic drugs. This approach was taken because adrenergic drugs have been shown to exhibit adverse effects on muscle circulation (Goodwin et al., 1972). A tracheotomy was performed immediately after induction of anaesthesia. At this time, animals were artificially ventilated by an

Figure 1. Schematic representation of medial gastrocnemius muscle-nerve preparation in the cat. The lumbar spinal column and the medial gastrocnemius muscle are fully exposed and the medial gastrocnemius nerve is fully denervated along its length (except for CP for the duration of the CEP recordings) to prevent noise artifacts from other nerve afferents. Compound evoked responses were recorded from dorsal and ventral roots preserved in continuity. Muscle nerve neurogram responses were recorded with a switchable electrode placed on the distal segment of the muscle nerve. Single afferent units were isolated in fine dorsal root filaments following dorsal rhizotomy.

Nerve-Muscle-Skin Preparation



infant respirator (3:1 air:oxygen mixture). This facilitated maintenance of stable circulation. In addition, the artificial ventilation allowed the experimenter to regulate the respiration gases to accommodate the cats' need for more or less oxygen and prevent acidosis or alkalosis of the blood.

The left medial gastrocnemius muscle was isolated with intact nerve supply, while all other peripheral nerve branches (except the common peroneal and superficial peroneal) with the same dorsal root origins (L_7 and S_1) were denervated. The wide denervation facilitated isolation by microdissection of functionally single sensory afferent axons in the L_7 and S_1 dorsal roots. Table 4 lists the denervated peripheral nerve branches and resected muscles. The Achilles tendon was kept intact while resecting the lateral gastrocnemius, soleus and plantaris muscle bellies. This conservative dissection approach minimized mechanical damage to the medial gastrocnemius tendon during tetanic activation of the parent muscle. The distal aspect of the semitendinosus muscle was lifted to prevent compression injury to the underlying medial gastrocnemius (MG), common peroneal (CP), and superficial peroneal (SP) nerves. In addition, light tension was applied to the distal aspect of a parallel trunk of the tibial nerve, as this provided slack in the MG nerve and helped avoid overstretching or strain of the nerve. The belly of the medial gastrocnemius muscle, distal to its origin, was separated from the surrounding fascia, to allow unrestricted muscle contraction and to guarantee optimal tetanic force recordings.

Once the MG muscle was completely exposed, the relation between muscle length and joint angle was determined. A reference marker was tied around the distal MG tendon

Table 4. Denervated nerves and resected muscles. Index of the muscles and the associated nerve pathways of the left hindlimb denervated during the medial gastrocnemius nerve muscle preparation.

Muscles	Nerve Branches	Root Supply
Gluteus Medius, Gluteus Minimus, Gemellus Superior, Tensor Fasciae Latae	Cranial Gluteal Deep branch to Gluteus Medius	Lumbar (L) $_7$ Sacral (S) $_1$
Gluteus Maximus, Caudofemoralis	Caudal Gluteal	L_7, S_1
Pyriformis	Pyriformis	L_7, S_1
Quadratus Femorus, Obturator Int.	Inferior Hemorrhoidal	L_7, S_1
Tail muscles	Pundental	S_1, S_2, S_3
Biceps Femoris	Caudal Femoral Cutaneous Hamstrings branch	S_1, S_2, S_3
Semimembranosus, Semitendinosus	Hamstrings branch	L_7, S_1
Tenuissimus	Tenuissimus	L_7, S_1
Lateral Gastrocnemius, Soleus	Caudal Cutaneous Sural	L_7, S_1
Popliteus, Plantaris, Flexor Digitorum Longus, Flexor Hallucis Longus, Tibialis Posterior	Tibial branch	L_7, S_1
Peroneus Longus, Tibialis Anterior, Extensor Digitorum Longus	Common Peroneal branch	L_7, S_1

and a corresponding marker was sewn into deep muscles surrounding the tibia when the MG muscle was at mid-physiological length (i.e. limb position while standing). MG muscle mid-physiological length corresponded to a knee flexion of 135° and an ankle dorsiflexion of 90°. A small fragment of bone and attached MG tendon were excised from the calcaneus and fixed to the moving shaft of an electromagnetic stretching device. The medial gastrocnemius nerve was dissected free over 2-3 cm and carefully cleaned from connective tissue to create optimal conditions for whole nerve neurogram recordings (see below Methods 2.4.2.5). In addition, the common peroneal and the distal cutaneous portion of the superficial peroneal nerve were exposed in 2 cm segments for subsequent electrical stimulation.

In parallel with the hindlimb dissection, a paravertebral muscle myotomy (L_1-S_3) and vertebral laminectomy (L_2-S_3) were performed. These surgical interventions generated additional space for the splitting plate and microdissection. The dorsal and ventral roots of L_7 and S_1 segments were carefully freed from connective tissue and mobilized. Initially, they were preserved in continuity to maintain circulation of the roots. The remainder of the exposed roots were cut to facilitate the arrangement of a cross-talk free montage of filaments for in-continuity recording of compound evoked potentials.

Subsequently, the animal was fixed to the stereotaxic frame with opposing stainless steel pins at the hip, and ipsilateral left knee and ankle joints. The skin in the lower back and left leg (symmetrically transected during the surgical procedure) was pulled vertically over the length of an overhanging frame (modified semi-circular shape), creating back and leg pools. During the placement of the stainless steel pins, the left hindlimb orientation

comprised a hip angle of 180°, knee angle of 135°, and an ankle angle of 90° (as noted above). This configuration allowed for an MG shortening and lengthening range of approximately 12 mm from the physiological mid-range position, and created large and managable back and leg pools. The back and leg pools were filled with paraffin oil to provide a non-conducting medium for the electrical stimulation of MG, CP and SP, and the isolated recording of bioelectric activity (i.e. compound evoked potentials, neurogram recordings, and single unit action potentials).

2.4.2.2 Tetanic force

The exposed medial gastrocnemius peripheral nerve was placed on one arm of a spring mounted silver wire stimulation/recording bipolar electrode while the other arm was positioned as a reference into a neighbouring deep muscle. First, the minimal threshold for electrical activation of MG alpha axons (T_α) was determined by stimulating the MG nerve at 10 Hz, using stimulus pulses of a 0.05 ms duration, while observing evoked potentials in ventral root recordings and muscle twitching in force recordings. Maximum tetanic contraction was then estimated, typically from six successive trials with repetitive stimulation at 30 Hz (over 2 s) of the medial gastrocnemius nerve at 10 x T_α. During tetanic force recordings, the muscle was kept at a fixed intermediate length corresponding to an ankle angle of 90°. The resulting force was measured with a commercial load-cell (Kistler Instrumente, AG Winterthur, Switzerland) mounted on the shaft of the electromagnetic stretching device and a custom built amplifier whose output was recorded with a digital volt meter.

2.4.2.3 Compound evoked potentials

Compound evoked responses to peripheral nerve stimulation were recorded antidromically and orthodromically in the ventral and dorsal roots, respectively. In both control and pyridoxine treated animals, the stimulus strength was calibrated exclusively as multiples of the minimal alpha threshold of the MG nerve, rather than the minimal absolute threshold of each individual nerve. The rationale for choosing this threshold was the expectation that, in dorsal root recordings, the thresholds of the largest functioning sensory axons in pyridoxine treated animals would be significantly higher than in controls, since the largest fibres would be selectively impaired after pyridoxine intoxication. Moreover, the magnitude of this effect was unpredictable, as the extent of large-fibre impairment could not be foreseen. These predictions were fully corroborated, as is illustrated in Results (Figures 22-25).

The L_7 and S_1 ventral roots were left intact and mounted in parallel on separate branches of a multi-branch recording electrode that was equipped with head-stage amplifiers close to the source of the signal. Before responses were recorded, the dominant projection of the MG nerve to either of the two spinal ventral roots, L_7 or S_1, was determined. Root dominance was determined as the root with the largest compound evoked potential at about 5 times α threshold. Systematic measurements were made only for the dominant root, typically the S_1 root for the medial gastrocnemius nerve. The α-motoneuron threshold was determined as the minimal electrical stimulation required to elicit an action potential in more than 50 % of the stimulations, as observed on a stimulus-triggered oscilloscope display. Average compound evoked potentials for 128 stimulations were recorded at stimulus strengths of 1, 1.1, 1.5, 2, 5, 7, 10, 15 and 20 times α threshold by an on-line digitizing Tekscope (sampling at 200 kHz) and saved on network computers

for later off-line analysis.

Following the ventral root antidromic compound evoked potential (CEP) recordings of the dominant root, orthodromic responses were recorded at the dorsal roots during electrical stimulation of the nerves. The dominant root recorded from during MG ventral recordings was also used for the dorsal root recordings, usually S_1. The L_7 dorsal root was typically used for the CP and SP compound evoked potentials. Dorsal root CEP recordings from MG, SP, and CP electrical stimulation were taken for the same stimulation strengths as for the ventral root recordings.

All CEP recordings were amplified (typical gain of 2,500 for control and 20,000 for treated animals), band pass filtered (10 Hz - 4 kHz), and transmitted from the digital oscilloscope to the Sun Sparc workstations (IPC, Sun Microsystems, Mountain View, CA) for later off-line analysis.

2.4.2.4 Vibration

Responses to whole muscle mechanical vibration were collected and averaged in a similar manner as the electrically evoked compound evoked potentials. Exceptions to the recording protocol were that (for greater ease of interpretation of responses, see Results 3.3.4) the sampling window spanned 2 stimulus cycles, the sampling rate depended on vibration frequency (200 kHz for 100 Hz; 40 kHz for 25 Hz), and, for improved signal to noise ratio, 1024 individual responses were averaged. The dominant root that was recorded from for MG CEP's, was placed on the multi-channel recording electrode while the stretching device was positioned so that the length of the MG muscle was -4 mm (0

mm being physiological mid-range length) to prevent muscle nerve conduction block. The muscle was vibrated using the electromagnetic stretcher with length feedback and second-order low-pass filter characteristics (corner frequencies at 50 and 100 Hz; Baumann and Hulliger, 1991). Repetitive sinusoidal command signals were generated with a custom built hybrid signal generator with 4096 x 4096 resolution (Banks et al., 1997). At each frequency of vibration (25, 33, and 100 Hz), the gain of the electromagnetic stretcher's input amplifier was adjusted to calibrate the largest amplitude of vibration (250 micrometers), relying on the output of the length transducer, which was linear within 1 % error. Vibration of smaller amplitudes (50 and 100 micrometers) were generated by linear attenuation of the sinusoidal commands, using custom built hardware. Standard TTL pulses generated by the signal generator demarcated sinusoidal cycles and were used to trigger the averaging device. The average of 1024 ensemble MG afferent responses to each vibration paradigm was suitably amplified (typical gain of 5,000 for control and 50,000 for treated animals), band-pass filtered (10 Hz - 4 kHz), and transmitted from the digital oscilloscope to the Sun Sparc workstations for later off-line analysis.

2.4.2.5 Muscle nerve neurogram recordings

Ensemble MG afferent activity was recorded during repetitive trapezoidal stretch of the parent muscle (at 0.125 Hz), using the spring mounted nerve electrode in recording configuration. To this end, the electrode head piece was connected to a custom built miniature head stage amplifier (with unitary gain), which was an integral part of the electrode, located less than 3 cm from the nerve. The headstage output signal was amplified and filtered in a cascade of three analogue amplifiers that provided band-pass

filtering (2 Hz - 4 kHz), global amplification between 10,000 and 100,000, and moving average filtering using a custom built 7th order Butterworth low-pass filter design (Platt, Hajduk, Hulliger and Easton, 1998). Individual neurogram responses to a single trapezoidal stretch of 8 mm peak-to-peak amplitude were digitized at 2 kHz using an LSI 11-73 computer. This computer system had a customized A/D interface with the options of offset correction and additional digital amplification. Typically, 10 consecutive responses were collected, with the muscle at a length of -4 mm, and transferred to Sun Sparc workstations for off-line averaging. Neurogram recordings were always made after L_7 and S_1 ventral rhizotomy, following the recording of the evoked compound responses in the ventral and dorsal roots. Ventral rhizotomies were performed before the muscle nerve neurogram recordings to knock out the β and δ fusimotor neurones. This surgical intervention prevented fusimotor drive from influencing the gain of the muscle spindle afferents.

2.4.2.6 Single afferent units

Microdissection of dorsal roots (L_7 and S_1) was used to isolate and characterize single MG afferents. The roots, initially preserved in continuity, were systematically screened one-by-one by examining and repeatedly subdividing small natural subdivisions (rootlets). The rootlets were cut sequentially, but only immediately before its screening began. This strategy maintained the stability and vitality of the rootlets over the duration of the recordings (potentially 12 hours), since rootlet fragility increased, and stability decreased, with increasing time after proximal rhizotomy. By doing so, single unit yield was appreciably increased, and possible bias due to axon-size dependent vulnerability minimized. For screening purposes, responses to both natural and electrical stimulation

were recorded with the same multi-branch silver electrode used for compound potential recordings.

The screening procedure was based on a combination of several monitoring techniques that were designed to facilitate detection and classification of single units, and to minimize the traditional bias of the isolation by microdissection for large mechano-sensitive afferents. The responses to muscle stretch that were recorded in multi-unit filaments were monitored using video and audio displays of the raw signal, and video displays of the low-pass filtered signal (50 ms moving window average, same 7^{th} order Butterworth filter as for the neurogram recordings). This signal processing detected the activity of stretch sensitive afferents hidden in non-stretch related multi-unit background activity. In addition, responses to electrical stimulation of the MG nerve (between 1 x T_α and 50 x T_α) were screened, using both single sweep displays of the responses to individual stimuli, and periodically updated displays of stimulus triggered averages continually computed over a fixed number (10-30) of the most recent response cycles (using a custom built averaging device). When MG afferent units were detected, the parent filament was further subdivided until all-or-none action potentials could unambiguously be identified and conduction latencies measured. Since the goal was to collect large samples of single units, a more time consuming systematic identification of receptor type (in terms of sensory modalities) was not always attempted. Yet, all units were broadly classified regarding mechano-sensitivity. In addition, for pyridoxine treated animals, whenever axons with conduction velocities above 30 m/s were isolated, they were classified as spindle or Golgi tendon organ afferents depending on their discharge characteristics during the rising phase of a maximal muscle twitch (Matthews, 1972),

while the dividing line between Ia and spindle secondary afferents was taken to be at 72 m/s. Given the time constraints, a more rigorous identification of primary and secondary spindle afferents on the basis of demonstrable innervation by dynamic fusimotor efferents was not realistic. Conduction distance was recorded at the end of the experiment so nerve conduction velocity could be calculated, thus, allowing for size classification of the single afferent units based on their conduction velocities.

2.5 Histological preparation

At the end of the acute electrophysiological experiment, peripheral nervous tissue samples (bilateral ventral and dorsal roots (L_6-S_1), medial gastrocnemius, common peroneal and superficial peroneal) were removed and stored in 2.5 % glutaraldehyde for subsequent histological analysis. The animals were then perfused with saline (5 minutes) and 4% formalin/saline solution (6 hours) using an intracardiac cannula. About 12 hours later, the cadavers were dissected. The brain and spinal cord were removed and kept in 4% formalin/saline solution for histological examination.

2.6 Analysis

2.6.1 Kinematic analysis

Two raw data files of the kinematic recordings (one from each camera) for each acquisition trial (treadmill locomotion and fall response) were preprocessed using the Expert Vision software (Motion Analysis Corp., Santa Rosa, CA). This preprocessing used a direct linear transformation method to determine the 3-dimensional positions of each of the markers in each frame of data. Gaps in tracked marker data were estimated by linearly interpolating between measured points. No further filtering was performed

during the tracking process. Such pre-processed data were kept in permanent files with the temporal sequences of 3-dimensional marker position coordinates (x, longitudinal; y, transverse; z, vertical). These files were used for all subsequent graphic displays of trajectories, for calculation of basic parameters of individual marker records (maxima, minima, excursion amplitude, etc.), and for the analysis of trajectory path length (see below Methods 2.6.1.1) using the Kintrak analysis software (Motion Analysis Corp.) and customized software on Sun Sparc workstations.

2.6.1.1 Locomotion

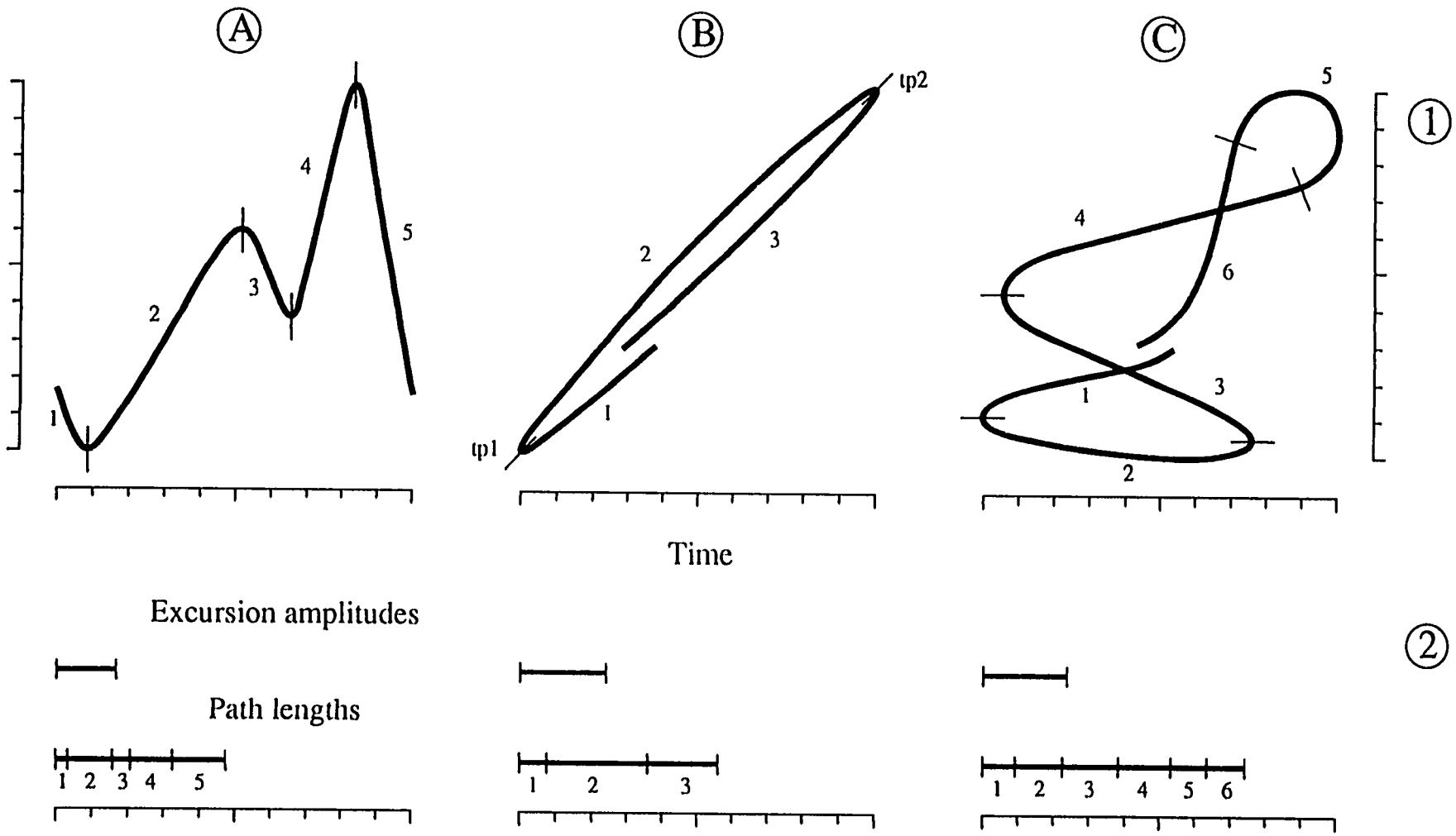
Preliminary analysis of treadmill locomotion using conventional joint angle analyses (e.g. joint angle displacement curves) did not portray the absolute magnitude of differences we observed, qualitatively, between control and pyridoxine recordings. One of the striking features of locomotion after pyridoxine treatment was the increased abnormality and variability of forelimb and hindlimb paw excursions in all 3-dimensions (see Results 3.2.1.2). Movement trajectories were represented as graphs of sequences of digitized coordinates, plotted as pairs or triplets in two or three dimensions. Ideally, for individual steps, such graphs should form closed loops. In practice this was rarely the case due to drifts in transverse and/or longitudinal position. These drifts led to offsets between start and end points in these coordinates (see Figure 6). Since the shape of these trajectories typically was complex, even for normal walking (see Figure 6.A), it was desirable to supplement univariate measures of trajectory size (e.g. excursion amplitude) by a more comprehensive multivariate measure. The path length of trajectories appeared to be a suitable measure since, for instance, during walking the path described by a paw is influenced by the cyclic motion of the limb and the concomitant postural alterations, and

since the shape and size of the trajectory are the result of coordinated movement in an appreciable number of joints.

Theoretically, multi-dimensional path length is defined as the integral of the RMS value of the first derivatives of the original variables in parametric representation (in the current application: x, y, z coordinates as a function of time). This can be approximated by calculating the sum of distances between successive sample points, where distance is defined as the RMS average of the increments of each coordinate. This approximation is, in fact, preferable over strict computation as a path integral, since differentiation of the coordinate functions would introduce appreciable noise, given the limited resolution of signals sampled at a finite rate (60/s), and since it would require piece wise continuous curve segments to be defined (e.g. by fitting suitable describing functions).

Path length was also calculated for one-dimensional signals plotted against time (individual coordinate functions or coordinate difference functions) to compare the power of discrimination (between pathological and normal walking) of one-dimensional with two- or three-dimensional measures of trajectory path length (cf. Figure 2.A.1 with 2.C.1, and see Figures 8-10). On the above definition of multidimensional path length, in the one-dimensional case, the path length is measured as the sum of the absolute differences between successive sample points, or as the sum of the distances between successive local minima and maxima (see Figure 2.A). All calculations of path length were carried out with customized software for this project, using C, and implemented on a Sun Sparc workstation.

Figure 2. Schematic illustration of path length analysis method. Column A: excursion profile of a single variable, plotted against time. Column B: simple phase diagram of two variables with similar temporal profiles and minimal phase shift (not illustrated), plotted against each other. Column C: phase diagram of complex shape, obtained by plotting two functions with very different temporal profiles against each other. Row 1: idealized profile (A) and phase diagrams (B,C) of kinematic recordings. Row 2: bar diagrams of excursion amplitude, minimum path length and measured path length of the graphs of row 1. Same scale (in arbitrary units) for all graphs of row 1. In row 2, same units, but scaled down for clarity (1:6). The graphs in row 1 were segmented arbitrarily to facilitate comparison with the measures of path length in row 2 (with small numbers identifying consecutive segments). In the 1-dimensional case (A), the path length is calculated as the sum of the absolute differences between adjacent minima and maxima (vertical bars). For the two-dimensional trajectories in B and C, the path length is calculated as the sum of distances between consecutive sample points. The 1-dimensional excursion amplitude simply is the difference between absolute maximum and minimum. The 2-dimensional excursion amplitude (B.2, C.2) is the root mean squared average of the excursion amplitudes in each dimension (for simplicity the same for both variables). The shortest possible path is that of a straight line trajectory between the extreme turning points (in B.1, from tp1 to tp2 and back). Hence the minimum theoretical path length is twice the 2-dimensional excursion amplitude. Note, that for the simple elliptic trajectory in B, measured and minimum path length are nearly the same. In contrast, for the complex trajectory in C, the measured path length is much longer, illustrating the sensitivity of the path length measure to complexity and distortion of shape.


A
B
C
1
2
3
4
5
tp1
tp2
6
Time
Excursion amplitudes
Path lengths
1 2 3 4 5
1 2 3
1 2 3 4 5 6
Measures (1:6)
①
②

The measurement of the path length was the method of choice since this measure is sensitive to both excursion amplitude and complexity of shape (e.g. distortion and presence of multiple loops). This feature of shape sensitivity is illustrated in Figure 2.B and 2.C, where the path lengths of idealized two-dimensional trajectories are compared, one with simple and one with complex shape. These trajectories were drawn so as to have identical excursion amplitudes in either dimension. For the narrow elliptic trajectory of Figure 2.B, the path length is practically identical with the minimum possible path length (i.e. twice the two-dimensional amplitude; see bar graphs in 2.B.2). In contrast, for the more complex trajectory of Figure 2.C, the effective path length is 1.65 times the minimum path length (bar graphs in 2.C.2).

The three dimensional coordinate system of x, y, z axes were analyzed with two different frames of reference. The first approach used 'external space' as the frame of reference. Therefore, limb motion was measured relative to a fixed frame of reference (i.e. treadmill). This frame of reference proved to be inadequate because it could not accommodate differences in drift along the transverse and/or longitudinal axes. As a result, a 'body referenced' coordinate system was used. This approach subtracts the forelimb trunk movement from the forelimb paw movement and the hindlimb trunk movement from the hindlimb paw movement, allowing analysis of limb motion to be independent of whole body drift motion.

Individual step cycles were demarcated separately for forelimb and hindlimb step sequences using interactive Kintrak analysis software on a Sun Sparc workstation. A step cycle was defined as the data between two consecutive peaks of negative vertical (z)

velocity of the wrist marker of the forelimb and the metatarso-phalangeal marker of the hindlimb. The peak of negative velocity was chosen as the empirically most reliable indicator of paw touch-down at the beginning of the stance phase of the step cycle. Visual displays of the original vertical and longitudinal coordinate records were used for the evaluation and, if necessary, adjustment of the demarcation settings generated by the velocity-peak detection algorithm.

2.6.1.2 Landing from falls

The vertical displacement of the lumbar spine marker was used to measure the magnitude of yield during landing from short falls. Yield was calculated as the difference between maximal and minimal vertical displacement during a 1 s period following time of foot contact.

The moment of paw contact during landing from a short fall was also identified interactively, again relying on the peak negative vertical velocity of the hind toe marker using the same Kintrak software. The only difference to the step cycle demarcation was the shape of the velocity profile, as landing was followed by much more extensive yield and, hence, a longer phase of negative velocity before the velocity returned to zero during stance.

2.6.2 Tendon tap reflex responses

Since the magnitude and presence of tendon tap responses during the relaxed condition were inconsistent with those recorded during the standing condition (see Results 3.3.1), only tendon tap reflex responses for the standing condition were analyzed systematically.

Digitized EMG records were transferred to the laboratory computer network using a serial interface. They were analyzed, cycle by cycle, by measuring mean root mean squared (RMS) signal magnitude during a fixed time window using customized software written in Matlab (Math Works Inc., Natick, Mass). RMS values of baseline EMG and of EMG during the response were calculated during non-overlapping windows. The size of a tendon tap response was calculated relative to its baseline EMG activity and defined as a ratio between the two. For each session, a mean and standard deviation was calculated from the relative response ratios.

Since the precise position of the EMG electrodes may have varied slightly between recording sessions, and since the duration of the response tended to increase with increasing severity of the neuropathy after pyridoxine treatment, the analysis windows were individually set for each session and animal. These window settings were called sessional global window settings. The baseline EMG analysis window fell into the period of the response latency. The reflex response window was set interactively by estimating mean onset and termination latencies from a display of the superimposed raw and/or rectified responses to all individual taps of a recording session (approx. 25 trials; see Figure 20.1, inset). The reliability of the interactive global window setting was established in pilot studies by comparing the results with those obtained from interactive window settings on a trial by trial basis. There were no differences between window setting approaches, and the global setting diminished the effects of any arbitrary preferences of the experimenter. For sessions where the tendon tap responses were minimal, inconsistent or absent, the average window settings of baseline and reflex response EMG, for that animal across all previous recordings, were calculated and used

instead.

2.6.3 Compound evoked potentials

To compare the electrically evoked compound responses between animals, all responses were normalized to convenient (and approximately average) conduction distances between the MG, CP, and SP nerve electrode and the root recording electrode at the level of the L_7 or S_1 ventral and dorsal roots. Normalization was performed by compressing or expanding the individual records with the appropriate scale factors (standard/measured conduction distance). The standard distance of 180 mm was used for the normalization of MG and CP CEP's, and the standard distance of 240 mm was used for the normalization of the SP CEP's. No attempts were made to measure the CEP size because the difference in CEP magnitudes between control and treated animals was equivalent to an all-or-none phenomenon. The controls showed normal CEP waveforms, but the pyridoxine treated animals experienced a total abolition of CEP responses.

2.6.4 Vibration responses

The magnitudes of the vibration responses were calculated in a similar manner to tendon tap responses, i.e. as a mean RMS estimate of the compound response waveform recorded in the dominant dorsal root (L_7 or S_1). However, for the vibration response, the absolute response magnitude was calculated with an analysis window that spanned the two cycles that were recorded for greater ease of interpretation of results.

2.6.5 Statistical tests

Statistical analyses of trunk crouching and paw clearance, path length, and landing from

falls data were carried out. Before landing from short falls were performed, it was assumed that there would be an increase in yield after pyridoxine treatment. Thus, a directional (one-tailed) two sample t-test was used to analyze the equality of the means (Woolson, 1987).

Path length analysis was applied to the kinematic recordings of treadmill walking trials. Recorded trials consisted of sequences of two or more consecutive step cycles. In pilot studies, the steps within an individual sequence were shown to be not independent. As such, the path length measures for individual step cycles could not be used as genuinely independent. Thus, for each sequence of steps, the mean path length was calculated. While this reduced the number of observations and thus the statistical power of any results, calculating mean path length per step sequence at least permitted statistical analyses to be performed. Since directionality (i.e. a consistent increase or decrease in observations) could not be assumed for the path length data, non-directional (two-tailed) tests were used.

To examine whether there was a change in the variance of the independent path length measures between control and treatment conditions, an F-test for the equality of variances was used (Woolson, 1987). It was found (see Results 3.2.1.2) that for the majority of measures there was a statistically significant change in variance ($p=0.05$). Therefore, a Cochrane-Cox t-test for equality of means was used. This is a modified t-test which compares the means of two normally distributed independent groups of data when the population variances are not assumed to be equal. This test tends to be conservative, i.e. if the test is carried out at $p=0.05$, the true level of significance may be less than in the

case where the variances of the populations are equal.

Graphic displays were generated with public domain (ACE/gr, P.J. Turner, Beaverton, Oregon) and commercial (FrameMaker 2.0, Frame technology Corp., San Jose) software packages.

2.6.6 Histological analysis

After removal of the nervous tissue samples, the cerebellums of a control animal and the animal with the most severe sensory deafferentation (as verified by the electrophysiological experiment) were placed in 70% ethanol overnight, dehydrated and embedded in paraffin. Coronal cerebellar sections were cut at 10 mircrometers and mounted onto glass slides. For both animals, the first series of sections was Nissl stained by using cresyl echt violet, while a second series of similar sections was stained using Mab *anti-zebrin II* (Brochu, Maler and Hawkes, 1990: for full methods of immunocytochemistry see Eiserman and Hawkes, 1993). Images were captured digitally and montages were prepared in Adobe Photoshop 4.

CHAPTER THREE: RESULTS

3.1 Overview

Overdoses of pyridoxine consistently elicited a severe sensorimotor syndrome in 11 out of 11 treated cats, following intraperitoneal injections of pyridoxine (350 mg/kg) on three or four consecutive days. An incapacitating motor dysfunction was observed within 3-4 days from the first injection. The symptoms of the resulting motor syndrome from pyridoxine intoxication included abnormal limb excursions, crouched gait and disrupted interlimb coordination. At the very height of the motor syndrome, most animals were unable to stand, walk on a treadmill or maintain stable posture upon landing from short falls.

Although the animals were unable to use their limbs in the coordinated manner necessary for walking or standing, they contracted their muscles vigorously in uncoordinated scrambling or crawling movements, or when reacting to undesired manipulation. For instance, when their hindlimbs were fully extended by an experimenter, they tended to react with energetic limb withdrawal or forceful kicking movements. Qualitative observations from these withdrawal responses and measurements of tetanic forces indicated that motor dysfunction was not due to a loss of strength arising from muscle or motoneuron dysfunction. Qualitatively, withdrawal responses elicited by moderate pinching of the animals' paws were as vigorous as before treatment, and gastrocnemius EMG activity during assisted standing appeared to be normal. Tetanic force in hindlimb MG muscle as well as antidromic compound potentials recorded in the ventral roots from MG nerve stimulation were within the range of normal values.

The terminal electrophysiological experiments revealed dramatic all-or-none effects on peripheral sensory and motor neurones for pyridoxine treated animals. The short latency components of dorsal root compound potentials from both muscle and cutaneous nerves were abolished. Muscle nerve neurogram responses to trapezoidal stretch were significantly reduced. Also, single unit recordings confirmed that large sensory axons are selectively impaired.

Clinically, vestibular righting and roll responses were unaffected. At the height of the motor syndrome, when the animals were tilted slowly in the sagittal plane, they responded appropriately with extension of the forelimbs, and when tilted in the frontal plane, with extension of the ipsilateral fore- and hindlimbs, in either case so as to counteract the tilting motion. During rapid roll rotations along the longitudinal body axis, the head maintained a horizontal position in the frontal plane, even for the largest trunk rotations (about 60°). Therefore, after pyridoxine treatment when the animals were experiencing severe motor deficits in limb movement, neither the head or neck control appeared to be abnormal.

At the height of the motor syndrome, most animals experienced difficulties maintaining an appropriate posture for food and drink intake. During this time, animals typically lost only 0.25 kg of body weight as food and liquid intake was assisted by an experimenter (see Methods 2.2.3). Throughout the research protocol, all animals displayed high levels of motivation and cooperation.

3.2 Chronic recordings

3.2.1 Locomotion

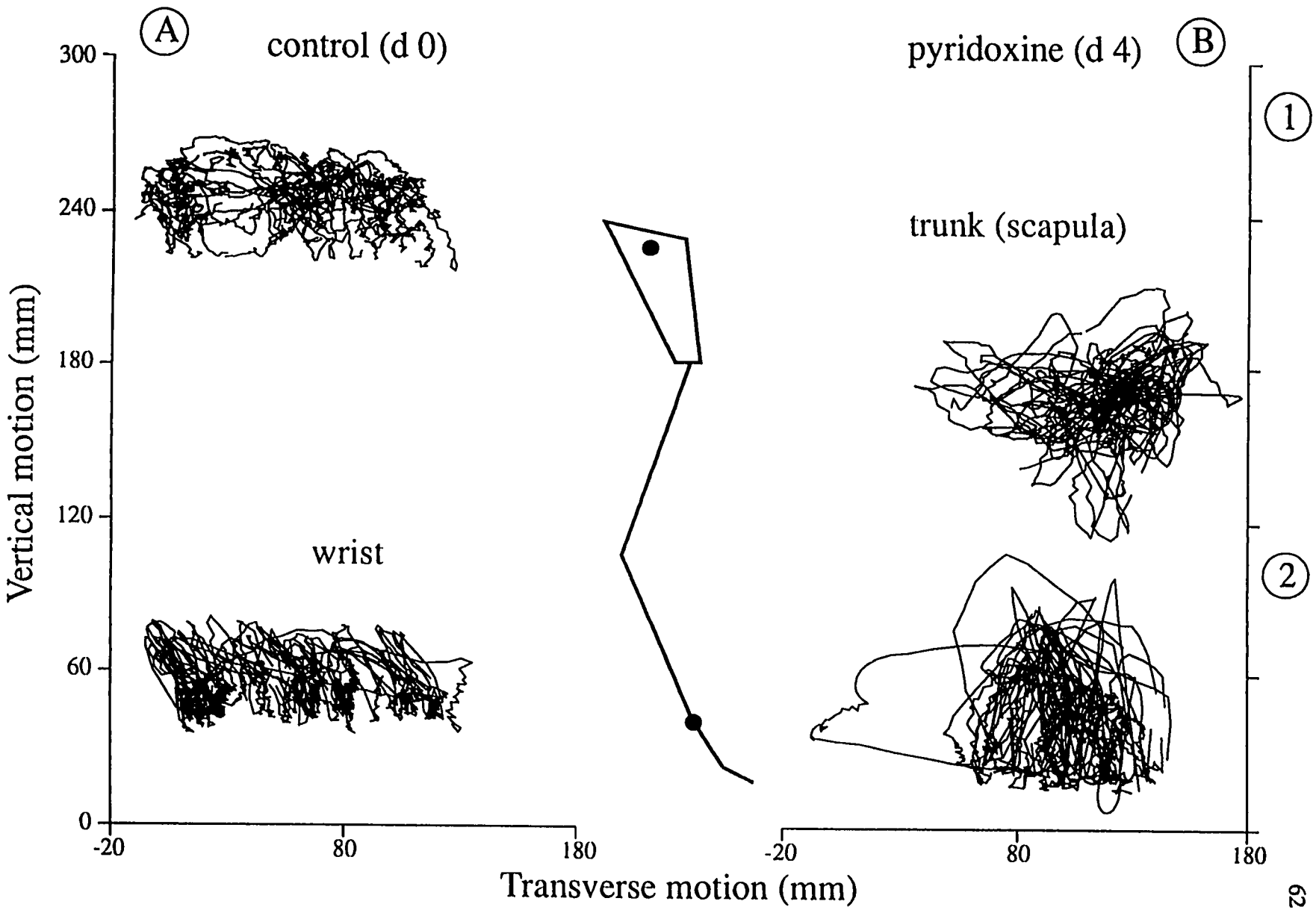
The motor deficits resulting from pyridoxine treatment spanned a wide range. The motor syndrome encompassed severe crouch, plantigrade foot contact, postural instability, abnormal limb excursions and erratic interlimb coordination during locomotion. Preliminary multivariate kinematic analysis indicated that during walking the basic cyclic pattern of individual limb movements was little affected (Bishop, Mah, Hulliger and Wojciechowski, 1996), while cadence, stride length and angular excursions exhibited greater variability. Before treatment, all animals comfortably walked on the treadmill at all 3 speeds (0.2, 0.4, and 0.8 m/s). At the height of the syndrome, during the first week after the manifestation of the motor impairment, not one animal was capable of walking at 0.8 m/s, with only 2 animals capable of walking at 0.4 m/s. In fact, 6 animals were unable to walk at the height of the syndrome.

3.2.1.1 Trunk posture and paw clearance

While the cyclic motion patterns of individual limbs appeared little affected, direct observation of animals during walking trials indicated that most animals demonstrated a crouched style of locomotion.

This phenomenon was seen in the vertical displacement profiles of the trunk and paw markers for forelimb and hindlimb. One animal that clearly exhibited a crouched style of gait after pyridoxine treatment was cat 8. Figure 3 illustrates the frontal plane trajectories in external coordinates of the forelimb trunk (scapula) and paw (wrist) during treadmill locomotion at 0.2 m/s. During the pre-treatment stage, cat 8's scapula

Figure 3. Frontal plane trajectories of forelimb motion in external coordinates during walking at 0.2 m/s. Data from cat 8. Column A: control recordings on day 0, before the commencement of pyridoxine treatment. Column B: recordings on day 4, during the last session before the syndrome reached its peak. Row 1: superimposed trajectories of forelimb trunk marker (scapula). Row 2: superimposed trajectories of the wrist marker. Inset, schematic representation of a forelimb skeleton (in sagittal view) indicating the topographic location of the markers. Note the decrease in mean vertical position of the scapula and wrist markers after pyridoxine treatment, reflecting the animal's crouched posture and plantigrade walking strategy (see also Results 3.2.1.1). This animal lost all walking capability within 12 hours after the recordings shown in column B.


A
control (d 0)
B
pyridoxine (d 4)
1
2
trunk (scapula)
wrist
300
240
180
120
60
0
-20
80
180
Vertical motion (mm)
Transverse motion (mm)

trajectory was characterized by an average height of 24 cm and its wrist clearance from the treadmill floor of about 4 cm (Figure 3.A). The average height of these frontal plane trajectories during 0.2 m/s walking was similar to those while standing for this animal. In contrast, after pyridoxine treatment, cat 8's average scapula height was 16 cm and the wrist clearance was only 2 cm (Figure 3.B). Therefore, all major joints of cat 8's forelimb demonstrated greater flexion throughout the entire step cycle after pyridoxine treatment.

A similar increase in flexion is seen for the hindlimb trunk (lumbar spine) and paw (ankle but not toe). The frontal plane trajectories of hindlimb motion in external coordinates for cat 8 show an average lumbar spine height of 29 cm, ankle clearance of 6 cm and toe clearance of 2 cm (Figure 4.A.1-3). Again, these heights are similar in magnitude to the marker positions during standing for this animal. After pyridoxine treatment, a crouched style of gait was also seen for the hindlimb (Figure 4.B.1-3) as the average lumbar spine height was 19 cm, ankle clearance was 3 cm, and toe clearance was 2 cm. The difference between control and pyridoxine hindlimb toe clearance was insignificant because the distance between the plantar surface of the foot and the marker position on the 5^{th} metatarsal was approximately 2 cm. Therefore, the vertical distance between the treadmill floor and the toe marker was approximately 2 cm, regardless if the walking was before or after pyridoxine treatment. The dramatic decrease of lumbar spine and ankle vertical height was verified by video recordings.

Figure 5 illustrates the effect of pyridoxine treatment on mean vertical trunk position and minimum vertical paw position (treadmill clearance) for all treated animals. Circles that

Figure 4. Frontal plane trajectories of hindlimb motion in external coordinates during walking at 0.2 m/s. Data from cat 8. Column A: control recordings on day 0, before the commencement of pyridoxine treatment. Column B: recordings on day 4, during the last session before the syndrome reached its peak. Row 1: superimposed trajectories of the hindlimb trunk marker (lumbar spine). Row 2: superimposed trajectories of the ankle marker. Row 3: superimposed trajectories of the toe marker. Note the decrease in the mean vertical position of the lumbar spine and ankle, but not toe, markers after pyridoxine treatment, reflecting the animal's crouched posture and plantigrade walking strategy (see also Results 3.2.1.1).

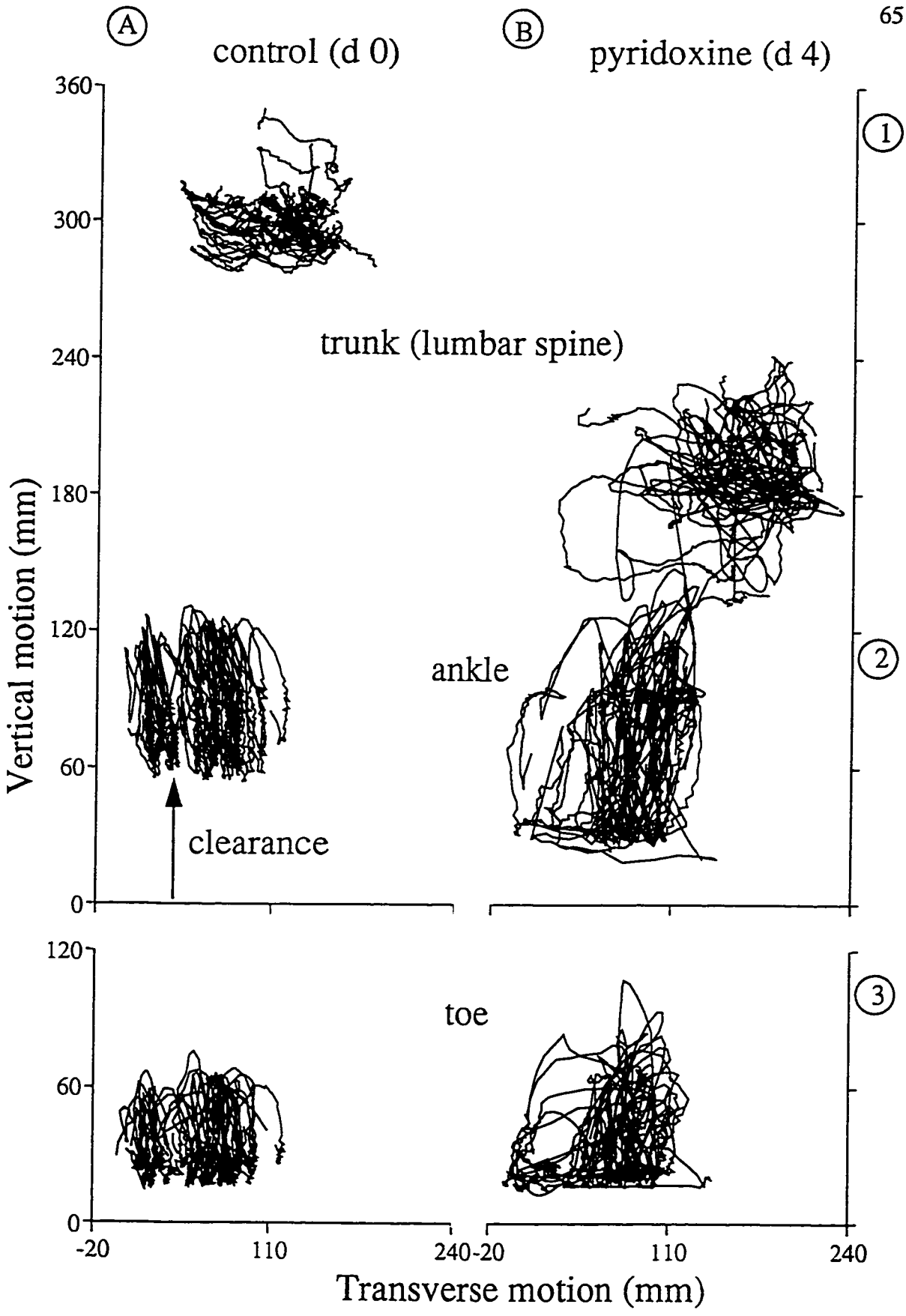
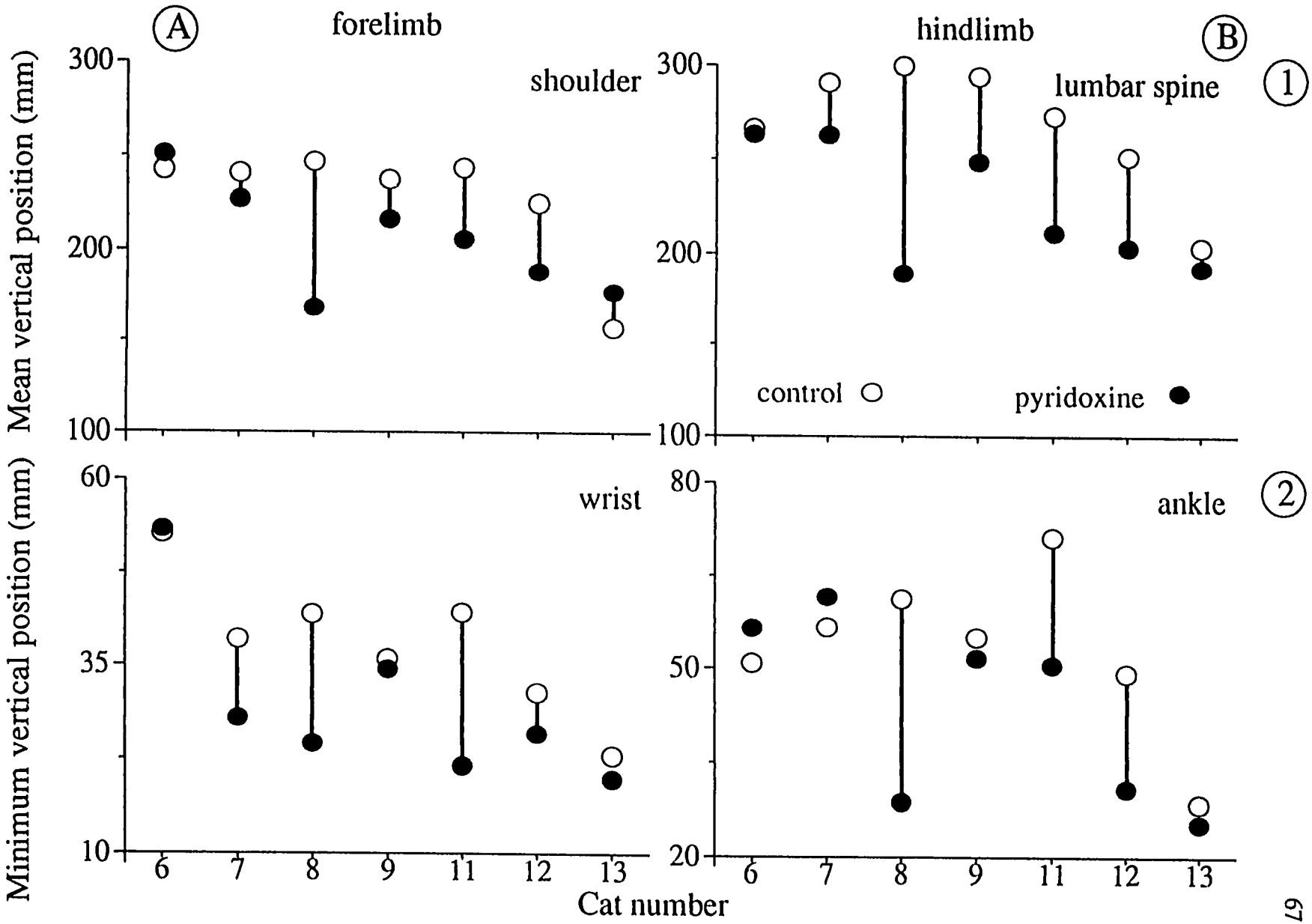

A
control (d 0)
B
pyridoxine (d 4)
360
300
240
180
120
60
0
Vertical motion (mm)
trunk (lumbar spine)
ankle
clearance
1
2
3
120
60
0
toe
-20
110
240
-20
110
240
Transverse motion (mm)

Figure 5. Effects of pyridoxine treatment on trunk posture and paw clearance during treadmill walking at 0.2 m/s. Data from cats 6-13. Column A: forelimb; column B: hindlimb. Row 1: mean vertical displacement of the trunk markers. Row 2: mean vertical position of the paw marker (A: wrist; B: ankle). Open circles, control recordings before pyridoxine treatment; filled circles, recordings during the last session (on days 3 or 4) before the ability to walk was lost. Vertical lines connecting open and filled circles indicate that the differences illustrated were statistically significant ($p<0.05$), indicating that most animals walked with a crouched posture and adopted a plantigrade walking strategy. The animals are identified along the horizontal axis by their serial numbers. Note that cat 8, whose paw and trunk trajectories are shown in Figures 3 and 4, showed the most extreme crouching and a particularly clear tendency of plantigrade walking.


A
forelimb
shoulder
300
200
100
B
hindlimb
lumbar spine
1
control
pyridoxine
60
35
10
wrist
80
50
20
ankle
2
6
7
8
9
11
12
13
Cat number
Mean vertical position (mm)
Minimum vertical position (mm)

are connected by lines indicate a significant difference between conditions ($p<0.05$). Both forelimb and hindlimb trunk markers exhibit a marked decrease in mean vertical position for 5/7 animals (Figure 5.A.1 and 5.B.1). In addition, 4/7 animals showed a significant decrease in minimal vertical position of the forelimb wrist (Figure 5.A.2). While 3/7 animals showed a significant decrease in minimal vertical position of the hindlimb ankle (Figure 5.B.2). Thus, indicating a trend towards a gait pattern with increased flexion in all major limb joints and a change in foot contact pattern from digitigrade to plantigrade locomotion (see Discussion 4.3.3). An outlier in both trunk and paw vertical positions was cat 13. This animal displayed small changes in vertical position of its trunk and paws before and after pyridoxine treatment. The lack of affect on overall limb posture was mainly due to the fact that this animal was timid and before pyridoxine treatment commenced, it walked with a crouched gait.

3.2.1.2 Path length of trunk and relative paw trajectories

After pyridoxine treatment, one of the striking features of locomotion was the increased instability of the animal. Most animals fell towards the sides of the treadmill when their trunks underwent large amplitude excursions or sways along the transverse axis. This swaying gait and instability was initially difficult to quantify. However, the increased instability was associated with an increased variability and abnormality of paw excursions in all three dimensions (see Discussion 4.2.5). Therefore, we developed the method of path length analysis, measuring, and then analyzing, the displacement in multiple combinations of the three movement axes (see Methods 2.6.1.1). This method was applied to the trunk and paw markers for both forelimb and hindlimb.

Marker trajectories, in any combination of the three axes, were irregularly shaped for both the control and treatment recordings. Figure 6 shows a series of typical frontal plane trajectories during 0.4 m/s walking for cat 11. The frontal plane trajectories were chosen as this plane of movement demonstrated the greatest significant difference between control and treatment locomotion (see later in Results 3.2.1.2). Both control (Figure 6.A) and after pyridoxine treatment (Figure 6.B) frontal plane trajectories demonstrate peculiar shapes that are difficult to interpret qualitatively. Yet, when the path lengths of the trajectories are calculated, there was often a large difference between control and after pyridoxine recordings (see path length scores in Figure 6).

Figure 7 shows the trunk and toe relative to trunk trajectories in the frontal plane. The superimposition of approximately 20 step cycles collected from one control animal illustrates the consistency of frontal plane excursions by these markers, and also the abnormal shapes of these control trajectories (Figures 7.A.1-2). A narrow spread with high density (darkness of trace) is seen in these control recordings. By comparison, the same animal after 4 days of pyridoxine treatment, walking at the same speed (0.4 m/s), shows a much larger cloud of data with greater excursions in both directions of transverse and vertical motion (Figures 7.B.1-2). Again, the phenomenon of crouched gait as seen in cat 8 (Figures 3 and 4) is seen in cat 11 walking at 0.4 m/s (cf. Figures 7.A.1 and 7.B.2). The increased variability of the locomotor pattern after pyridoxine treatment is evident by the decreased density of the trajectories (Figures 7.B.1 and 7.B.2). The differences in variability are more clearly illustrated by Figure 7.3 displaying the calculated path lengths of trunk and relative paw frontal plane motion. The measured variability is illustrated graphically by the rectangular boxes, which are defined, for each

Figure 6. Representative path length measures for frontal plane trajectories during 0.4 m/s walking. A representative sample of cat 11 trajectories during control recordings (Column A) and after pyridoxine treatment (Column B). Rows 1-2: illustration of the range of trajectory size, shape and location. Row 2: superimposed schematic illustration of the method of path length integral calculation. The arrows indicate the direction of motion during a single step cycle, from paw contact to next paw contact. Note that, characteristically, none of the trajectories formed a closed loop. The integral symbols indicate that path length was calculated as the cumulative sum of distances between consecutive samples (see Methods 2.6.1.1). Numbers indicate the calculated path length magnitudes (in mm) for each of the trajectories illustrated. The relatvie displacement was calculated by subtracting trunk marker coordinates from concomitant paw marker coordinates. Note the large variability in, and complexity of, trajectory shapes for both control and pyridoxine recordings. Also, note that despite similarities in trajectory complexity, the pyridoxine path lengths were, on average, larger.

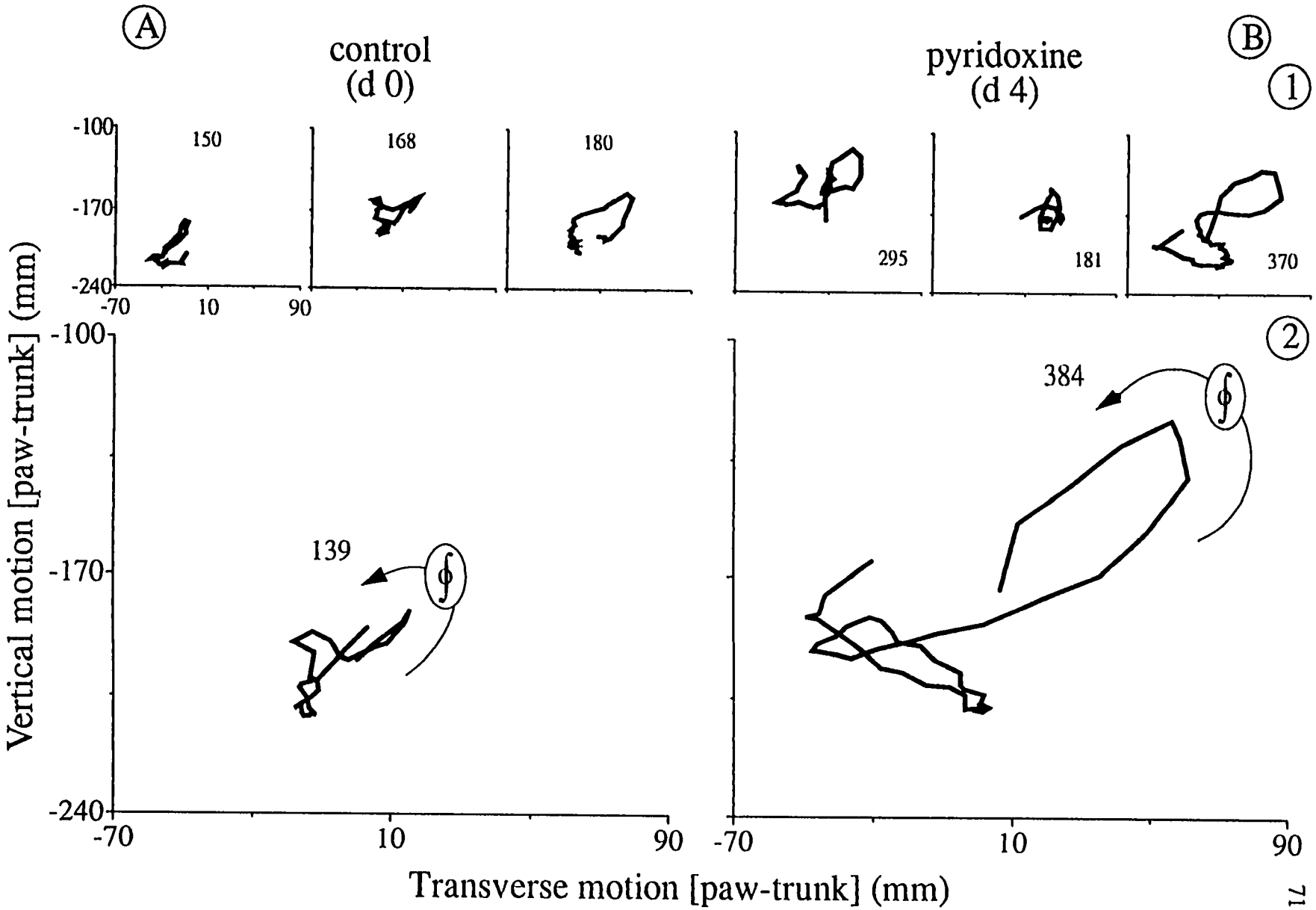
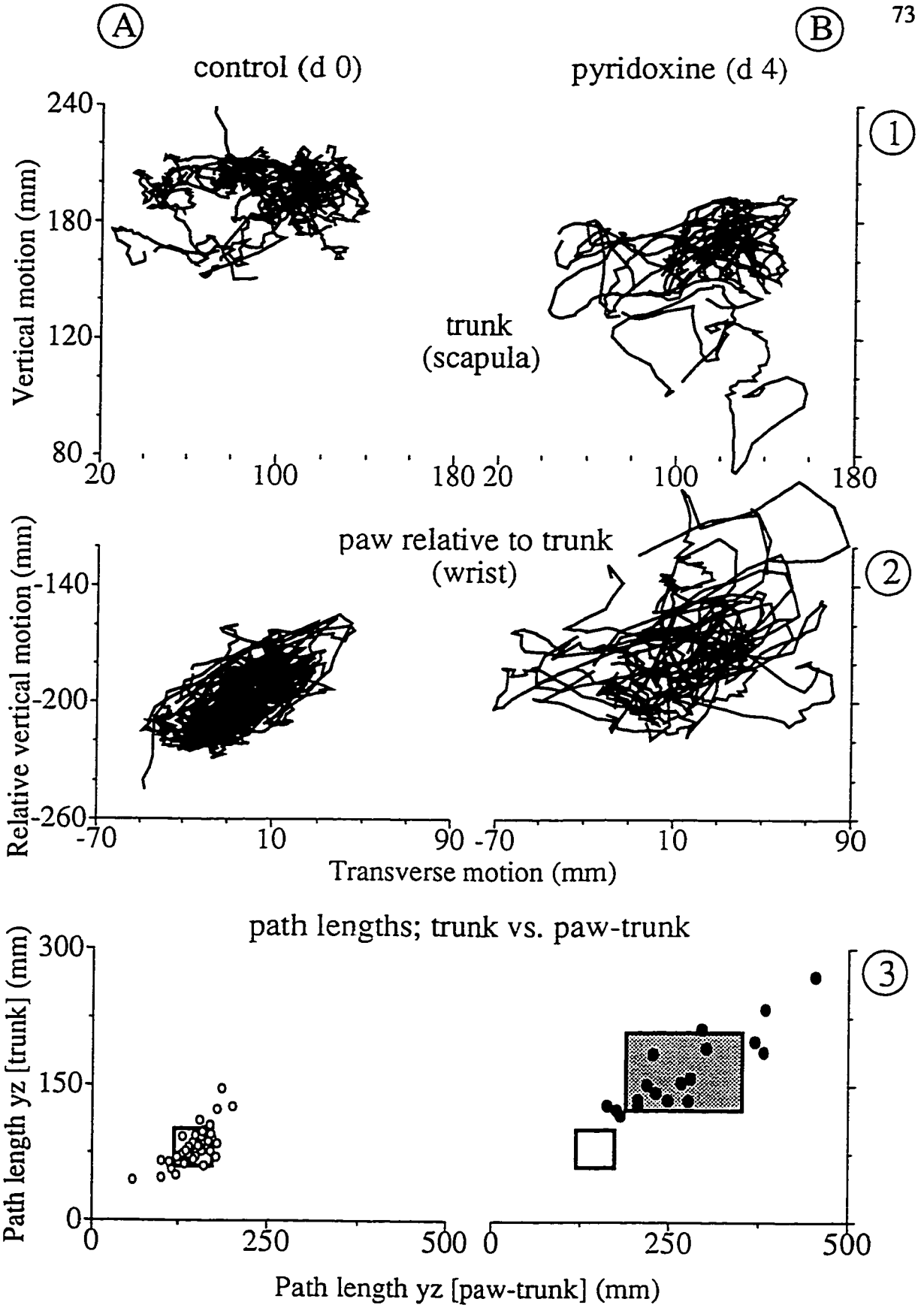

A
control
(d 0)
B
pyridoxine
(d 4)
1
2
150
168
180
295
181
370
384
139
-100
-170
-240
-70
10
90
Vertical motion [paw-trunk] (mm)
Transverse motion [paw-trunk] (mm)

Figure 7. Frontal plane trajectories of forelimb motion and their calculated path lengths during 0.4 m/s treadmill walking. Data from cat 11. Column a: control recordings on day 0. Column B: pyridoxine recordings on day 4. Row 1: superimposed trajectories of the forelimb trunk marker (scapula). Row 2: superimposed trajectories of the wrist relative to trunk (see Methods 2.6.1.1). Row 3: superimposed individual step cycle path lengths and sessional variance rectangles. Note the increased spread of trajectories and path length scores (variance rectangles), and the increase in mean path length after pyridoxine treatment.

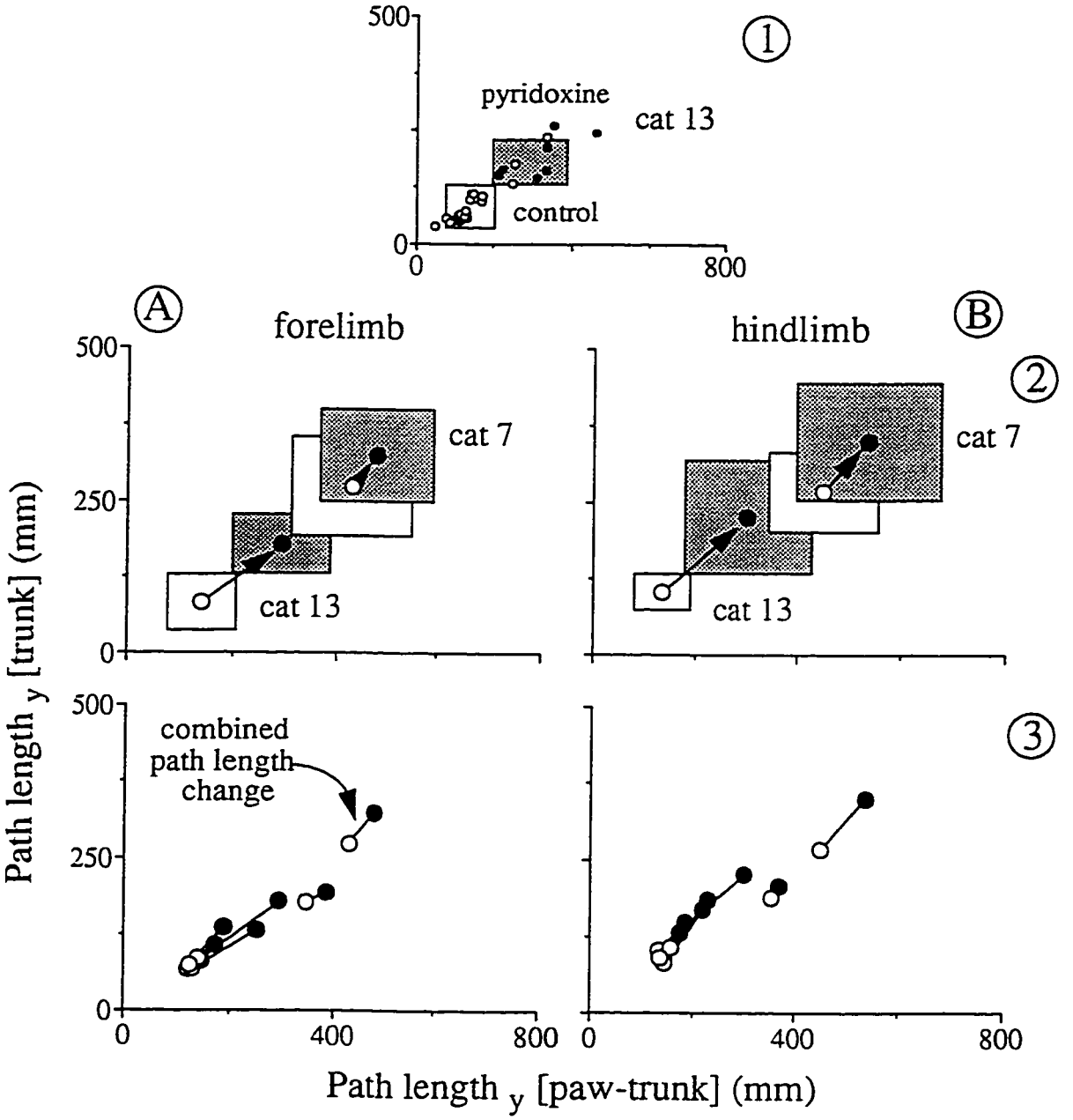

A
B
control (d 0)
pyridoxine (d 4)
1
Vertical motion (mm)
240
180
120
80
trunk
(scapula)
20
100
180
2
paw relative to trunk
(wrist)
Relative vertical motion (mm)
-140
-200
-260
-70
10
90
Transverse motion (mm)
path lengths; trunk vs. paw-trunk
3
Path length yz [trunk] (mm)
300
150
0
250
500
Path length yz [paw-trunk] (mm)

variable, by the lines of mean path-length +/- 1 standard deviation (in short, 'variance rectangle'). The dispersal of control data points is very narrow, and this results in a smaller variance rectangle (Figure 7.A.3). In addition, the cloud of data points for the control locomotion is located in the bottom left corner of the figure, indicating a relatively short path length in the frontal plane for both the trunk and paw relative to trunk markers. However, for the same animal, at the height of the motor syndrome, the dispersal of data points is much greater along both axes, and the resulting variance rectangle is much larger (Figure 7.B.3). The larger amplitude of the traces in Figures 7.B.1-2 resulted in longer path lengths, therefore, resulting in a range of data points located more to the right and top of Figure 7.B.3, demonstrating greater path lengths for both the trunk and paw relative to trunk.

The choice of a two-dimensional trajectory path length measure was initially determined by the visual impression from direct observation, that movement variability and irregularity was particularly pronounced in the frontal plane. However, this choice remained to be corroborated by statistical analysis of data from all animals and by comparing the power of resolution of one-, two- and three-dimensional path length estimates. The results of this systematic comparison are shown in Figures 8 through 10. In contrast to the data of Figures 6 and 7, where walking at 0.4 m/s was examined, the systematic evaluation of path length analysis was done for recordings at 0.2 m/s, since, during the critical window just before the maximum development of the motor syndrome (on day 3 or 4), the majority of animals were not capable of walking at the higher speed.

Figure 8 illustrates the analysis of path-length changes for one-dimensional measures (cf.

Figure 8. Trunk and relative paw path length changes for 1-dimensional transverse motion during 0.2 m/s treadmill walking. Column A: forelimb data. Column B: hindlimb data. Row 1: individual step cycle path lengths and variance rectangles for cat 13 forelimb. Row 2: two examples, cats 7 and 13, of mean path length and variance rectangles for control (opaque circles and boxes) and pyridoxine (black circles and shaded boxes) recordings. Row 3: mean path lengths for all treated animals for control (opaque circles) and pyridoxine (black circles) recordings. Lines connecting circles indicate the combined path length change. See text for statistical significance of 1-dimensional path length calculations (Results 3.2.1.2).


500
0
0
800
1
pyridoxine
cat 13
control
A
forelimb
B
hindlimb
2
500
250
0
cat 7
cat 13
cat 7
cat 13
3
combined
path length
change
500
250
0
0
400
800
0
400
800
Path length y [trunk] (mm)
Path length y [paw-trunk] (mm)

Methods 2.6.1.1). The analysis is based on the scatter plots of trunk movement path-length vs. relative paw movement path length, whose rationale was developed in Figure 7. Figure 8.1 shows path length estimates for all steps from an individual animal, with control and post-treatment data and associated variance rectangles assembled in a single graph to facilitate comparison. The calculations were carried out both for the forelimb (8.A) and hindlimb (8.B) segments. Transverse motion (in the frontal plane), rather than vertical or sagittal motion, was chosen for the illustration, partly to facilitate comparison with the results of two-dimensional analysis (Figure 9), and partly because preliminary analysis of the alternative directions indicated that the separation of pathological from normal conditions was most effective for transverse movement components. Figure 8.2 shows graphic summaries of forelimb and hindlimb path-length data, for clarity only for two animals. Mean values are shown as circles and the variability estimates as variance rectangles (as defined above). Open symbols illustrate control walking and filled symbols illustrate pathological walking. It can be seen that for both animals (cats 7 and 13) mean trunk and paw path-length increased during pathological walking, both for the forelimb and hindlimb segment. This is illustrated by the vectors (arrows) that connect the means (circles) of the control with those of the pathological condition, with the angles of these vectors relative to the horizontal (between 35° and 60°), reflecting the relative magnitudes of the increase in both means with pathology. The magnitude (length) of each vector is a measure of combined path-length change, i.e. a global index of trunk and paw movement trajectory alteration.

The changes in mean one-dimensional path length observed for all 7 animals of this study are shown in Figure 8.3. It may be seen that in all cases the trunk and paw

trajectory path lengths increased, although the size of this effect varied between animals. A statistical analysis of the difference between path length means of pathological and normal walking was carried out, separately for the trunk and paw measures for both forelimb and hindlimb, using a 2-tailed Cochrane-Cox t-test on means of the means of step sequences (see Methods 2.6.5). None of the trunk path length estimates of Figure 8.A.3 (forelimb) and only 2/7 of the estimates of 8.B.3 (hindlimb) differed significantly at the 95% confidence level. In contrast, 3/7 forelimb and 6/7 hindlimb paw path length estimates revealed significant differences. For the two representative animals of row 2, only the hindlimb paw path length estimates differed significantly.

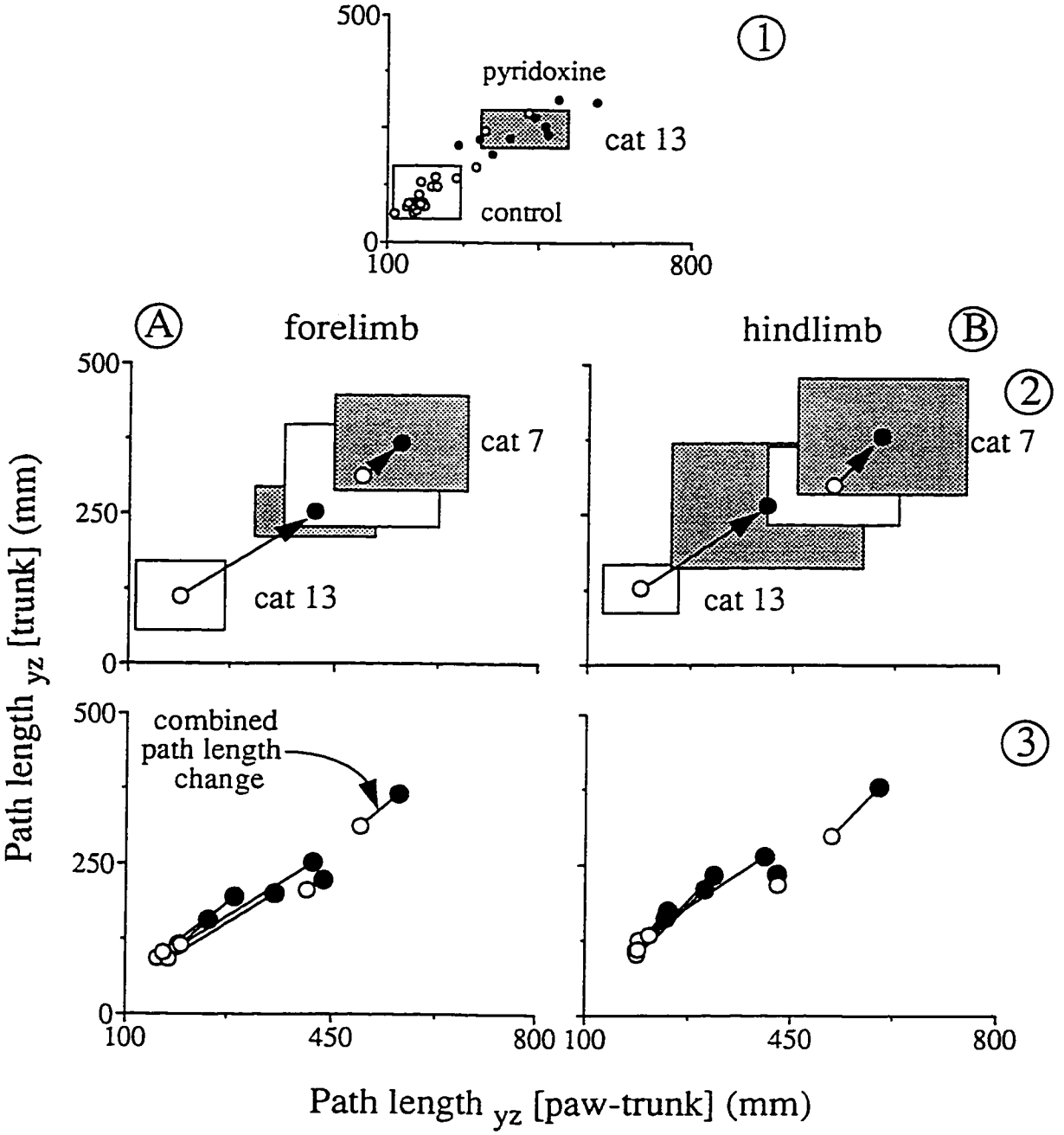
For the present data, the increase in trajectory path length mainly reflects increased irregularity and excursion amplitude, but not a significant increase in step length. This is in line with a notion of ataxic gait being associated with greater variability of movement. One might predict that the variance of the path length estimates should also increase, since appreciable fluctuations (rather than a systematic increase) in step length were observed in several animals, with frequent occurrence of abnormally large or abnormally small steps. The illustration of the variability of the path length measures of Figure 8.2 (variance rectangles) indicates that this was true in some, but not all cases. In the case of cat 7 (Figure 8.2) the differences in variance were marginal and not significant statistically. In contrast, for cat 13 all four estimates of mean variance differed significantly (trunk and paw, both limbs). Overall, in 12/28 cases the variances differed at the 95% level of significance. However, in two of these cases the variance of path length estimates was significantly reduced, rather than increased. Thus, we had to use the Cochrane-Cox t-test, rather than the standard t-test, when we compared the means of the

means of sequences (above).

Figure 9 summarizes the results of the analysis of path length alterations for two-dimensional measures, using the same format as for Figure 8 and illustrating, in row 2, data from the same two representative animals (cats 7 and 13). Comparison with Figure 8 shows that, in the representative scatter plot individual step path length measures of row 1, the separation of the clusters of the two conditions and of the variance rectangles was clearly increased (data from cat 13). Similarly, in row 2, there is a greater tendency of separation of the variance rectangles (especially for cat 13) and the size of the vectors of combined path length change is increased in relation to the size of the variance rectangles. The statistical analysis of the difference between the means of the means of sequences of path lengths of normal and pathological walking revealed a clear increase in the incidence of significant differences: 5/7 forelimb trunk and 6/7 hindlimb trunk path length estimates of Figure 6.3 differed significantly at the 95% level, i.e. 79% for the two-dimensional measures, compared with 14% for the one-dimensional measure. For the relative paw path lengths, 4/7 for both forelimb and hindlimb were different at a significant level, i.e. 57% (same as for the one-dimensional measure). Thus, two-dimensional analysis of trajectory path length clearly revealed greater power of separation than one-dimensional analysis.

In contrast, the analysis of the variability of the means of sequences of path lengths depicted in Figure 9 revealed the same finding as for the one-dimensional analysis, in that in 13/28 cases the variances differed at the 95% level of significance. However, again, in two of these cases the variance of path length estimates was reduced, rather

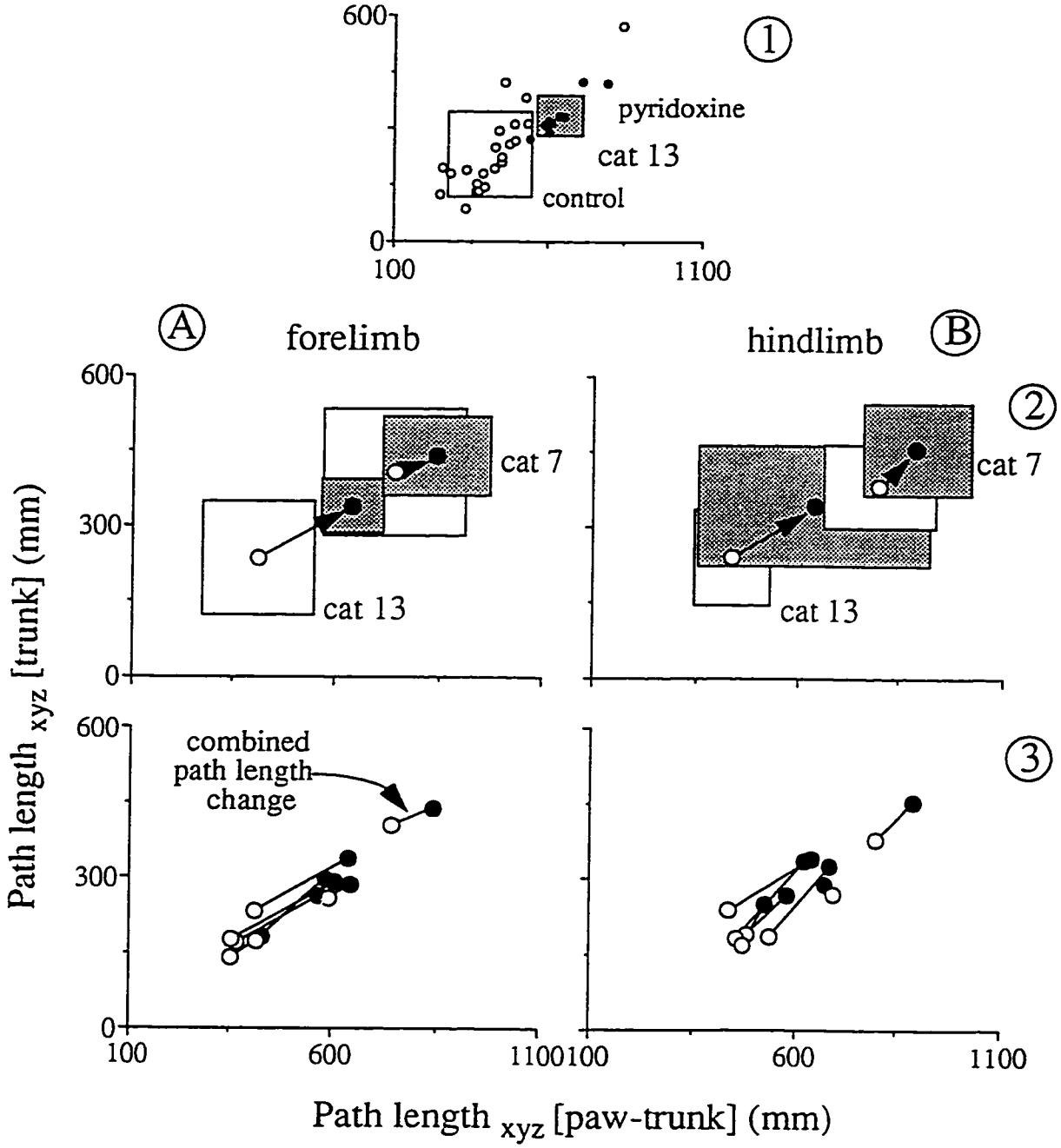
Figure 9. Trunk and relative paw path length changes for 2-dimensional trajectories in the frontal plane during 0.2 m/s treadmill walking. Column A: forelimb data. Column B: hindlimb data. Row 1: individual step cycle path lengths and variance rectangles for cat 13 forelimb. Row 2: two examples, cats 7 and 13, of mean path length and variance rectangles for control (opaque circles and boxes) and pyridoxine (black circles and shaded boxes) recordings. Row 3: mean path lengths for all treated animals for control (opaque circles) and pyridoxine (black circles) recordings. Lines connecting circles indicate the combined path length change. See text for statistical significance of 2-dimensional path length calculations (Results 3.2.1.2).


500
0
100
800
pyridoxine
cat 13
control
①
A
forelimb
hindlimb
B
②
cat 7
cat 13
③
combined
path length
change
Path length yz [trunk] (mm)
Path length yz [paw-trunk] (mm)
250
450

than increased.

Given the increased power of separation of conditions of two-dimensional analysis, it was an obvious step to extend the analysis to three dimensions, and to include trajectory movement in the sagittal plane. Formally, the motivation was to examine whether the added degree of freedom added to the power of separation. Empirically, the motivation was derived from the observation of an appreciable increase in the variability of step length along the longitudinal axis, at least for some animals after pyridoxine treatment (Bishop, Hulliger, Foweraker and Davis, 1998). Following the format of Figures 8 and 9, Figure 10 illustrates the observations on three-dimensional path length analysis. The general finding was that three-dimensional analysis did not further enhance the power of separation. If anything, the separation of conditions was statistically less effective. This is illustrated by the representative variance rectangles of path length measures in Figure 10.1 (cat 13; forelimb path length measures; same recording session and ensemble of steps as in Figures 8.1 and 9.1), where the variance rectangles of normal and pathological walking are much closer together than in Figure 9.1. This tendency is also evident by comparing the degree of separation of the variance rectangles of Figure 10.2 with those of Figure 9.2 (especially cat 13). Similarly, the incidence of statistically significant differences in path length means was slightly lower than for two-dimensional analysis: 3/7 forelimb trunk and 6/7 hindlimb trunk path length estimates of Figure 10.3 differed significantly at the 95% level, i.e. 64% for the three-dimensional measures, compared with 79% for the two-dimensional measure. For the relative paw path lengths, 5/7 forelimb and 4/7 hindlimb means were different at a significant level, i.e. 64% compared with 57% for the two-dimensional measure. Thus, three-dimensional analysis of trajectory

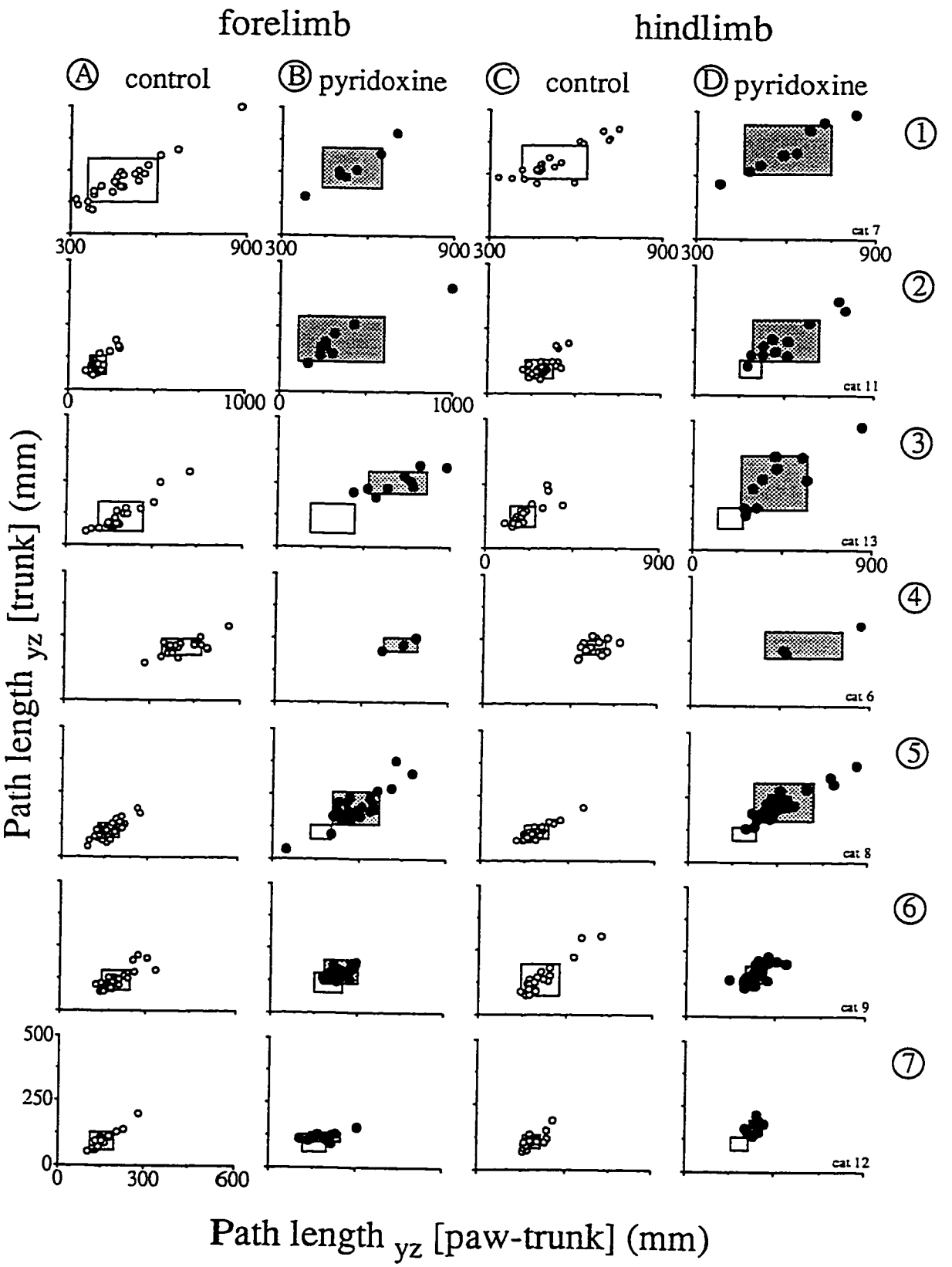
Figure 10. Trunk and relative paw path length changes for 3-dimensional trajectories during 0.2 m/s treadmill walking. Column A: forelimb data. Column B: hindlimb data. Row 1: individual step cycle path lengths and variance rectangles for cat 13 forelimb. Row 2: two examples, Cats 7 and 13, of mean path length and variance rectangles for control (opaque circles and boxes) and pyridoxine (black circles and shaded boxes) recordings. Row 3: mean path lengths for all treated animals for control (opaque circles) and pyridoxine (black circles) recordings. Lines connecting circles indicate the combined path length change. See text for statistical significance of 3-dimensional path length calculations (Results 3.2.1.2).


600
0
100
1100
1
pyridoxine
cat 13
control
A
forelimb
hindlimb
B
2
cat 7
cat 13
cat 7
cat 13
3
combined
path length
change
Path length xyz [trunk] (mm)
Path length xyz [paw-trunk] (mm)

path length failed to further enhance the power of separation between the normal and pathological conditions. Initially, this finding was surprising, since separate analysis of longitudinal motion revealed several distinct abnormalities (see Bishop et al., 1998). However, since the longitudinal component of path length was about twice as large as the combined frontal plane components (cf. scales of Figures 10.3 with 9.3), the speed related variability of longitudinal path length (both normal and pathological) simply masked the pathology-related differences that were detected with two-dimensional frontal plane analysis. Finally, the analysis of variability of the path length measures revealed the same inconsistent pattern as for one- and two-dimensional analysis, in that significant differences were found in 12/28 instances, 5 of these showing the above mentioned paradoxical decrease in variance. Therefore, frontal plane trajectories, the summation of movement along both y and z axes, were used to show the differences between control and after pyridoxine treatment walking.

Figure 11 illustrates the trunk and paw relative to trunk path length changes for frontal plane trajectories of all treated animals. The individual path length scores for each step cycle during the pre-treatment stage (control) are similar for each animal and their low variability is indicated by the small size of the background variance rectangle (with the exception of cat 7, Figures 11.A.1 and 11.B.1). In comparison, after pyridoxine treatment, the individual path length scores for most animals have increased in magnitude and variability. Those path lengths that are significantly different in mean, or standard deviation, or both, are represented by the superimposition of the control standard deviation box. This allows the direct comparison of the effect pyridoxine treatment has on path length magnitude (means), and variability, in those animals with the most severe

Figure 11. Path lengths of frontal plane trajectories of trunk and relative paw motion during 0.2 m/s treadmill walking. All figures: superimposed individual step cycle path lengths and variance rectangles. Column A: forelimb trunk (scapula) and relative paw (wrist - scapula) control recordings. Column B: forelimb trunk and relative paw pyridoxine recordings. Column C: hindlimb trunk (lumbar spine) and relative paw (toe - lumbar spine) control recordings. Column D: hindlimb trunk and relative paw pyridoxine recordings. Rows 1-7: recordings from cats 6-13. Superimposed variance rectangles from control recordings onto pyridoxine recordings indicate a significant difference in either mean path lengths or path length variabilities, or both.


forelimb
hindlimb
Ⓐ control
Ⓑ pyridoxine
Ⓒ control
Ⓓ pyridoxine
①
②
③
④
⑤
⑥
⑦
cat 7
cat 11
cat 13
cat 6
cat 8
cat 9
cat 12
300
900
0
1000
500
250
600
Path length yz [trunk] (mm)
Path length yz [paw-trunk] (mm)

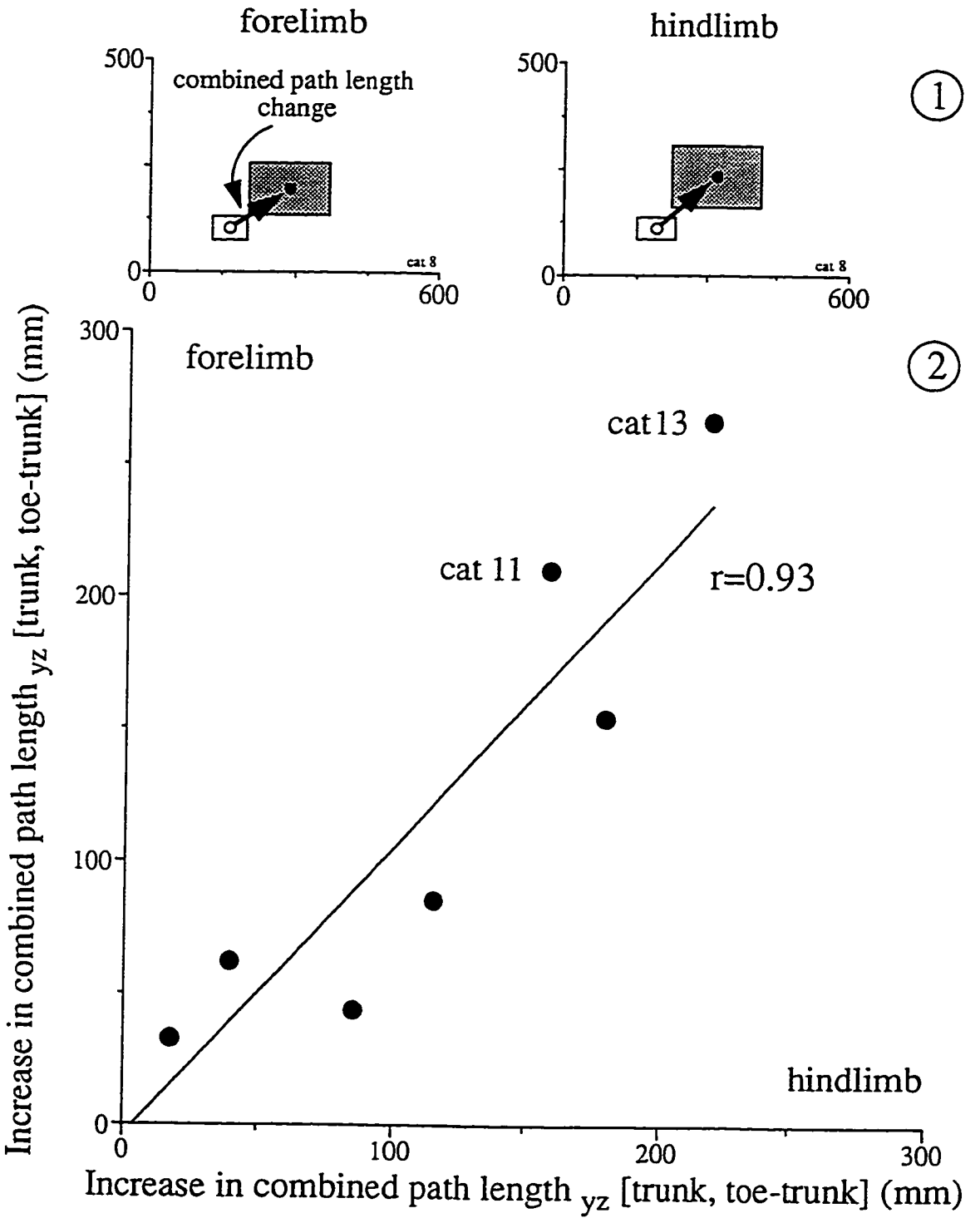
limb trajectory abnormalities.

Both forelimb and hindlimb individual path length scores show significant differences in mean scores and variability between control and after pyridoxine treatment recordings. The next logical question was to determine whether there was a difference in path length increase and variability between the forelimb and the hindlimb. Figure 12 shows the comparison between forelimb and hindlimb path length changes in the frontal plane. The top two panels (Figure 12.1) demonstrate that the increase in combined path length in the frontal plane is calculated as a vector between the mean control and pyridoxine treated path lengths. Increases in combined path length for the forelimb were matched by increases in the combined path length of the hindlimb (regression r= 0.93; Figure 12.2) Therefore, the effect of pyridoxine appeared to be evenly distributed between the forelimb and the hindlimb (see Discussion 4.6).

3.2.1.3 Interlimb coordination

The motor deficits during treadmill locomotion were not restricted to frontal plane movement abnormality. Equally striking abnormalities of entirely different nature were seen for movements in the sagittal plane, although not consistently in all animals. During normal walking the cyclic motion of forelimbs and hindlimbs is strictly coordinated, with a consistent phase relation between the limbs and with the hindlimb typically leading the forelimb. Close observation of the locomotion data in the sagittal plane after pyridoxine treatment revealed a disruption in interlimb coordination as the forelimb and hindlimb of several animals showed inconsistent phase relations. This was confirmed by careful

Figure 12. Comparison of path lengths of frontal plane trajectories of forelimb and hindlimb motion during 0.2 m/s treadmill walking. Row 1: illustration of method for calculating the increase in combined path length for forelimb and hindlimb data (data from Figure 11.5). Row 2: increase in combined path length from control to pyridoxine recordings for forelimb versus that for hindlimb. Note, the regression line has a slope of 0.93, indicative of a strong correlation between forelimb and hindlimb increases in combined path length.


forelimb
hindlimb
500
combined path length change
0
cat 8
600
1
2
300
200
100
cat13
cat 11
r=0.93
Increase in combined path length yz [trunk, toe-trunk] (mm)
Increase in combined path length yz [trunk, toe-trunk] (mm)

inspection of video recordings and by the analysis of temporal profiles of the longitudinal motion of forelimb and hindlimb markers. Locomotion after pyridoxine treatment was characterized by abnormal limb excursions in all three dimensions. Since treadmill speed was relatively constant, the variability of longitudinal paw motion (Figure 10) indicated that the forelimb and hindlimb could be experiencing variable step cycle durations. Yet, only 2/8 treated animals showed clear differences in their interlimb coordination when compared with control recordings because of the high variability of baseline recordings in the other 6/8 animals.

Figure 13 illustrates cat 7's forelimb wrist (hatched line) and hindlimb toe (thick line) longitudinal displacement during walking at a low speed (0.2 m/s) before pyridoxine treatment. Treadmill motion was backward and is shown by the sloping straight line (with arrowhead) indicating the path of an object resting on the treadmill. During stance, the paws rested on the treadmill and travelled backwards (downward sloping segments of the two limb traces), while during swing they are brought forward, ahead of the treadmill motion (upward sloping segments). In these displays, touchdown occurs at the maxima (peak) and takeoff at the minima (trough) of the marker motion profiles. This simple graphic display of forelimb and hindlimb marker position profiles permitted convenient analysis of the phase coordination of forelimb and hindlimb stepping movements.

The effect of pyridoxine treatment for two animals that showed the greatest variability in gait patterns is displayed in Figure 14. These two animals, cats 11 and 13, also showed the largest combined path length increases for forelimb and hindlimb trajectories

Figure 13. Longitudinal motion for forelimb and hindlimb during 0.2 m/s treadmill locomotion. Illustration of the temporal profiles of longitudinal displacement of the critical markers for analysis of interlimb coordination. Note the treadmill motion and limb motion during stance are shown in downward direction. Data from control recording of cat 7 during 0.2 m/s treadmill walking.

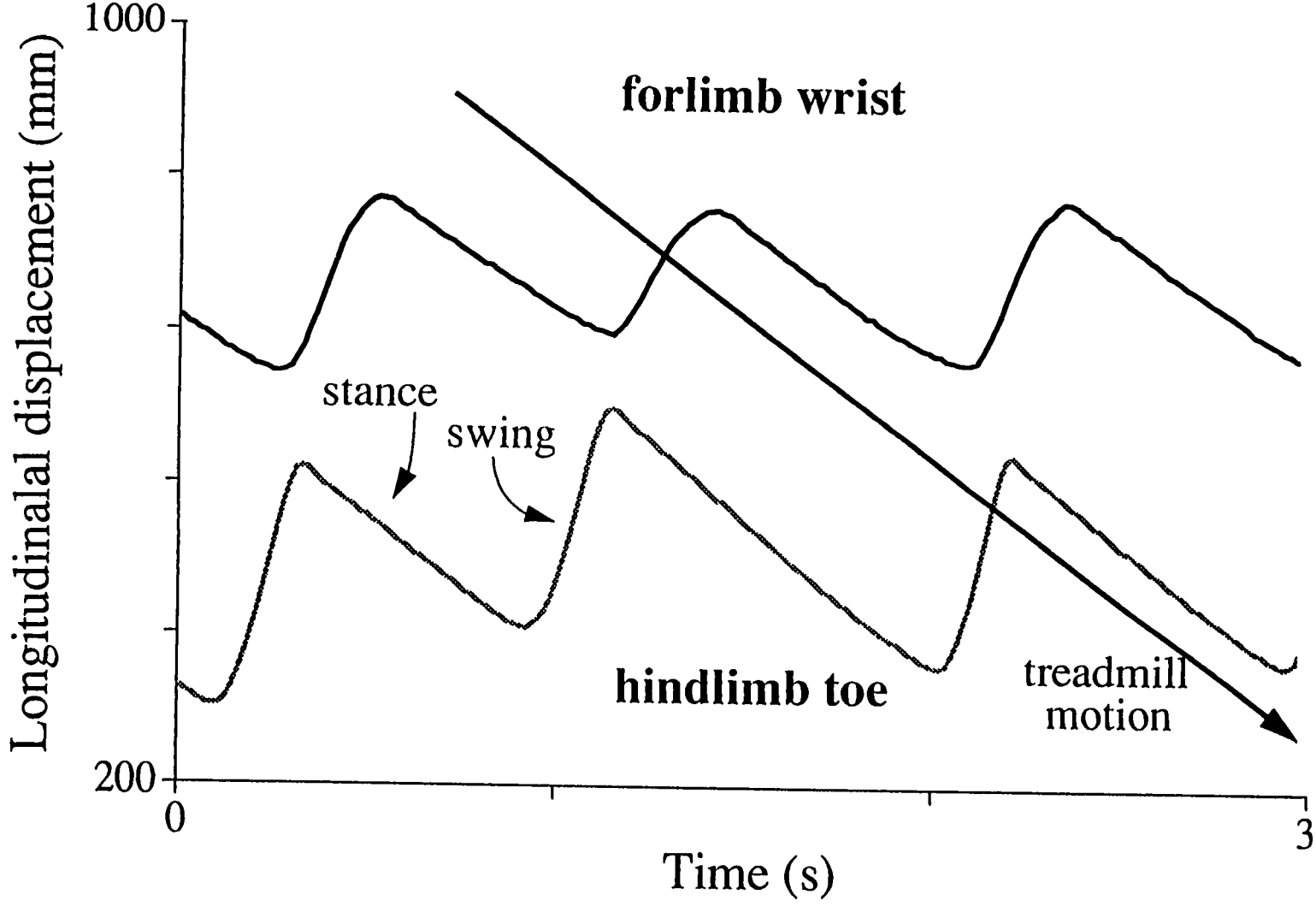
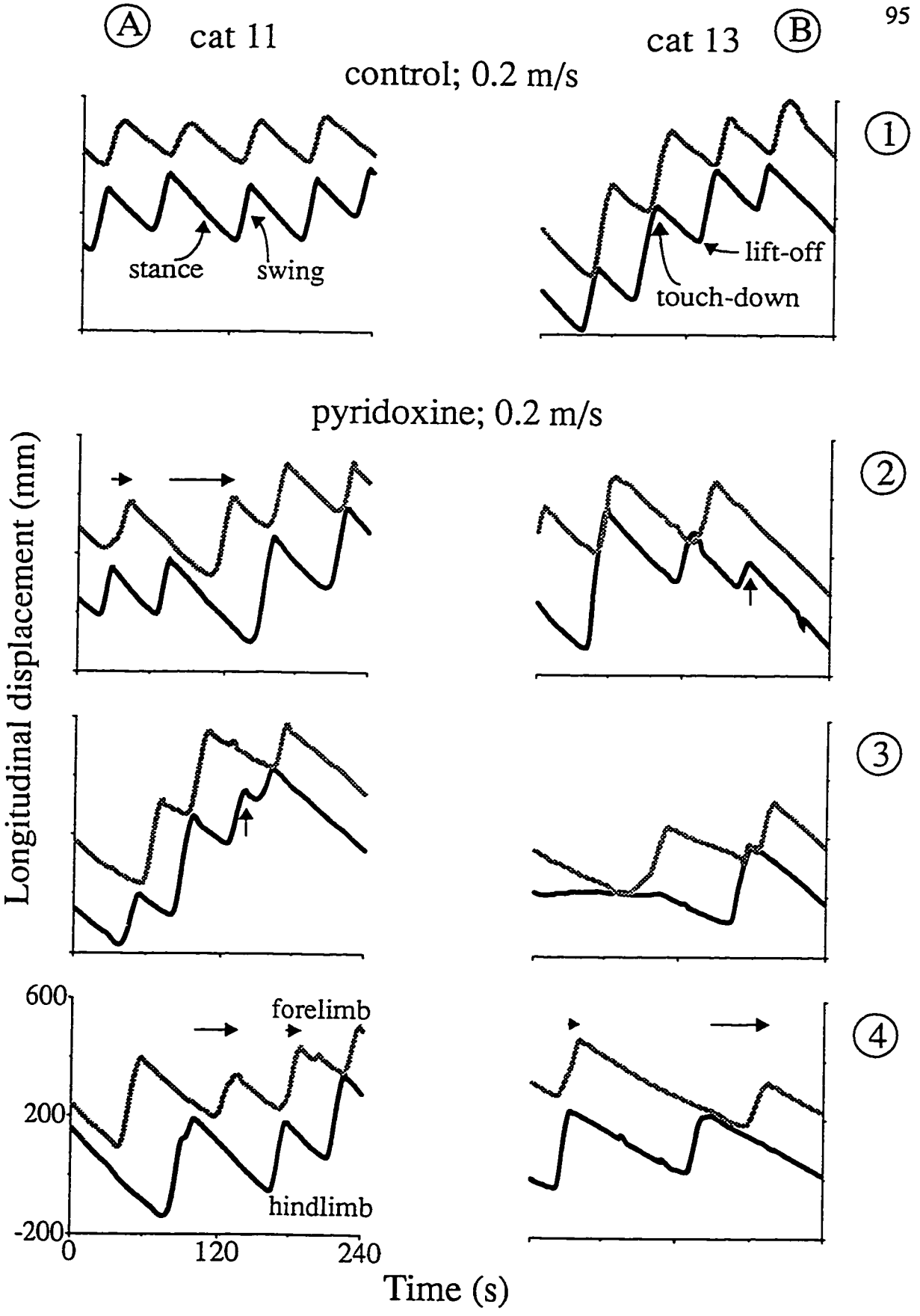

1000
200
0
3
Longitudinalal displacement (mm)
Time (s)
forlimb wrist
stance
swing
hindlimb toe
treadmill
motion

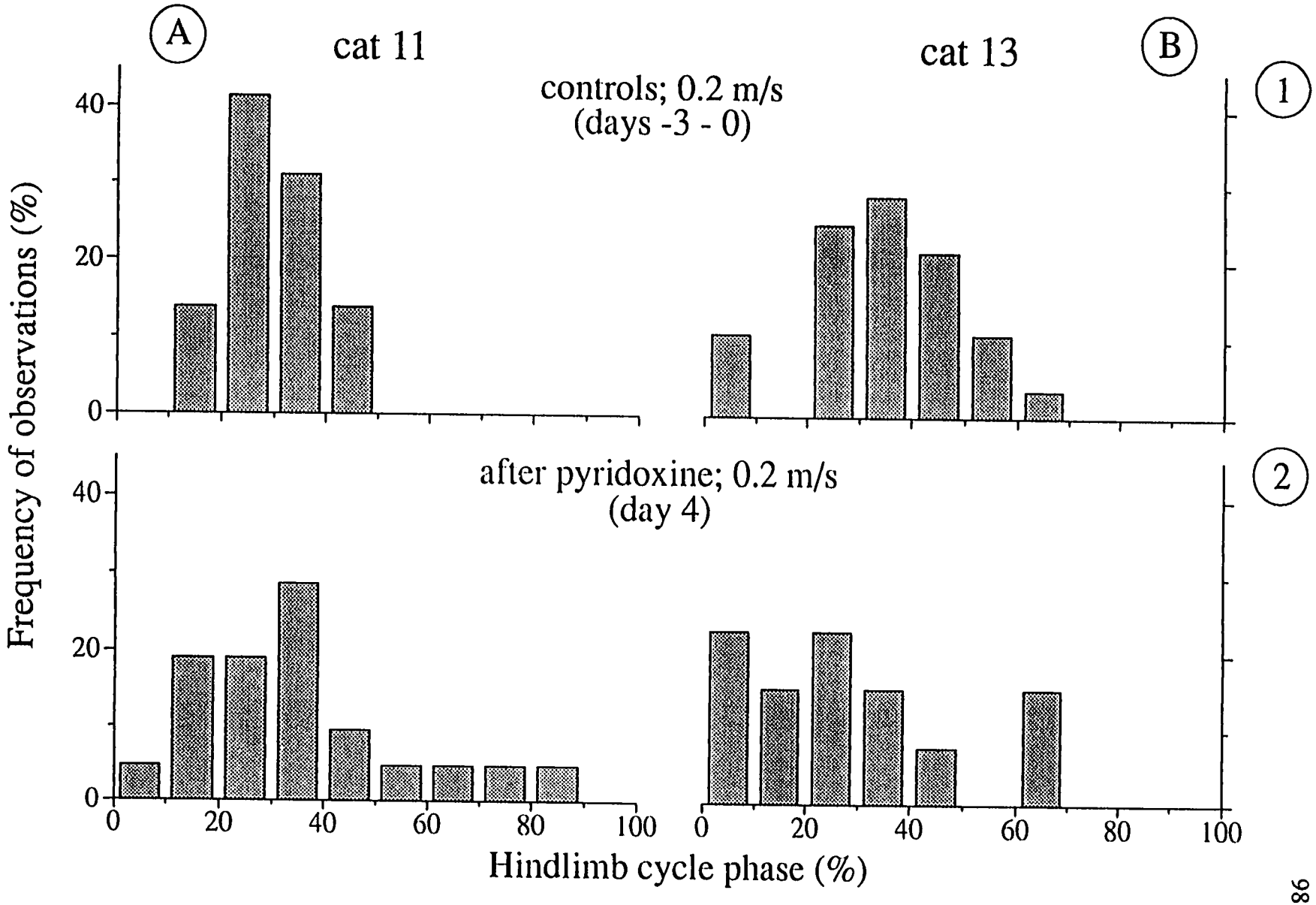
Figure 14. Longitudinal forelimb and hindlimb paw marker motion during 0.2 treadmill walking. Column A: individual 4 s walking trials recorded from cat 11. Column B: individual 4 s walking trials recorded from cat 13. Row 1: representative traces from control recordings. Rows 2-4: representative traces from pyridoxine recordings. Note, in the pyridoxine recordings, large phase shifts between one step cycle and the next (horizontal arrows in A.2, A.4 and B.4), and instances where two hindlimb cycles took place during a single forelimb step cycle (vertical arrows in A.3 and B.2). Also note that both control recordings have a strict 1:1 forelimb-hindlimb step cycle relation.


A
cat 11
cat 13
B
control; 0.2 m/s
stance
swing
lift-off
touch-down
1
pyridoxine; 0.2 m/s
2
3
4
Longitudinal displacement (mm)
600
200
-200
forelimb
hindlimb
0
120
240
Time (s)

as shown in Figure 12.2. During the pre-treatment period (Figures 14.A.1 and 14.B.1) both cats 11 and 13 displayed a consistent phase locked relationship between forelimb and hindlimb step cycles for 0.2 m/s walking. Both the peaks (toe contact) and troughs (toe lift-off) are synchronized between the hindlimb toe and forelimb wrist and there was a strict 1:1 cycle relationship between forelimb and hindlimb (Figure 14.A). In contrast, for walking at the same speed after pyridoxine treatment, forelimb and hindlimb step cycle coordination was more erratic and phase locking was less consistent, as large phase shifts occurred between one cycle and the next (horizontal arrows in Figures 14.A.2,4 and 14.B.4). In addition, there were instances where two hindlimb cycles took place during a single forelimb cycle (vertical arrows in Figures 14.A.3 and 14.B.2) and vice versa (not illustrated, but cf. Hulliger, Bishop, Djupsjobacka and Zanussi, 1998, for harness supported locomotion).

The phase relation between forelimb and hindlimb step cycles was analyzed by systematic measurements of the forelimb cycle demarcation (foot contact) relative to the concomitant hindlimb cycle and by expressing it as a percentage of the normalized hindlimb step cycle. Figure 15 shows the representative cross-cycle phase histograms for the two animals shown in Figure 14 during control and pyridoxine locomotion. The control recordings for both animals show a smooth unimodal phase relationship with the peak of forelimb contacts occurring at about 30 % of the hindlimb step cycle duration (Figures 15.A.1 and 15.B.1). At the height of the motor syndrome, both animals show a much broader or flattened distribution of phase correlations (Figures 15.A.2 and 15.B.2). This display shows that forelimb contact occured at almost any point in the hindlimb step cycle, inferring that the respective forelimb and hindlimb CPG's were operating

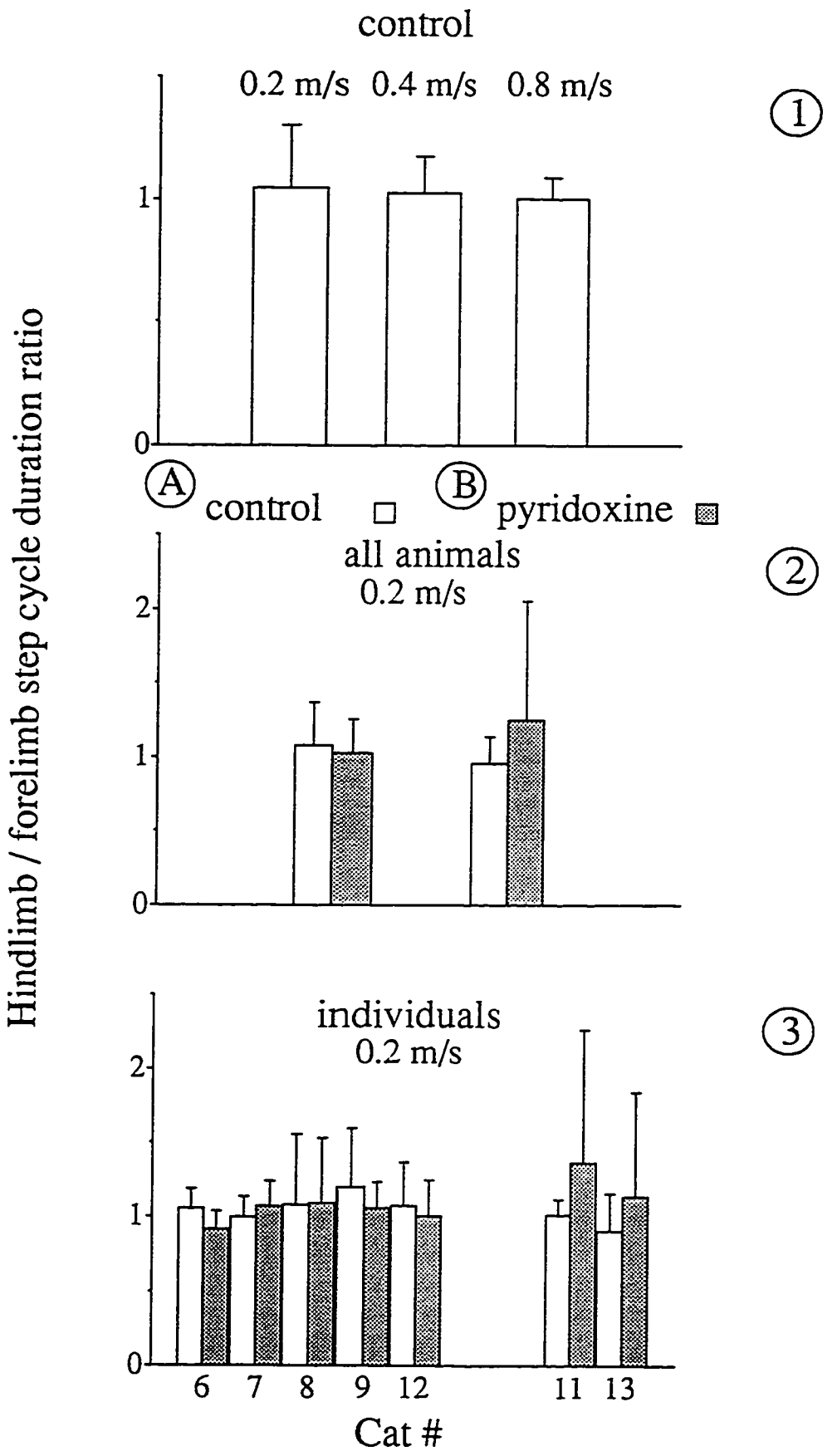
Figure 15. Cross-cycle phase histograms of forelimb-hindlimb step cycle coordination during 0.2 m/s treadmill walking. Column A: data from cat 11. Column B: data from cat 13. Row 1: histograms from control data. Row 2: histograms from pyridoxine data. Note the wide distribution of phase relations for pyridoxine recordings in contrast to the strong phase coupling between the forelimb and hindlimb for control recordings.


A
cat 11
B
cat 13
controls; 0.2 m/s
(days -3 - 0)
1
after pyridoxine; 0.2 m/s
(day 4)
2
Frequency of observations (%)
40
20
0
0
20
40
60
80
100
Hindlimb cycle phase (%)

independently (see Discussion 4.3.4). However, this figure shows the phase correlations for the two animals that experienced the greatest disruption in interlimb coordination. The other treated animals failed to significantly express disrupted interlimb coordination when statistically looking at all step cycles collected, even though step cycle variability and forelimb-hindlimb desynchronization did occasionally occur.

Not only was interlimb phase correlation disrupted in 2/8 animals, but so was the individual forelimb and hindlimb step cycle durations for these animals. As noted in Vilensky and Patrick (1984), locomotor pattern variability increases with decreasing speed of locomotion. This is exactly what we saw. Figure 16 shows the average ratios, between hindlimb step cycle duration and forelimb step cycle duration, and their respective standard deviations for all animals during control locomotion at three different speeds. As a measure of variability, the standard deviations increase as we decrease locomotion speed from 0.8 m/s (fast walk) to 0.4 m/s (comfortable walk), and from 0.4 m/s to 0.2 m/s (slow walk) (Figure 16.1). A consequence of tight phase coordination during normal walking is that fore- and hindlimb cycle durations are practically identical and the resulting step cycle ratios are close to 1. Keeping with the separation of animals used in Figures 14 and 15, we see that step cycle duration ratios are relatively normal for baseline and the height of motor syndrome for the animals that did not experience significantly disrupted interlimb coordination (Figure 16.A.2). For this group of animals, the average step cycle ratio was close to 1 with very little deviation for both control and after pyridoxine treatment recordings. The panel below (Figure 16.A.3) displays the individual step cycle duration ratios that make up this average. Again, the mean ratios were close to one and accompanied by a low standard deviation for each animal of this

Figure 16. Step cycle duration ratios between forelimb and hindlimb step cycles. Row 1: mean control step cycle duration ratios at 0.2, 0.4, and 0.8 m/s treadmill locomotion. Row 2: mean step cycle duration ratios at 0.2 m/s for control and pyridoxine recordings for a group of cats with little effect (cats 6-9, 12; A) and a group of cats with a large effect (cats 11 and 13; B). Row 3: mean step cycle duration ratios at 0.2 m/s for each individual cat. Note that cats 11 and 13, as shown in figures 14 and 15, show the greatest difference in control and pyridoxine ratios and variance. The mean step cycle duration ratio increase to 1.4 (cat 11) and 1.2 (cat 13) indicates that, on average, hindlimb step cycle duration was longer that of the forelimb.


control
0.2 m/s 0.4 m/s 0.8 m/s
1
0
A
B
control
pyridoxine
all animals
0.2 m/s
2
1
0
individuals
0.2 m/s
2
1
0
6 7 8 9 12 11 13
Cat #
Hindlimb / forelimb step cycle duration ratio
1
2
3

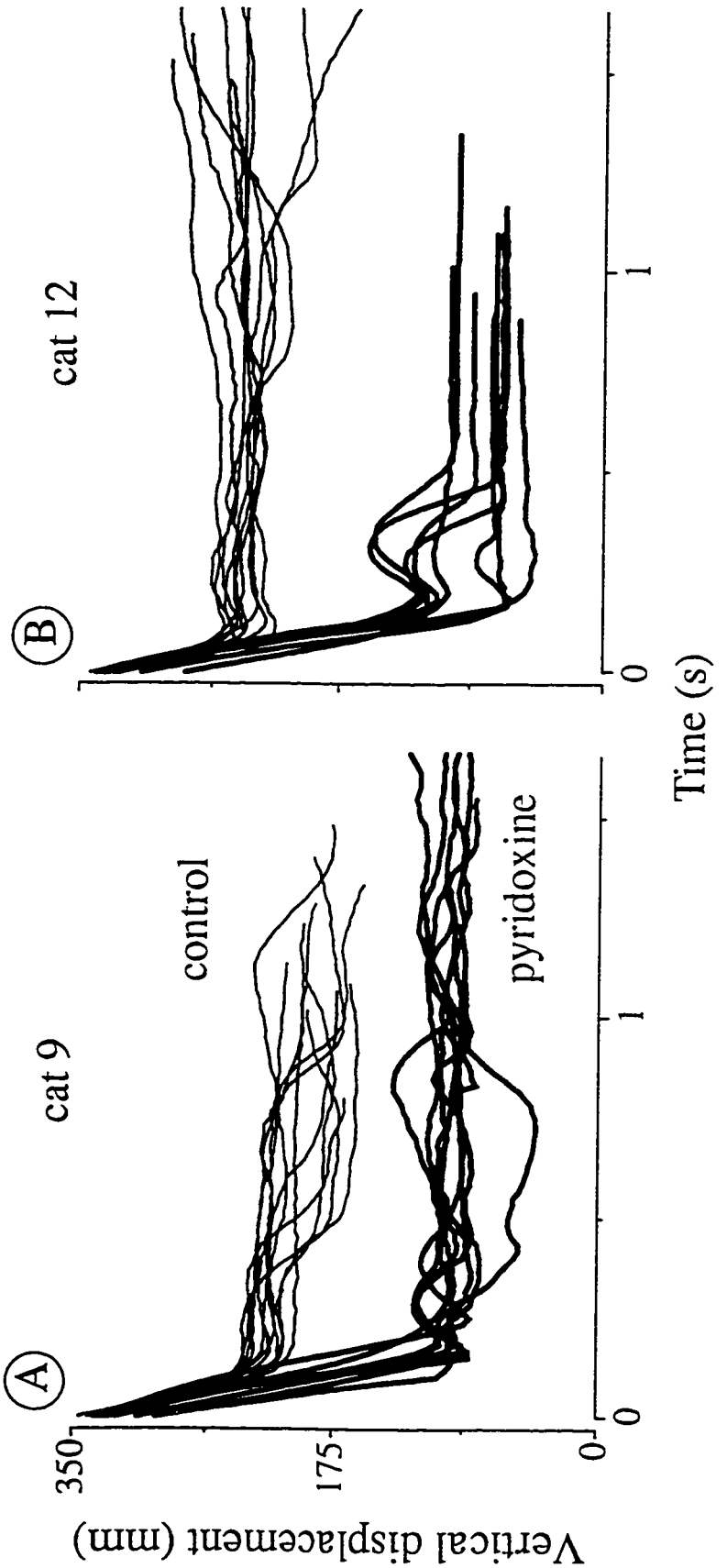
group during control and after pyridoxine treatment recordings. However, the data from the two animals with severely disrupted interlimb coordination shows considerable variability in the calculated ratios together as a group (Figure 16.B.2) and as individuals (Figure 16.B.3). The greater standard deviation scores are indicative of the greater range of ratios between forelimb and hindlimb step cycle durations, with forelimb step cycles being 1/3 to 3 times the duration of the hindlimb step cycle. For both these animals together (Figure 16.B.2) and alone (Figure 16.B.3), the mean cycle duration ratio after pyridoxine treatment was noticeably different from 1.0, in fact, their ratios were greater than 1. Therefore, on average, during episodes of decoupling, the hindlimb walked at a higher cadence than the forelimb for these two animals.

3.2.2 Landing from falls

In response to short falls (15 cm), animals typically prepare for landing by fully extending all four limbs. Upon contact, they yield and absorb the inertial impact by partial flexion in the principle joints of each limb for a period of about 100-150 ms. This is quickly followed by moderate re-extension of the limbs, resulting in a body height equal to that for their normal standing. With the kinematic recording arrangements of this study, this sequence of events during landing was conveniently monitored by displaying the temporal profiles of the vertical displacement of the lumbar spine marker.

Figure 17 illustrates the yield upon landing and subsequent stabilization at an intermediate standing height for two representative animals, cats 9 and 12. Several responses were superimposed for before and after treatment, with all displays aligned to the moment of

Figure 17. Vertical displacements of lumbar spine during landing from short falls. Column A: data from cat 9 during control (thin lines) and pyridoxine (thick lines) recordings. Column B: data from cat 12 during control (thin lines) and pyridoxine (thick lines) recordings. In the control recordings, the steady state displacement of the lumbar marker indicates the animal's standing size. Note, after pyridoxine treatment, both animals failed to stand, and landed on their trunk as the average vertical displacements of 100 mm was equal to the vertical trunk diameters.

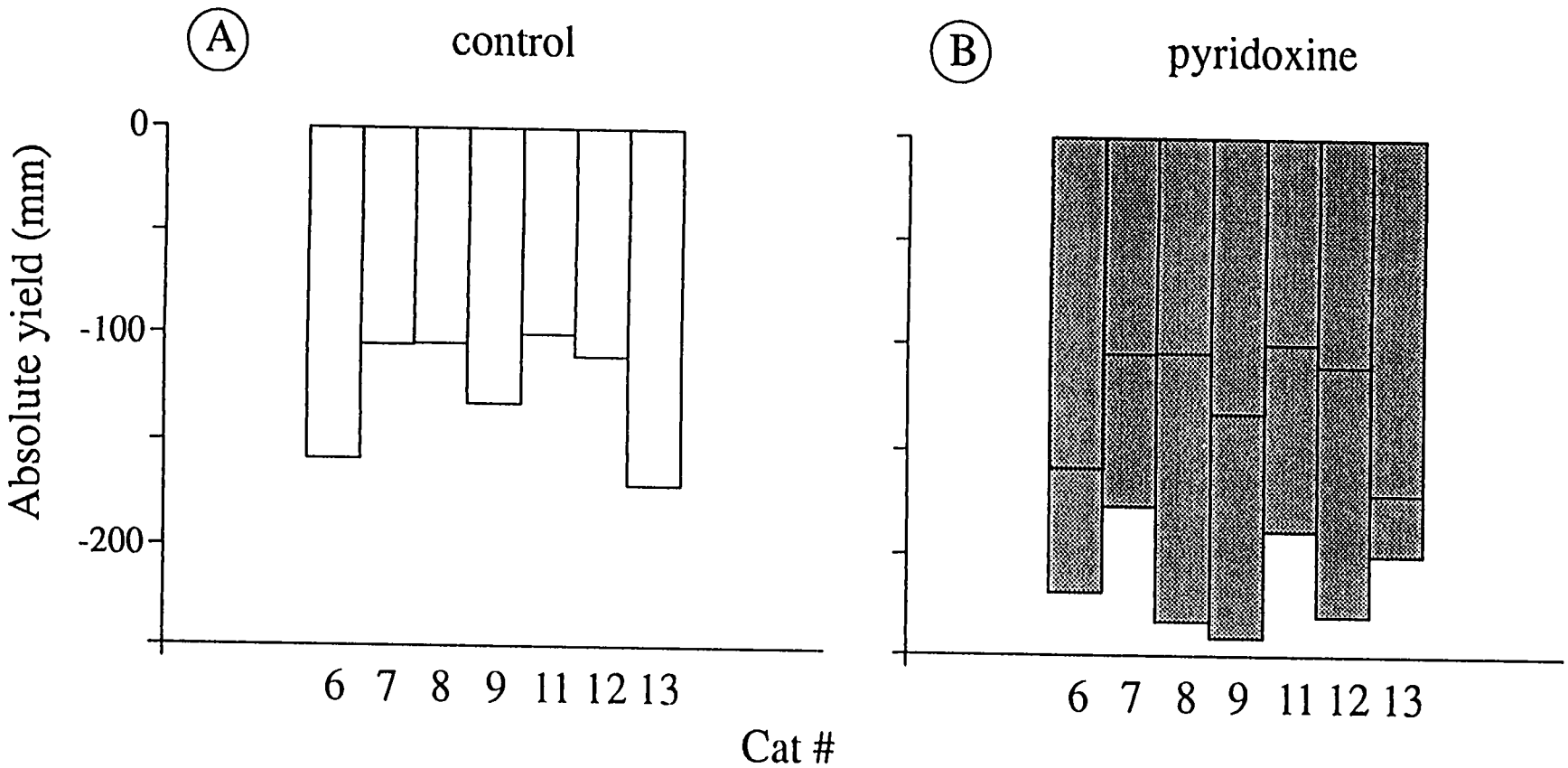

A
cat 9
control
pyridoxine
350
175
0
0
1
B
cat 12
0
1
Time (s)
Vertical displacement (mm)

first paw contact as determined by a maximum negative velocity criterion (see methods 2.6.1.2). Before pyridoxine treatment was initiated, the initial yield upon landing was minimal (Figures 17.A and 17.B, thin lines). It can be seen that in the normal landing response the yield after contact amounted to a vertical downward displacement of the trunk by 100 mm, and that the animals ended up standing as indicated by the steady displacement of the marker above the floor, 220 mm (in A) and 250 mm (in B). This difference in vertical standing position of the lumbar spine marker directly relates to the differences in size between cat 12, the larger animal, and cat 9. At the height of the motor syndrome (thick lines), both the duration and amplitude of the yield phase were significantly increased in each animal. In fact, after pyridoxine treatment, the animals failed to absorb the inertial impact and landed on their trunks, unable to stand. The residual displacement of 8-15 cm after the landing response reflects the height of the animal's trunk, with the variation between trials being attributable to different degrees of body rotation while lying.

Figure 18 demonstrates the comparison of the average steady state (>500ms) vertical displacements of the lumbar spine marker and the change in yield (see Methods) for the treated animals between control (18.A) and height of motor syndrome (18.B) recordings. The average yield for the control recordings was about 110 mm while the average yield for the same animals after pyridoxine treatment was 200 mm. For each animal, the difference in yield, seen by the superimposition of the control over the pyridoxine recordings, was statistically different ($p<0.01$).

3.3 Electrophysiology

Figure 18. Yield during landing from short falls. Data from all 7 animals tested. Column A: control fall responses. Column B: pyridoxine fall responses. Note the superimposed trace of the baseline measurements on column B, indicating the significant increase in yield for all animals ($p<0.05$).


A
control
B
pyridoxine
Absolute yield (mm)
0
-100
-200
6 7 8 9 11 12 13
Cat #
6 7 8 9 11 12 13

3.3.1 Tendon tap reflex testing

Figure 19 shows lateral gastrocnemius EMG responses to Achilles tendon taps. Under control conditions, crisp EMG responses to taps were consistently elicited when the animal was relaxed and resting on the lap of the experimenter and when the animal was standing and supporting most of its body weight, although their amplitude varied considerably (Figure 19). This variability was partly attributable to the lack of standardization of the mechanical stimulus (tap), which was applied manually. During control conditions, responses typically had a highly reproducible latency of 7-8 ms. This latency respresents the time required for the propagation of an action potential from the muscle spindle receptor to MG muscle fibres through an Ia afferent and alpha motor neurone mediated monosynaptic pathway. In some of the animals, tendon tap responses while the animal was relaxed and resting on the lap of the experimenter disappeared even before clear clinical signs of the sensorimotor syndrome were detectable (Figure 19.B.1), but this was not a consistent phenomenon and therefore not further pursued (see Methods). In contrast, tendon tap responses during standing were reduced, but not abolished, by the time the first symptoms of the syndrome were apparent (Figure 19.B.2). The EMG responses to tendon taps when the animal was relaxed and resting on the lap of the experimenter and when the animal was standing and supporting most of its body weight disappeared completely by the time the syndrome was fully developed (Figure 19.C).

The time course of tendon tap response decline after pyridoxine treatment for the entire sample of 8 cats for whom reflex testing during standing was performed is shown in Figure 20. Control recordings taken on 3 separate occasions before commencement of

Figure 19. Achilles tendon tap responses as recorded electromyographically from the lateral gastrocnemius muscle. Successive recordings (25 trials) from cat 7 of raw surface EMG superimposed and aligned by the tap impact pulses that were monitored with a transistor switch mounted on the tendon. Column A: control recordings on day 0, before commencement of pyridoxine treatment. Column B: pyridoxine recordings on day 3 when the first motor deficits appeared. Column C: pyridoxine recordings on day 4, at the time when the full syndrome was manifest. Note the gradual disappearance of the tendon tap reflex responses as the motor syndrome unfolded. Also note, that on day 3, the responses were almost clearly absent for the relaxed condition, whereas the response was clearly present for the standing condition recorded in the same session.

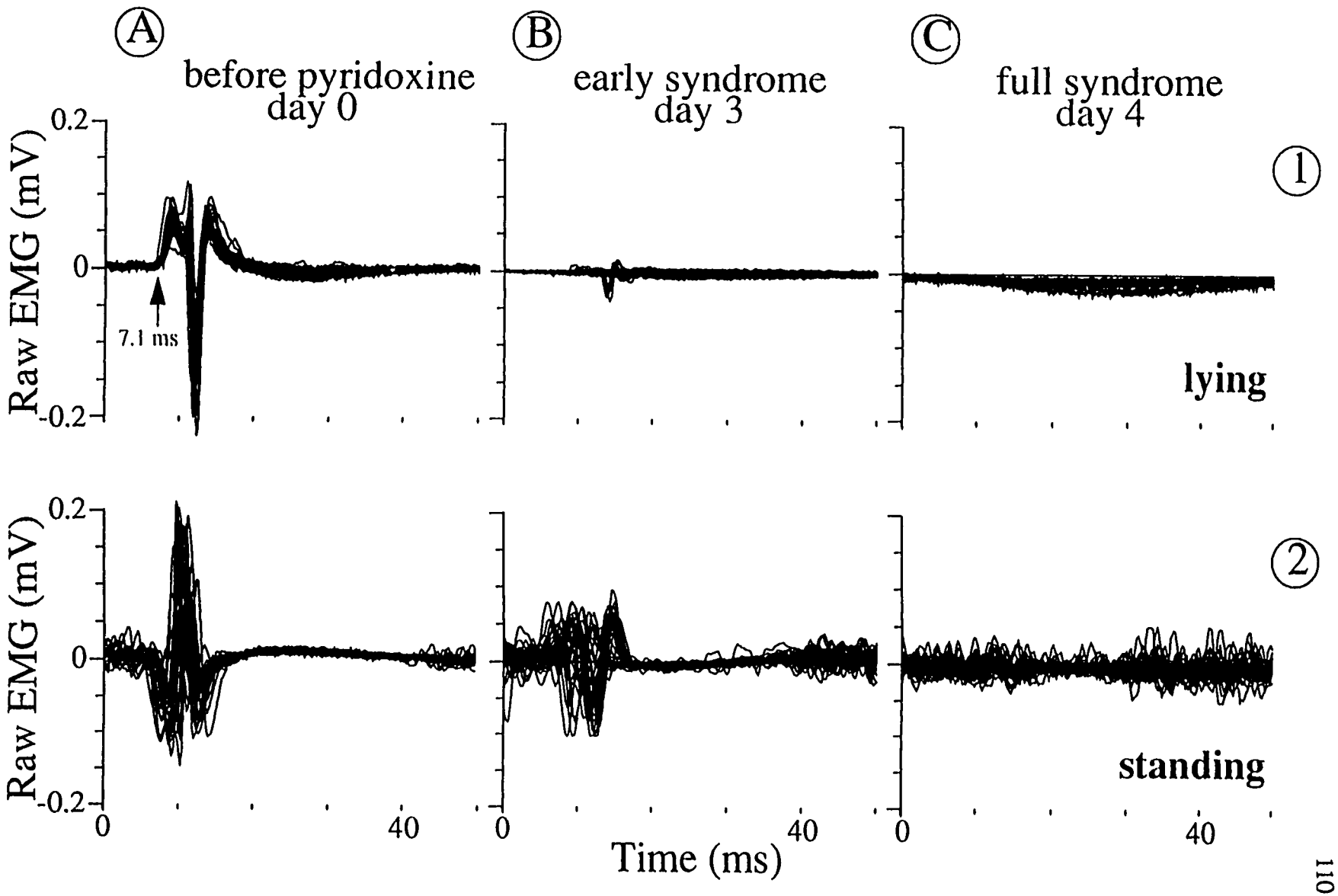
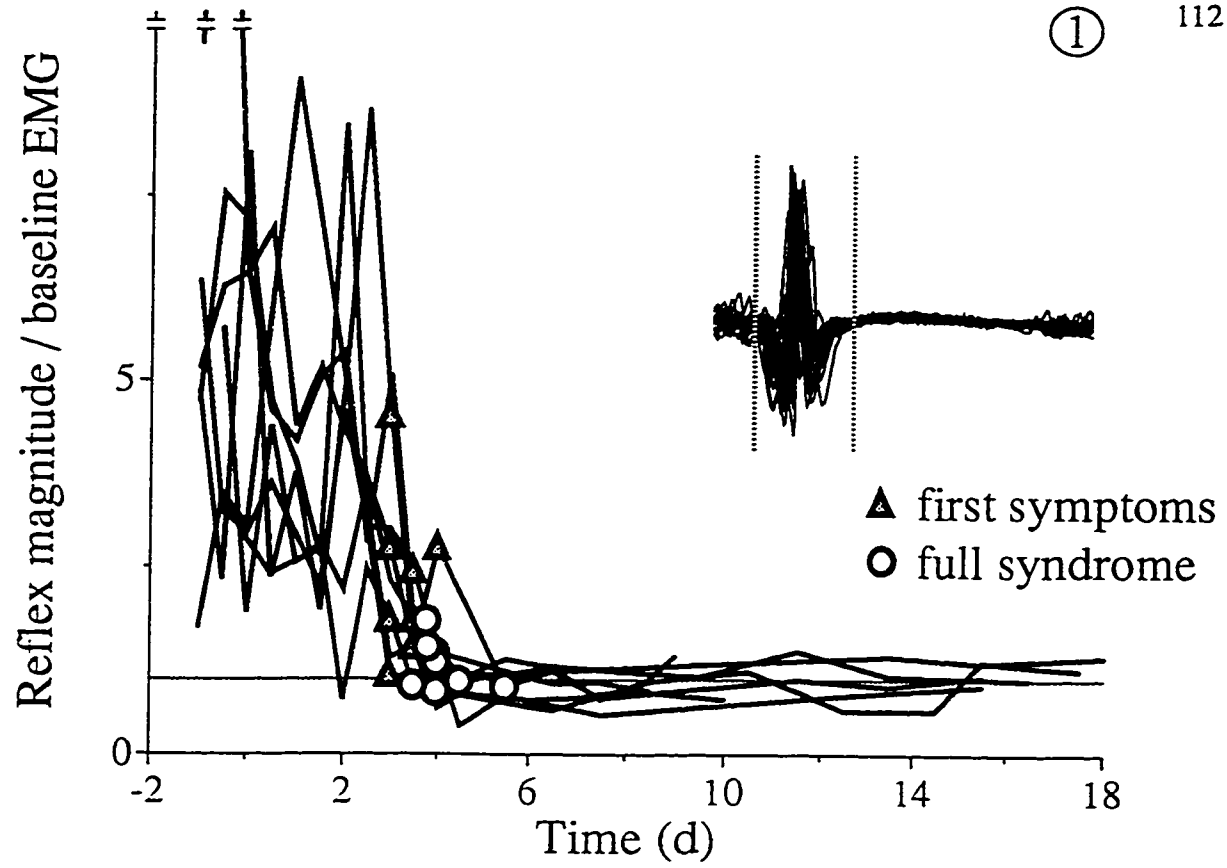
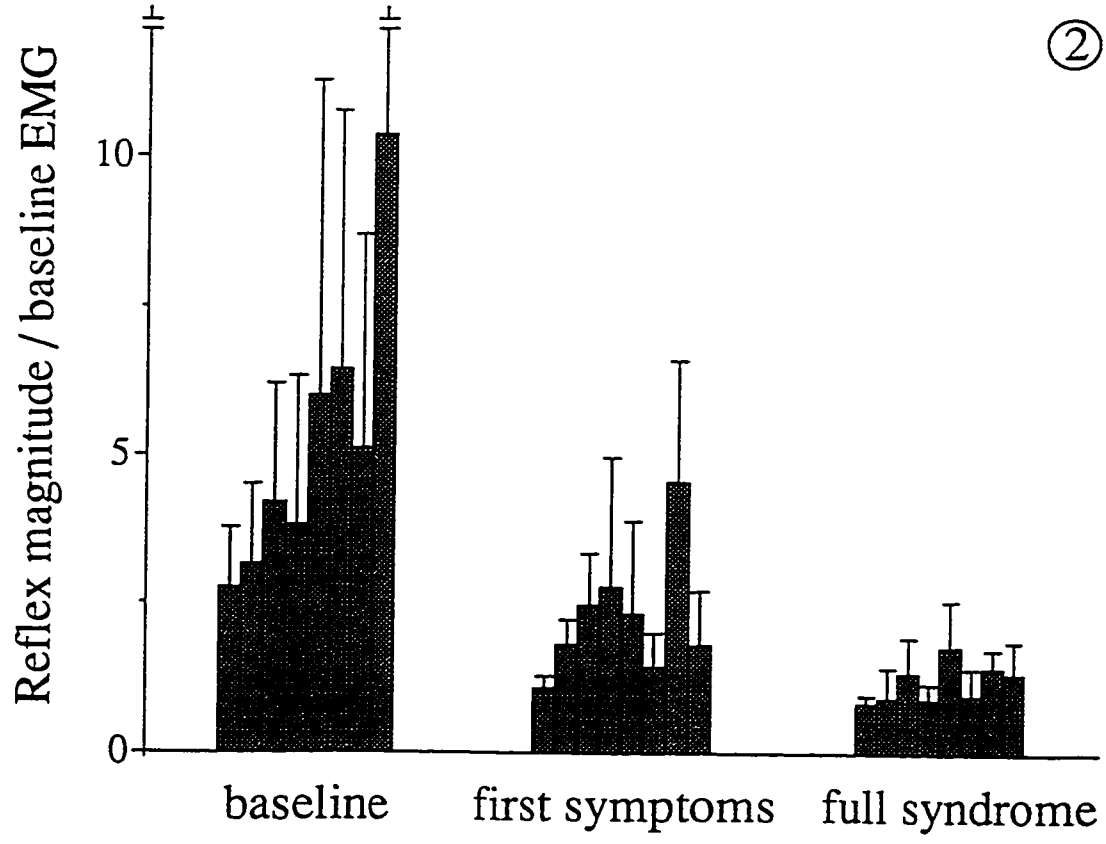

A
before pyridoxine
day 0
B
early syndrome
day 3
C
full syndrome
day 4
1
2
lying
standing
Raw EMG (mV)
0.2
0
-0.2
7.1 ms
0
40
Time (ms)

Figure 20. Average RMS-EMG responses to Achilles tendon taps recorded from the lateral gastrocnemius muscle. Data from all tested animals for the duration of the recording protocol. Row 1: average RMS-EMG for the entire sequence of recordings. Row 1 inset: typical window setting for response activity used in the analysis. Row 2: average RMS-EMG relative response activity during the periods of control, first symptoms and full syndrome recordings. Note the significant decrease in relative response activity for all animals between control and full syndrome recordings. Also note in Row 1, the similarity in time course of tendon tap response disappearance across animals.


①
Reflex magnitude / baseline EMG
5
0
-2
2
6
10
14
18
Time (d)
first symptoms
full syndrome


②
Reflex magnitude / baseline EMG
10
5
0
baseline
first symptoms
full syndrome

pyridoxine treatment were pooled and their average response for all animals was 6.5 as a ratio between the baseline activity and the response activity (Figure 20.2; see Methods 2.6.2). Even though these reflex responses were normally still present when the first motor symptoms were manifest (triangles in Figure 20.1), the response magnitudes declined to an average ratio of 2 (first symptoms in Figure 20.2). Reflex responses were abolished by about the fourth day after treatment when motor deficits had reached their maximum (open circles in Figure 20.1), resulting in a relative response activity ratio of 1 (full syndrome in Figure 20.2). The maximum syndrome was typically manifest on day 3 or 4 after the first injection of pyridoxine. Thereafter, tendon tap responses remained totally suppressed, although the majority of the animals progressively recovered from the impairment and regained some locomotor capability during the subsequent two weeks. For the majority of treated animals, the first symptoms were apparent at day 3 and the full syndrome was manifest by day 4 (Figure 20.1). The relative response activity levels calculated for these specified time frames were also consistent in magnitude as shown in Figure 2.2 for the first symptoms and full syndrome phase across all animals. The decrease in relative response activity is accompanied by a decrease in the standard deviation across trials. Thus, the decrease in response activity was consistent within a session of tendon tap trials for all individual animals.

To elucidate the state of the muscle contractility and functionality, withdrawal reflexes were monitored during the tendon tap reflex testing sessions. For all animals that could not stand at the height of the motor syndrome, EMG recordings from the gastrocnemius muscles displayed qualitatively normal activity during this reflex (not illustrated).

3.3.2 Tetanic force

The measurement of tetanic force was taken to illustrate the effects of pyridoxine treatment on muscular contractility and motor endplate viability. Clinically, it was striking that even at the height of the sensorimotor syndrome, muscle force was unimpaired.

Tetanic muscle force of the MG muscle was measured in the terminal experiment for the majority of the treated animals (8) 21 days after the first injection, and in 3 cases between 9 and 11 days after the start of the treatment. Figure 21 shows that the tetanic forces varied considerably between individuals, but that there was no systematic difference between control and pyridoxine treated animals. This observation was true regardless of whether absolute forces (Figure 21.1) or relative (i.e. body mass normalized) forces (Figure 21.2) were compared. The display of cumulative probabilities of normalized force in Figure 21.3 further emphasizes that there was no systematic difference between control and pyridoxine treated animals. The absence of a detectable difference was readily confirmed in a Kolmogorov-Smirnov test. Data from 2 of the treated animals was not included because of faulty force measurement equipment and inaccurate recordings.

3.3.3 Compound evoked potentials

Responses to graded electrical stimulation of peripheral nerves revealed drastic differences between control and pyridoxine treated animals in dorsal, but not in ventral, root recordings. Figure 22 shows averaged compound potentials elicited in a control animal by MG and SP nerve stimulation at intensities expressed in multiples of MG alpha axon threshold (T_α; see Methods 2.4.2.3). The short-latency (around 2.5 ms) antidromic

Figure 21. Effects of pyridoxine on tetanic force measured from the medial gastrocnemius muscle. Data from all animals. Row 1: average tetanic force compared with the mass of the cat. Row 2: relative tetanic force (tetanic force divided by the mass of the animal) compared with the mass of the cat. Row 3: the cumulative probability of the relative force compared with the relative force. Note that the pyridoxine treated animals produced similar tetanic forces as those produced by control animals.

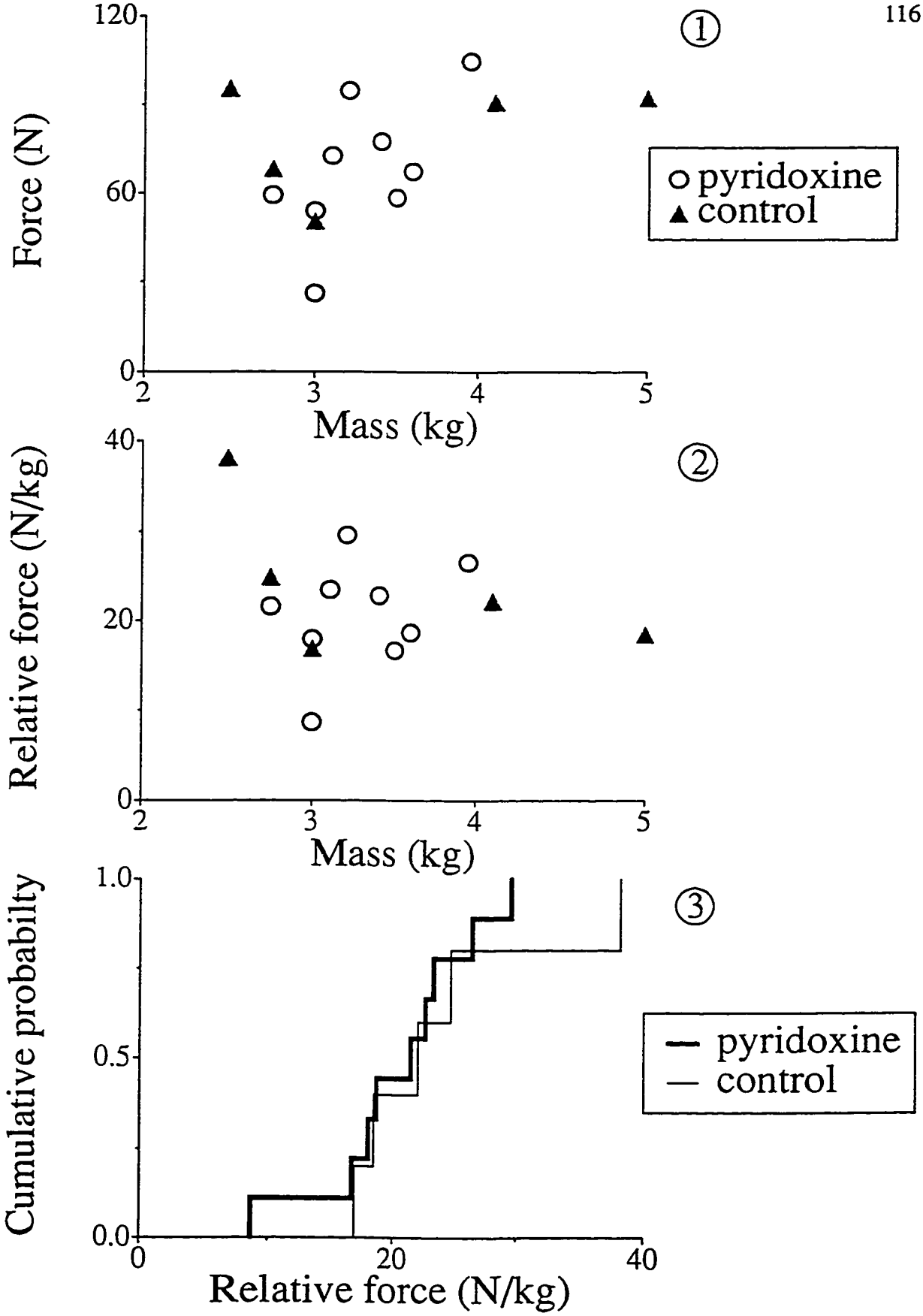
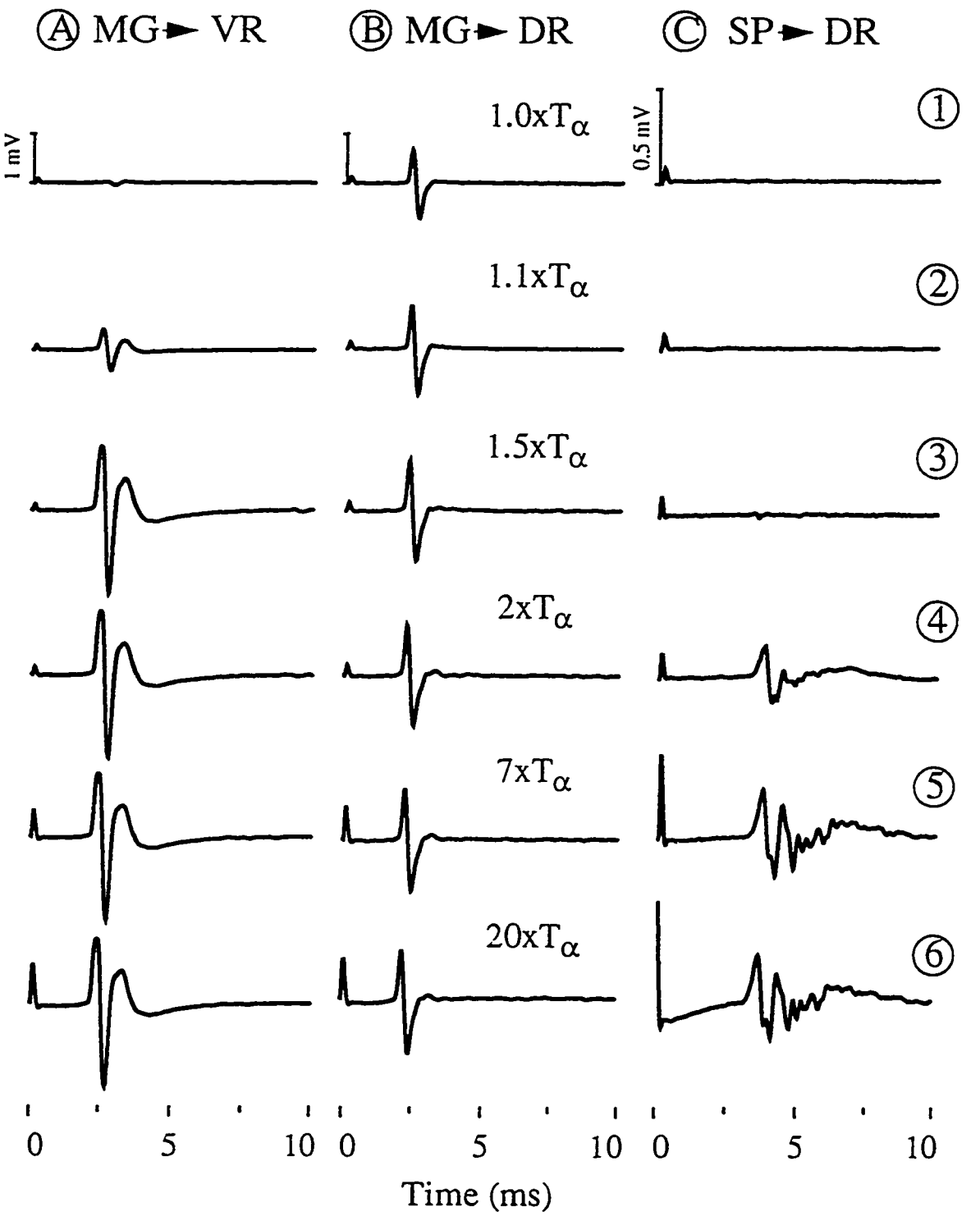

①
120
60
0
Force (N)
2
3
4
5
Mass (kg)
pyridoxine
control
②
40
20
0
Relative force (N/kg)
2
3
4
5
Mass (kg)
③
1.0
0.5
0.0
Cumulative probabilty
0
20
40
Relative force (N/kg)
pyridoxine
control

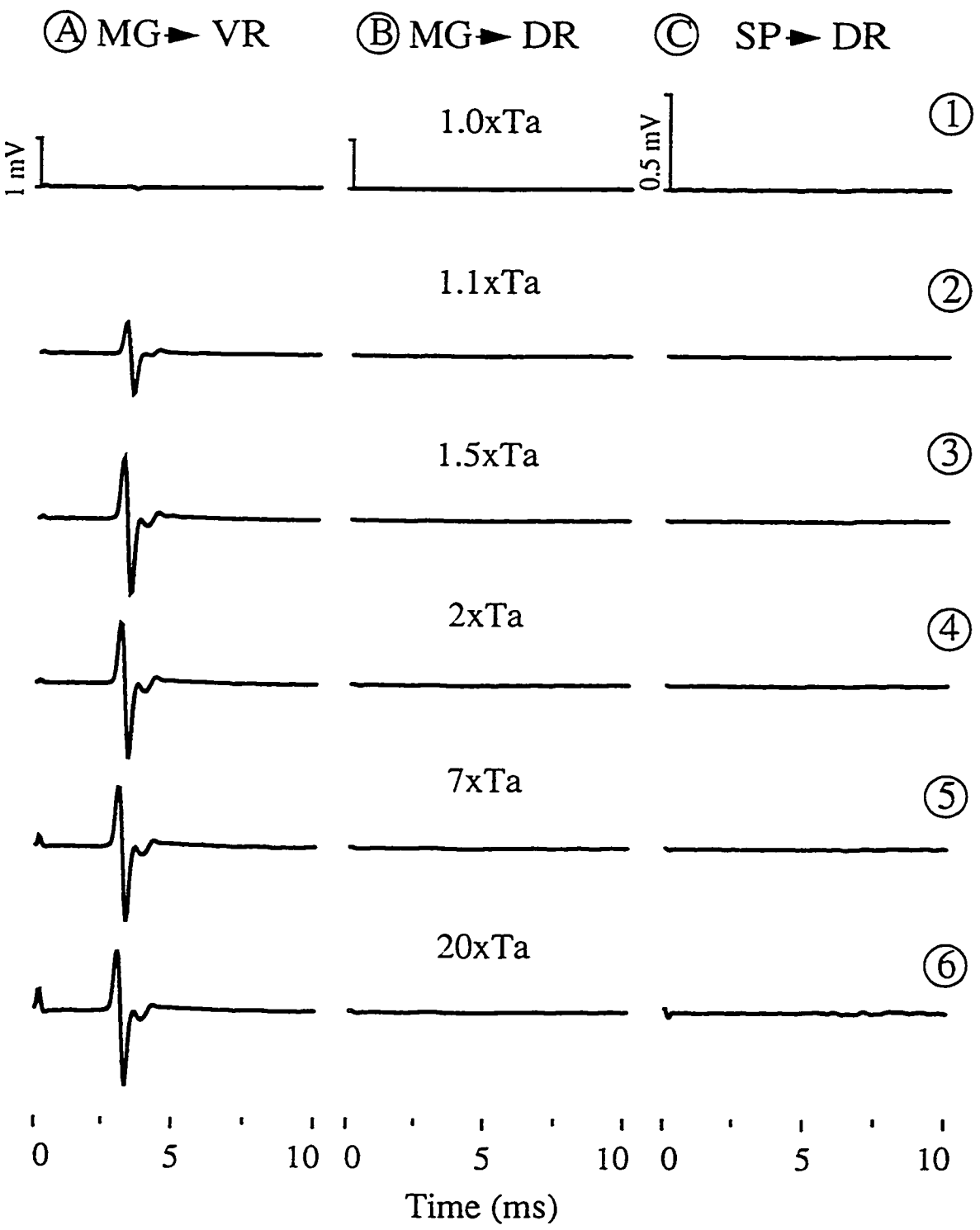
Figure 22. Averaged compound evoked potentials recorded in the spinal roots of a representative control animal in response to electrical stimulation of peripheral nerves. Column A: compound evoked potentials recorded at the ventral root (S_1) during medial gastrocnemius stimulation. Column B: compound evoked potentials recorded at the dorsal root (S_1) during medial gastrocnemius simulation. Column C: compound evoked potentials recorded at the dorsal root (L_7) during superficial peroneal stimulation. Rows 1-6: potentials resulting from increases in electrical stimulus intensity as a factor of alpha threshold. Note the large potential recorded in the dorsal roots from MG stimulation at 1.0 x T_α compared to A.1 or C.1. Also note the change in scale in column C.


Ⓐ MG► VR
Ⓑ MG► DR
Ⓒ SP► DR
1 mV
0.5 mV
1.0xTα
1.1xTα
1.5xTα
2xTα
7xTα
20xTα
①
②
③
④
⑤
⑥
0
5
10
Time (ms)

response of alpha axons recorded in the S_1 ventral root reached a maximum magnitude between 1.5 and 2.0 x T_α (Figure 22.A). In control dorsal root recordings, appreciable orthodromic short-latency group I responses were present with stimulation of the MG nerve at 1.0 x T_α (Figure 22.B). The group I volley was maximal at 1.5 x T_α. As in the case of the ventral root recordings, longer latency components were small. Even the group II (spindle secondary) component was inconspicuous, albeit well identified (latency 3.0 ms, threshold at 1.5 x T_α, equivalent to 2.0 x Ia threshold, not illustrated). Control dorsal root volleys elicited by SP nerve stimulation were consistently smaller, had higher thresholds (typically at 1.5 x T_α) and occurred at longer latency, the latter due to the longer conduction distance as well as the slower conduction velocity of the fastest SP axons (Figure 22.C). Although the SP responses of Figure 22.C were magnified for clarity (x 2), it is evident that longer latency components in the group II range (4-8 ms) were more pronounced.

Figure 23 shows compound potentials elicited in a separate, pyridoxine treated, animal using the same protocol of stimulation as in Figure 22. The ventral root responses in Figure 23.A showed short-latency components of comparable magnitude with those of the control animal of Figure 22.A. In contrast, it is strikingly evident that the dorsal root short latency low-threshold responses to MG and SP nerve stimulation were abolished (Figure 23.B and 23.C). It is clear that the missing response components were not simply shifted to longer latencies, as might be the case if axonal conduction velocity has been merely slowed, nor is it likely that thresholds of activation were simply increased, as no response components of comparable size were detected up to 20 x T_α.

Figure 23. Averaged compound evoked potentials recorded in the spinal roots of a representative pyridoxine treated animal in response to electrical stimulation of peripheral nerves. Column A: compound evoked potentials recorded at the ventral root (S_1) during medial gastrocnemius stimulation. Column B: compound evoked potentials recorded at the dorsal root (S_1) during medial gastrocnemius simulation. Column C: compound evoked potentials recorded at the dorsal root (L_7) during superficial peroneal stimulation. Rows 1-6: potentials resulting from increases in electrical stimulus intensity as a factor of alpha threshold. Note the absence of significant potential change for both medial gastrocnemius and superficial peroneus sensory nerves. Also note that the ventral root recording of motor neurone potentials were unaffected and of the same magnitude as the control animal in Figure 22. Note change of scale in column C.


Ⓐ MG► VR
Ⓑ MG► DR
Ⓒ SP► DR
1.0xTa
1 mV
0.5 mV
①
1.1xTa
②
1.5xTa
③
2xTa
④
7xTa
⑤
20xTa
⑥
0
5
10
0
5
10
0
5
10
Time (ms)

Figures 24 and 25 illustrate the generality of these findings in compilations of recordings from all control and pyridoxine treated animals that were examined in terminal experiments. For better comparison, all responses were normalized to convenient (and approximately average) conduction distances of 180 mm (MG) and 240 mm (SP), by compressing or expanding the individual records with appropriate scale factors (standard / measured conduction distance; see Methods 2.6.3). In Figure 24, the display of superimposed ventral and dorsal root compound responses elicited in control animals by MG and SP nerve stimulation at 2, 5 and 20 x T_α illustrates the consistency of the observations of large short-latency components followed by generally only very small components at latencies beyond 3 ms (MG) and 4 ms (SP). Figure 25 shows the corresponding compound responses to nerve stimulation recorded in 8 pyridoxine treated animals. There was no evident difference in the antidromic ventral root potentials between treated and control animals (cf. Figures 24.A with 25.A). In contrast, in the dorsal roots, the short-latency components were dramatically reduced, in fact nearly completely abolished, with two exceptions. First, in response to SP nerve stimulation, a single trace stands out at all three stimulation intensities (arrows in Figures 25.C.1-3). These were all from the same animal which, in single unit recordings, turned out to be the animal that clearly had the least extensive sensory deprivation in terms of the axonal conduction velocities that were affected by the neuropathy (see Figure 29.B.4). This animal was also observed to have the least dramatic motor deficits as it was able to walk throughout the experimental protocol, even at the height of the motor syndrome when all other animals could not. Secondly, small response components were consistently present, at short latency (2.5 ms), in the orthodromic dorsal root responses to MG nerve stimulation (arrow in Figure 25.B.1). Several observations from independent control

Figure 24. Averaged compound evoked potentials recorded in the spinal roots of all control animals in response to electrical stimulation of peripheral nerves. Column A: compound evoked potentials recorded at the ventral roots (L_7-S_1) during medial gastrocnemius stimulation. Column B: compound evoked potentials recorded at the dorsal roots (L_7-S_1) during medial gastrocnemius simulation. Column C: compound evoked potentials recorded at the dorsal root (L_7) during superficial peroneal stimulation. Rows 1-3: potential resulting from increases in electrical stimulus intensity as a factor of alpha threshold. Note the consistent size of potentials across all control animals for all conditions.

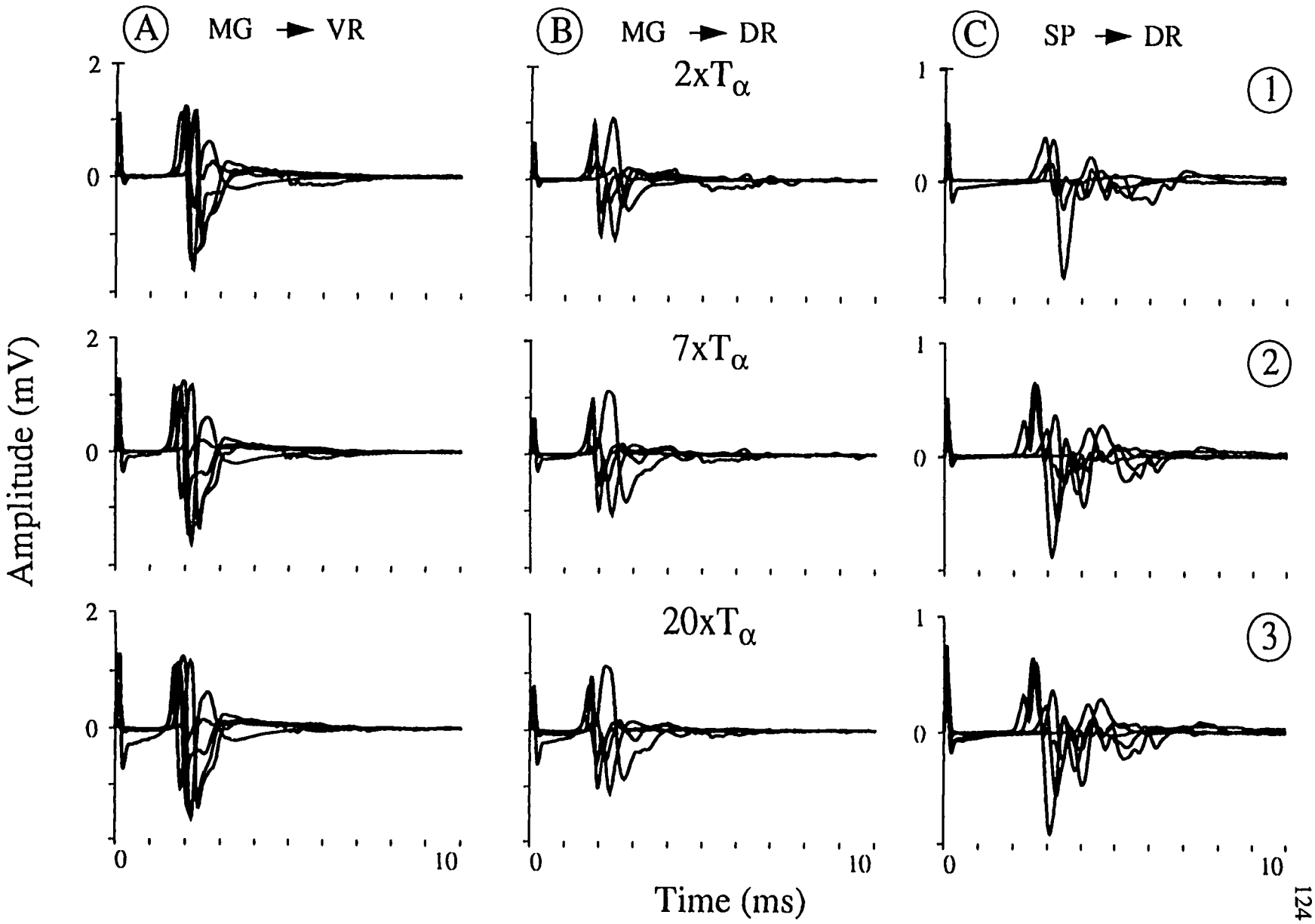
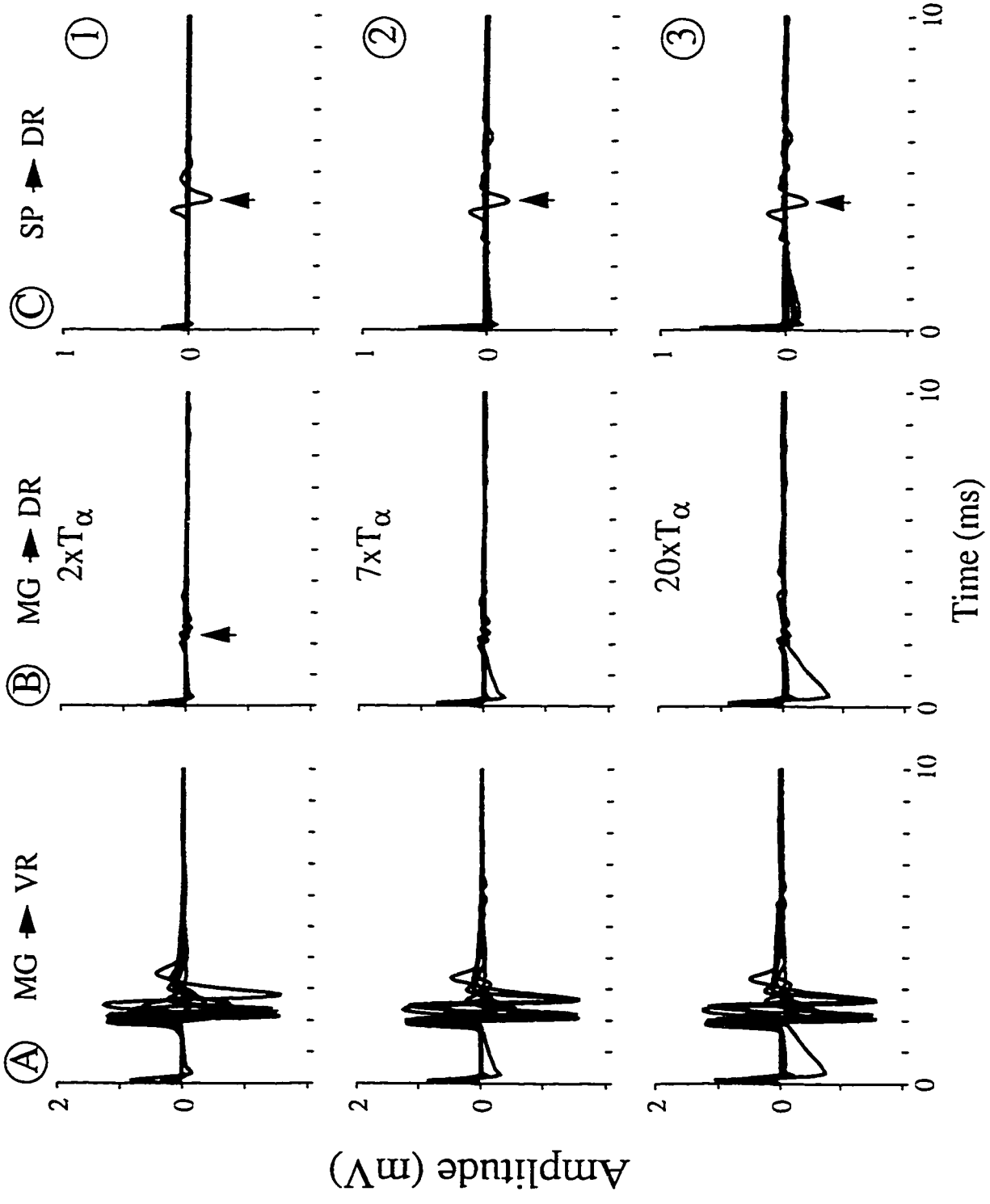

A MG → VR
B MG → DR
C SP → DR
2xTα
7xTα
20xTα
1
2
3
Amplitude (mV)
Time (ms)
0
10
2
1

Figure 25. Averaged compound evoked potentials recorded in the spinal roots of all pyridoxine treated animals in response to electrical stimulation of peripheral nerves. Column A: compound evoked potentials recorded at the ventral roots (L_7-S_1) during medial gastrocnemius stimulation. Column B: compound evoked potentials recorded at the dorsal roots (L_7-S_1) during medial gastrocnemius simulation. Column C: compound evoked potentials recorded at the dorsal root (L_7) during superficial peroneal stimulation. Rows 1-3: potentials resulting from increases in electrical stimulus intensity as a factor of alpha threshold. Note the unchanged magnitude of ventral root potentials and the abolition of potentials for both dorsal root potentials. Arrows in column C indicate the presence of cat 11's evoked potential, and column B indicate the presence of ventral root cross-talk artifact (see Discussion 4.4.3).


Ⓐ MG → VR
Ⓑ MG → DR
2xTα
7xTα
20xTα
Ⓒ SP → DR
①
②
③
2
0
1
0
10
Time (ms)
Amplitude (mV)

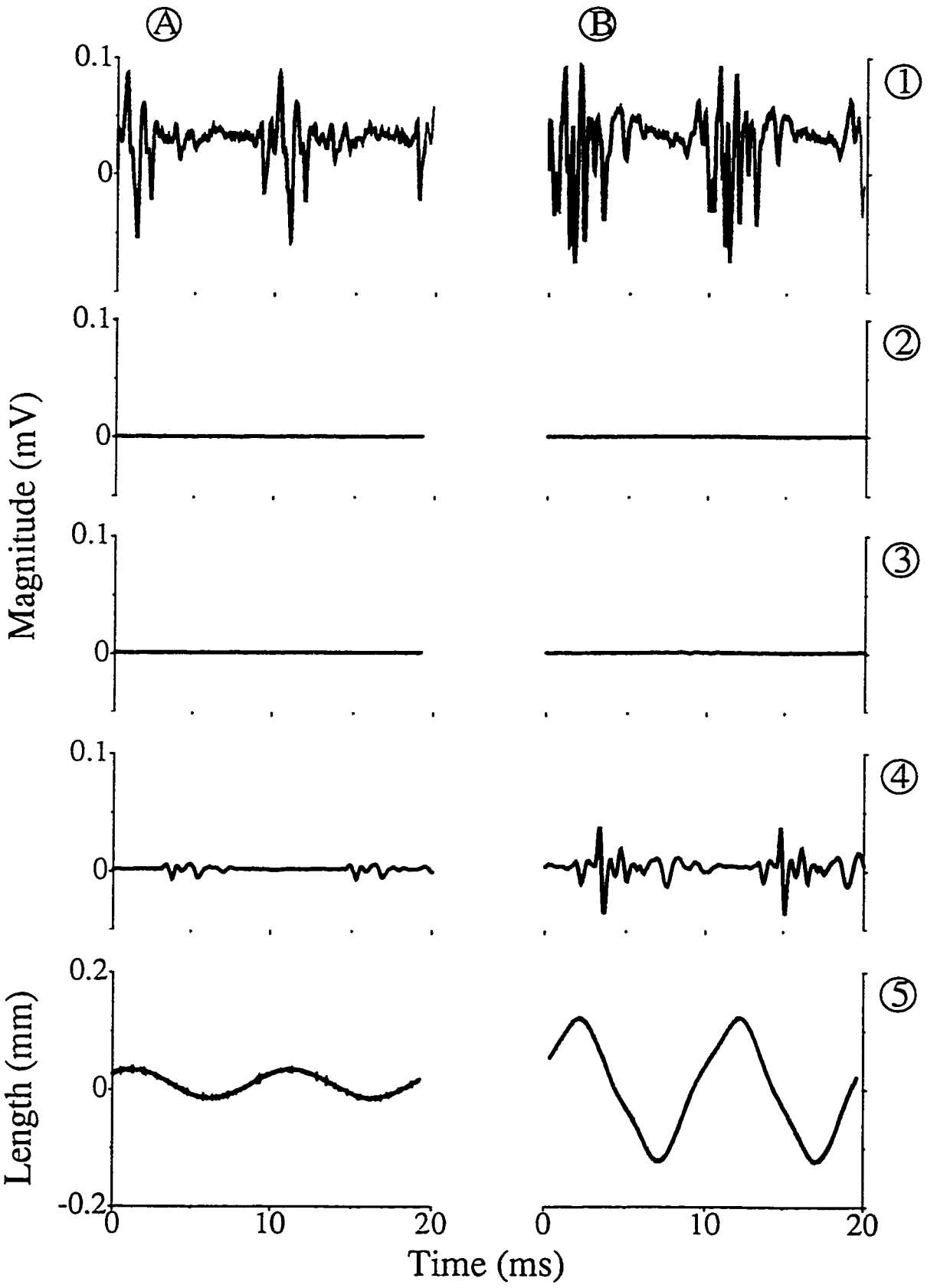
measurements identified these components as artifacts due to cross-talk from the ventral roots, where large compound potentials were present, at the same time, in response to the stimuli (see Discussion 4.4.3).

This illustrates that the ventral roots (mostly motor neurones) were unaffected by the pyridoxine treatment, whereas the sensory nerves were dramatically affected by the treatment and demonstrated a substantial drop in potential magnitude. Thus, on CEP evidence, pyridoxine intoxication caused a selective deficit in sensory axons, probably exclusively in large sensory axons.

3.3.4 Vibration

In control animals, compound responses to whole MG muscle vibration at 100 Hz showed complex profiles with multiple peaks that spanned more than 180° of the stimulus cycle. For clarity, this is illustrated in Figure 26 by displaying averaged double, rather than the conventional single, cycle histograms. The wide phase distribution of the response was not due to the spread of conduction latencies among the afferent fibres that contributed to these vibration responses, since MG neurogram recordings to the same vibratory stimuli revealed response profiles of practically identical width (not illustrated). In pyridoxine treated animals, the compound responses to vibration recorded in the dorsal roots were in most cases entirely abolished (as in Figure 26.2) or at least very strongly attenuated (Figure 26.3). In one exceptional case, relatively small responses were present after pyridoxine intoxication (Figure 26.4). The animal that revealed these responses was the same animal illustrated in Figures 29.B.4 and 25.C (with the outlier SP compound

Figure 26. Dorsal root compound response to 100 Hz vibration of the medial gastrocnemius muscle tendon. Column A: response to 50 micrometer peak to peak vibration magnitude. Column B: response to 250 micrometer peak to peak vibration magnitude. Vibration responses recorded from a representative control animal (e362; Row 1), the most severely affected animal (cat 12; Row 2), an animal exhibiting intermediate levels of syndrome (cat 13; Row 3), and from the animal with the least severe motor deficits (cat 11; Row 4). Row 5: input signals that indicate the length of the muscle at the tendon as it undergoes mechanical stretch. Note the dramatic decrease in vibration response for the pyridoxine-treated animals. Also note that cat 11 did exhibit some response to vibration even though no group I afferents were found upon single unit recordings (see Discussion 4.4.5).


Ⓐ
Ⓑ
0.1
0
①
②
③
④
⑤
Magnitude (mV)
Length (mm)
0.2
-0.2
0
10
20
Time (ms)

responses), i.e. the animal that also showed the least extent of large-fibre deafferentation.

In Figures 26.1 and 26.4 it is evident that the compound responses to vibration were appreciably larger for larger vibration amplitudes (c.f. the responses to 250 mm with those to 50 mm vibration). Since the threshold of vibration amplitude to elicit a Ia response is 5 micrometers, the increased responses observed between 50 and 250 micrometer vibration amplitudes is most likely indicative of increased activation of the mechanosensitive Ib and II afferents.

The results of the quantitative analysis of the magnitude of the response to vibration at different amplitudes at 25 and 100 Hz are shown in Figure 27. Mean values (across all control and all pyridoxine treated animals) are plotted against peak-to-peak amplitude of vibration. The main finding was that, in line with the observations from individual animals (as shown in Figure 26), the size of the vibration response for pyridoxine treated animals was consistently strongly reduced, regardless of amplitude or frequency. The fact that vibration responses at 25 Hz were reduced as much as they were at 100 Hz renders it very unlikely that the failure of sensory afferents to respond to 100 Hz vibration after pyridoxine treatment was merely attributable to some frequency dependent conduction block.

3.3.5 Muscle nerve neurogram

Figure 28 further emphasizes that the absence of low-threshold short-latency responses in compound evoked potentials was not due to some obscure change in axonal

Figure 27. Magnitude of dorsal root compound response to vibration of medial gastrocnemius muscle tendon. Column A: full cycle RMS of 25 Hz vibration response. Column B: full cycle RMS of 100 Hz vibration response. Note the dramatically decreased compound response of pyridoxine treated animals for both 25 Hz and 100 Hz vibration.

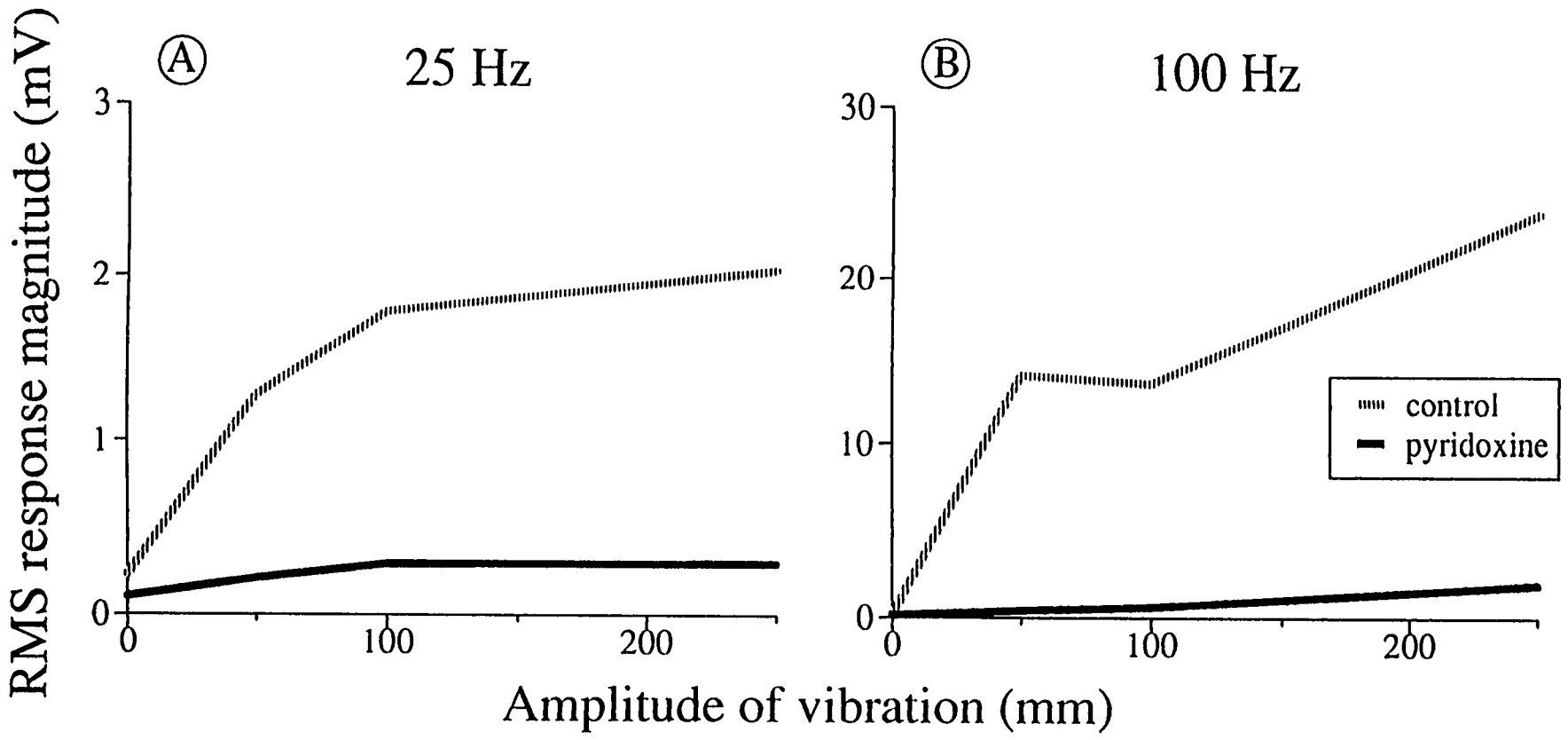
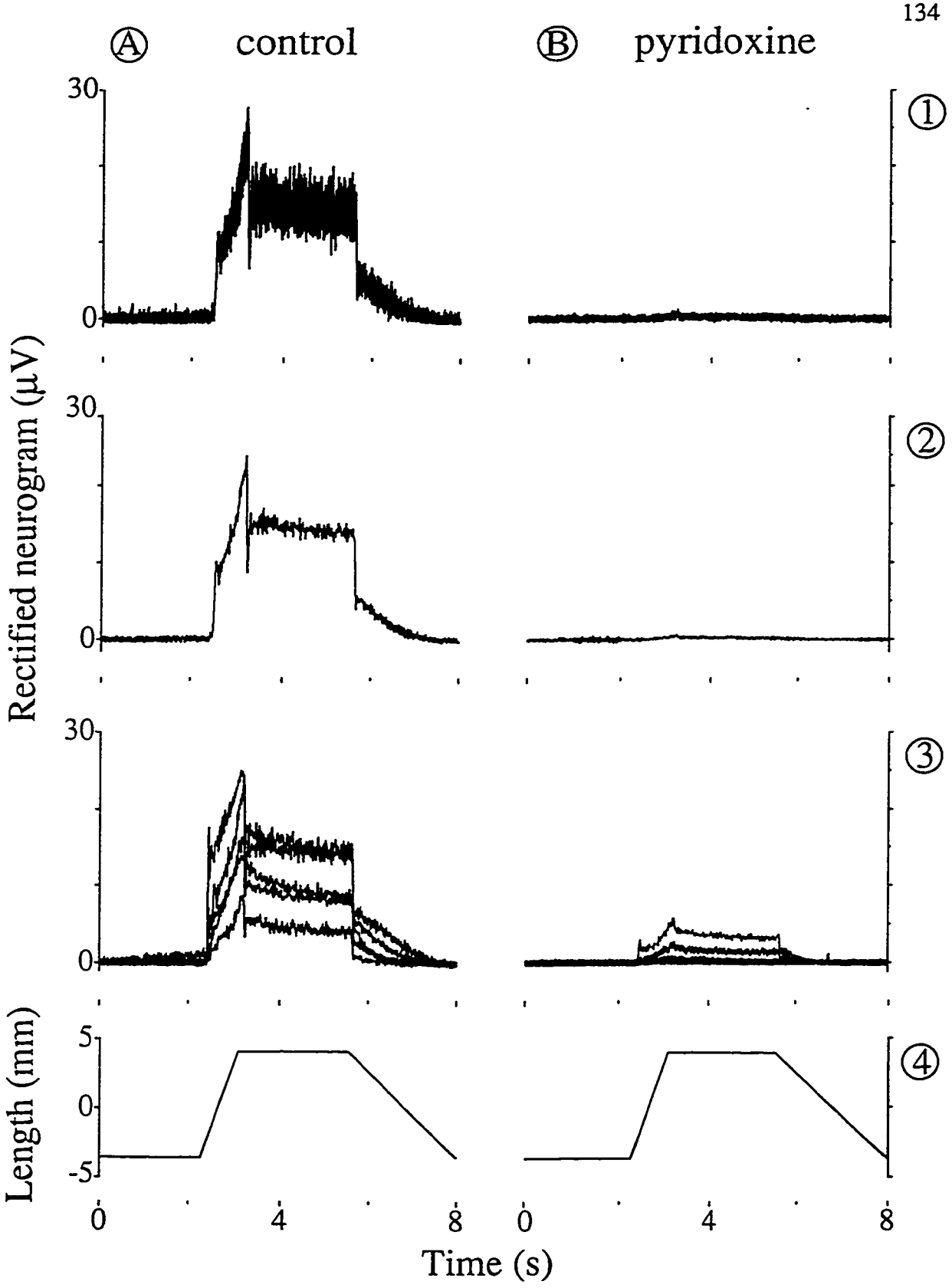

RMS response magnitude (mV)
Ⓐ
25 Hz
Ⓑ
100 Hz
3
2
1
0
30
20
10
0
100
200
0
100
200
control
pyridoxine
Amplitude of vibration (mm)

Figure 28. Medial gastrocnemius muscle nerve neurogram responses to trapezoidal stretch. Column A: data recorded from control animals; responses apparently dominated by spindle Ia afferents (see Discussion 4.4.4). Column B: data recorded from pyridoxine treated animals; significant reduction and abolition of the dynamic features. Row 1: superimposed traces of 10 recordings from one representative animal for both the control and pyridoxine treated animals. Row 2: average trace calculated from the series of traces in row 1. Row 3: superimposed average traces from all control and pyridoxine treated animals. Row 4: schematic illustration of conventional trapezoidal stretch. Note the dramatic decrease in neurogram stretch responses for pyridoxine treated animals.


Ⓐ control
Ⓑ pyridoxine
30
0
①
②
③
④
Rectified neurogram (μV)
Length (mm)
5
0
-5
0
4
8
Time (s)

excitability alone, but instead, that it appears to reflect a genuine inability of low-threshold afferents to respond to any form of stimulation. Ensemble MG afferent responses to natural stimulation were monitored by recording the MG nerve neurogram during large amplitude (8 mm peak-to-peak) trapezoidal stretch of the parent muscle. In control animals, these ensemble responses were remarkably sensitive indicators of the responsiveness of stretch sensitive sensory afferents (Figure 28.A). Judging from the presence of a distinct dynamic transient and overshoot during the dynamic phase of the ramp stretch, and the presence of an initial burst at the beginning and a clear deceleration response at the end of the ramp stretch, these responses were dominated by spindle primary (Ia) afferents.

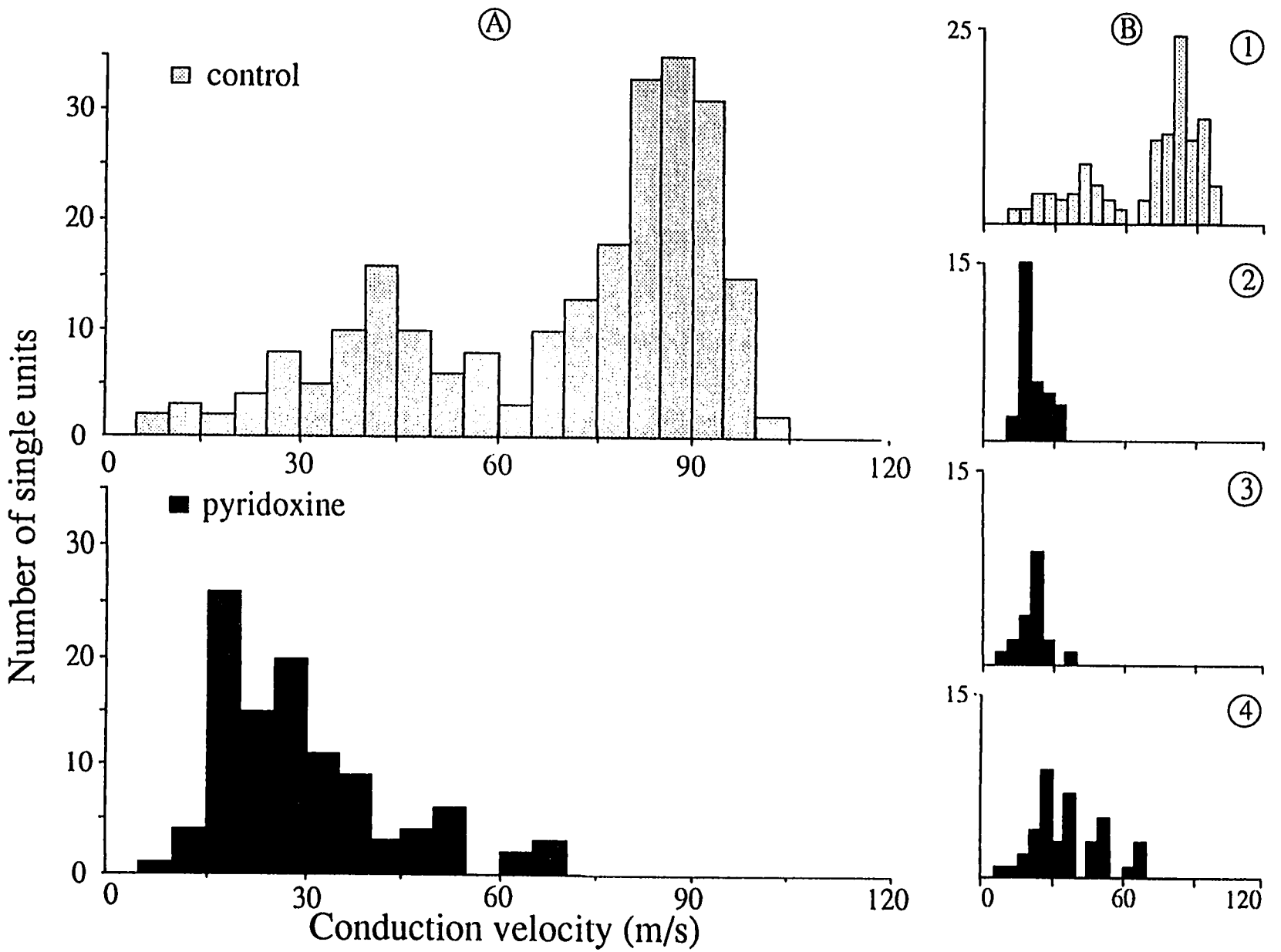
After pyridoxine, the neurogram ensemble responses were strongly attenuated (cf. Figures 28.A.2 and 28.B.2) and the characteristic features of Ia response were abolished. This is further evident in the comparison of the superimposition of all averaged responses, calculated individually for each control (Figure 28.A.3) and pyridoxine treated (Figure 28.B.3) animal. Figure 28.B.3 again reveals a distinct outlier in the family of generally depressed ensemble responses following pyridoxine intoxication. As in the case of Figures 25 and 26, this response was recorded in the animal, which judging from the evidence of Figure 29.B.4, had experienced the least extensive sensory deprivation. Neurogram responses to this form of stretch were consistent within animals (28.A.1, 28.B.1), but varying between animals of the same class, control versus pyridoxine treated (28.A.3, 28.B.3).

3.3.6 Conduction velocity - single afferent units

At the end of the terminal experiments, the dorsal roots were systematically screened for single MG afferents to estimate single unit conduction velocity spectra (see Methods 2.4.2.6). Figure 29.A (top panel) shows the classical bimodal distribution of MG afferent conduction velocities for control animals with a local minimum at 65 m/s (i.e. close to the conventional and arbitrary group I / II dividing line of 72 m/s). In contrast, two and three weeks after pyridoxine treatment, only five axons with conduction velocities at or above 60 m/s were encountered. All five had conduction velocities below 70 m/s and were classified as slowly conducting Golgi tendon organ afferents. In all eight experiments on pyridoxine treated animals with single unit recordings, not a single spindle primary (Ia) afferent was found but considerable numbers of group III and IV afferents were isolated (Figure 29.A, bottom panel).

The response of single stretch sensitive MG afferents was monitored in displays of instantaneous firing rate. An appreciable number of afferents recorded from pyridoxine treated animals, classified as group II afferents on conduction velocity evidence, revealed characteristic spindle secondary response profiles to trapezoidal stretch. In contrast, none of the single afferents isolated in pyridoxine treated animals revealed response properties typical of spindle primary afferents, with the characteristic indicators of pronounced dynamic sensitivity (dynamic overshoot, deceleration response, abrupt silence during shortening; c.f. Figure 12.A). This strongly suggests that the absence of rapidly conducting group Ia afferents reflected a genuine deficit and not merely a distortion of the normal conduction velocity spectrum due to some global slowing of axonal impulse propagation.

Figure 29. Conduction velocity of medial gastrocnemius afferents. Column A: cumulative spectra from five control animals (top) and seven B6 treated animals (bottom). Column B: individual conduction velocity profiles for a control (e351; Row 1), the most severely affected pyridoxine animal (cat 12; Row 2), an intermediate animal (cat 13; Row 3) and the least affected pyridoxine animal (cat 11; Row 4). Note that the majority of control medial gastrocnemius afferents have conduction velocities of 80 m/s or higher, whereas not one pyridoxine treated afferent had a conduction velocity greater than 70 m/s.


Ⓐ
control
pyridoxine
Number of single units
Conduction velocity (m/s)
0
30
60
90
120
10
20
30
Ⓑ
①
②
③
④
25
15

Finally, it bears emphasis that the extent of deafferentation (in terms of axonal conduction velocity) varied appreciably between animals. In Figures 29.B.2 to 29.B.4 examples of spectra obtained from individual pyridoxine treated animals are illustrated. In Figures 29.B.2 and 29.B.3 the extent of the deprivation was severe. In either case, the sensorimotor deficits and locomotor inabilities were very severe, clinically. In contrast, the animal of Figure 29.B.4 was the individual with the clinically least severe motor syndrome. This animal also showed the least severe deafferentation. Figure 29.B.1 illustrates a conduction velocity spectrum from a representative control animal. Again, a clear bimodal distribution is shown with a minimum at 65 m/s and a large number of isolated single afferents with conduction velocities in the range of group I afferents (about 90 m/s).

3.4 Correlation between motor and sensory deficits

In effort to relate the sensory deficits (cause) with the motor deficits (effects) several attempts were made plotting separate or cumulative motor deficits against the sensory deficits. The best indication of the extent of sensory deprivation was given by the individual cats' single afferent unit conduction velocity spectra. The different maximal single afferent conduction velocities amongst pyridoxine treated animals seemed to qualitatively correspond with the differences in motor deficits. For example, cat 11 showed the greatest single afferent conduction velocity of 68 m/s (see Figure 29.B.4) and was the only animal that was able to walk throughout the treatment and post-treatment periods. The measure of the maximum single afferent conduction velocity was plotted against forelimb frontal plane path length increase, hindlimb frontal plane path length increase, the increase in yield in response to landing from short falls, the extent of

crouching and the extent of plantigrade walking strategy, separately or in combination. However, not one of these trials succeeded in illustrating the degree of correlation that was thought to exist between these measures qualitatively. Possible reasons for the lack of a strong correlation between motor and sensory deficits are considered in the discussion (see Discussion 4.2.1 and 4.7).

CHAPTER FOUR: DISCUSSION

4.1 Main findings

The main kinematic finding was that sensory deprivation induced by pyridoxine intoxication elicited massive motor deficits. The animals developed a complex sensorimotor syndrome characterized by crouched gait, increased instability, abnormal limb excursions and disrupted interlimb coordination. At the height of the syndrome, most animals were unable to walk or stand for several days. They also lost their ability to adjust to motor disturbances, as illustrated by their inability to stand in response to landing from short falls.

The main electrophysiological finding was that 1 or 2 weeks after the maximal motor syndrome, short-latency responses of sensory afferents in the group I range were no longer encountered. In the majority of animals, this was accompanied by a loss of the rapidly conducting group II afferents. In contrast, both tetanic force and antidromic ventral root compound potentials, as elicited by stimulation of the MG nerve, were normal. This clearly indicated that overdoses of pyridoxine produced a highly selective large-fibre sensory neuropathy.

4.2 Critical discussion of methods

4.2.1 Time course of experimental procedures

The recordings of motor deficits and of the extent of sensory deprivation after pyridoxine treatment were separated by 1 to 2 weeks. Motor deficits were shown in treadmill locomotion and landing from short falls which were recorded at the height of the motor

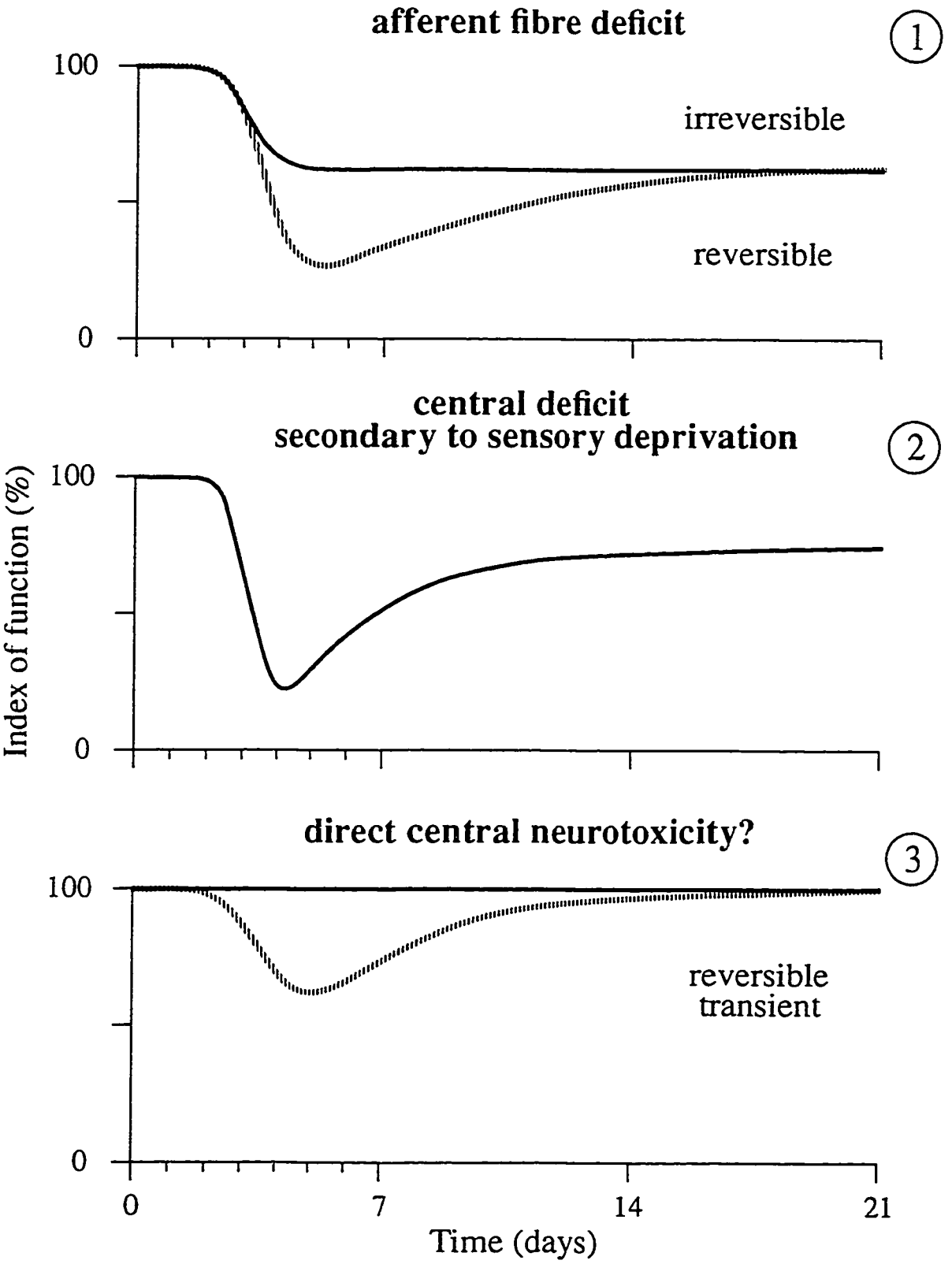
syndrome (typically day 4). In contrast, the extent of sensory deprivation was investigated during the terminal acute electrophysiological experiment, typically conducted on day 9-21 (Table 1). The presence of a 1 to 2 week gap between sensory deficit (cause) recordings and the motor deficit (effect) recordings made a correlation between the two sets of data difficult.

The terminal acute electrophysiological experiment was conducted 1 to 2 weeks after ending pyridoxine treatment rather than at the height of the motor syndrome for three main reasons. Firstly, the electrophysiological experiment was postponed until 1 to 2 weeks after the maximal syndrome developed to allow time for the manifestation of neuronal degeneration to occur. At the end of the electrophysiological experiment, the pyridoxine-induced neuronal degeneration was examined in histological samples of various nervous tissues (see later in Discussion 4.9). Secondly, the presence of a 1 to 2 week gap enabled continued recordings of treadmill locomotion, landing from falls and tendon tap reflex responses as the animals recovered motor function. The complete analysis of the data during recovery was not within the scope of this Masters project. However, preliminary results of recovery period data showed a decrease in magnitude and variance of trunk and relative paw path lengths during slow treadmill locomotion, and a decrease in yield during landing from short falls, while tendon tap reflex reponses remained absent. Thirdly, at the height of the motor syndrome, the animals did not maintain proper levels of eating and drinking. As a result, before experimenter aid was effective, the animals' health was severely compromised by weight loss (mainly dehydration). In this state an animal would not be able to withstand the demands of a 24 h electrophysiological experiment. Therefore, treated animals were allowed to recover for

a period of 1 to 2 weeks to regain proper levels of hydration and body weight.

As noted above, since electrophysiological recordings were not made at the height of the motor syndrome, several possibilities exist as to why such severe motor deficits are experienced, and why no correlation could be made between the motor deficits at the height of the motor syndrome and the sensory deficits recorded in the terminal electrophysiological experiment. Figure 30 shows some of these possibilities. First, it is clear from earlier deafferentation studies (Goldberger, 1988a, 1988b; Mott and Sherrington, 1895; Taub, 1976) that the loss of sensory feedback does disrupt normal motor function. The results from preliminary histological examination of the peripheral nerves (not illustrated) and from the electrophysiological experiments support the notion that pyridoxine created an irreversible afferent fibre deficit (Figure 30.1). This indicates the simplest possible explanation of the observed motor deficits as the sensory loss was induced rapidly and irreversibly (solid line), and remained constant after termination of the pyridoxine treatment. However, at present, it can not be ruled out that sensory loss was partly reversible (hatched line), so that it might have been more pronounced at the height of the motor syndrome. It was surprising that all animals, but one, became unable to walk at the height of the motor syndrome. The severity of the initial motor deficits may be due to an underestimation of the functional loss of sensory feedback at this stage (transient decrease in feedback function at days 4 to 5; hatched line in Figure 30.1). A large transient increase in pyridoxine (or its metabolites) could elicit a transient and reversible neurotoxic effect that impairs the function of the sensory neurons at the height of the motor syndrome more extensively than subsequently recorded in the terminal electrophysiological experiment.

Figure 30. Schematic illustration of potential pyridoxine-induced peripheral and central deficits. Row 1: potential reversible and irreversible afferent fibre deficits. Row 2: potential central deficits that are secondary to the pyridoxine-induced sensory deprivation. Row 3: potential direct central neurotoxicity. Note the index of function is an hypothetical scale with 100% representative of normal function. See text for details (Discussion 4.2.1).


afferent fibre deficit
1
100
irreversible
reversible
0
central deficit
secondary to sensory deprivation
2
Index of function (%)
100
0
direct central neurotoxicity?
3
100
reversible
transient
0
0
7
14
21
Time (days)

Secondly, it is very likely that an indirect central deficit that is secondary to the pyridoxine-induced sensory deprivation contributed to the motor deficits (Figure 30.2). The abrupt removal of sensory feedback is likely to lead to acute central deficits. With the prolonged sensory deprivation, the acute central deficits may be superseded by plastic adjustments leading to more permanent alteration of CNS function. As a result, the motor commands for and supraspinal regulation of locomotion may be inaccurate and lead to the abnormal locomotor patterns observed in the pyridoxine treated animals.

Thirdly, the results from preliminary histological examination of the central nervous tissue (see Discussion 4.9) and from the electrophysiological experiments support the notion that pyridoxine did not have any effect on the CNS. Yet, it is possible that the excess pyridoxine (or its metabolites) may have had a transient and direct neurotoxic effect on the CNS. The direct neurotoxic effect could have been reversible in character and elicit a transient decrease in normal central function (hatched line in Figure 30.3). Preliminary metabolic analyses 3 weeks after pyridoxine treatment using NMR spectroscopy revealed a depression of neuronal metabolism in the peripheral but not the central nervous system (Sutherland, Hulliger, Tyson and Bishop, unpublished observations). Therefore, if there are any direct central neurotoxic effects, it would appear at best/worst to be transient.

What Figure 30 indicates is that there are numerous reasons why a correlation could not be drawn between the motor deficits studied at the height of the motor syndrome and the sensory deficits that were recorded in the terminal electrophysiological experiment 1-2 weeks later. In addition, for a correlation to be drawn between the motor deficits and

the sensory deficits at any stage of the testing protocol, the kinematic recordings of behavioral motor deficits (e.g. in locomotion) need to be supplemented with functional testing of the sensory and motor nerves and of supraspinal centres. Therefore, while it is the most parsimonious interpretation inferred from the results of the terminal electrophysiological experiments that the motor deficits recorded at the height of the motor syndrome are due to the large-fibre sensory loss alone, the notion of an exclusively peripheral neuropathy can only be accepted tentatively. For the absence of direct neurotoxic effects on the CNS and transient peaks in peripheral sensory dysfunction are to be ruled out more directly, the same recordings would have to be made at earlier stages in the treatment and testing periods.

4.2.2 Chemical versus surgical deafferentation

It was confirmed that it was possible to produce a non-invasive, selective sensory neuropathy in the cat, using pyridoxine instead of surgical deafferentation. This allowed data to be collected throughout the entire time course of the treatment and recording protocol, as the motor deficits became manifest. Hence, pyridoxine induced deafferentation allowed for better recording resolution of the unfolding motor deficits, and a better estimate of the importance of sensory feedback in motor control compared with the surgical deafferentation where motor deficits are studied only after the sensory loss is produced (see also Introduction 1.3.1.2). Pyridoxine induced deafferentation appeared to be selective for the large sensory fibres, not affecting the group III and IV afferents. Therefore, pyridoxine intoxication at specific dose levels permitted selectivity of sensory deprivation that mirrored the sensory neuropathies in patients that had no vibration, pressure or kinesthetic sensation nor any tendon reflexes, while pain and temperature

sensations were unaffected (Cooke, Brown, Forget and Lamarre, 1985; Rothwell et al., 1982).

The time course of motor deficit development resulting from pyridoxine-induced deafferentation contrasts the time course of traditional surgical deafferentation. Deafferentation by dorsal rhizotomy is traumatic and complicated by the resulting lesion of the axial musculature that is important in the postural control of an animal (see Introduction 1.3.1.2). Therefore, after surgical deafferentation the abnormal locomotion may be produced by the effects of the surgical trauma and removal of axial muscles and/or by the effects of sensory loss. It is also difficult to know when the trauma has fully subsided, so that residual motor deficits can be ascribed to the sensory loss. Further, during recovery from the surgical trauma the animal may begin to compensate for the loss of sensory information. Therefore, the motor behavior that is observed after the recovery may underestimate the significance of sensory feedback in motor control.

Two drawbacks with pyridoxine induced deafferentation are the lack of control over the produced sensory deficits and the fact that the mechanism of neurotoxicity is unknown. With i.p. administration of pyridoxine at fixed dose levels, one cannot adjust the dosage effectively so as to produce a pre-defined extent of deafferentation. With interperitoneal injections, it is not known how much is directly effecting the nervous tissue. The amount of pyridoxine taken up by the circulatory system and its time course is dependent upon many factors such as the efficiency of the lymphatic and circulatory systems in soaking up the excess pyridoxine from the interstitial fluid and the accuracy of the

pyridoxine injection (i.e. not in a fat pad or some other abdominal organ). According to Cox, Murray and Boone (1962), approximately 70% of the administered pyridoxine is excreted by rats within 5 hours, while the remainder is excreted much more slowly with a half-life of 17 days. Thus, depending on the accuracy of the injections, the excretion process may be normal or delayed and lead to variability about pyridoxine neurotoxic effects. In addition, excessive intake of pyridoxine must exceed the kidney's capacity to take up and excrete pyridoxine metabolites and these excesses may have neurotoxic effects on a number of different tissues including the peripheral nervous tissue. In fact, muscle tissue may be a site of pyridoxine storage and toxicity and result in impaired muscular function and thus motor deficits. In contrast, it can be seen during surgical rhizotomies which nerves are or are not being lesioned.

Regarding the unknown mechanism of pyridoxine's neurotoxicity, it is not clear whether pyridoxine itself or its metabolites are toxic. Therefore, it is not known which compound should be controlled, for example by measuring serum concentrations (see later in Discussion 4.8).

As an indication of the extent and time course of sensory deprivation while motor deficits were unfolding, tendon tap reflex responses while standing were monitored daily. The presence and/or magnitude of the reflex response provided insight into the pyridoxine effects on peripheral nerves, in particular the components of the monosynaptic stretch reflex involving muscle spindle primary afferents and alpha motoneurones of the medial gastrocnemius. In addition, the presence and/or magnitude of the background tonic EMG provided insight into the functioning of the alpha motoneurones, the neuromuscular

junction, and muscle fibre action potential propagation. Typically, the animals failed to respond to the tendon taps at the same time that motor deficits were manifest, while tonic EMG activity was present throughout the entire recording protocol. Thus, tendon tap reflex and tonic EMG monitoring did provide insight into the extent and time course of selective sensory deprivation during the treatment period.

4.2.3 Dosage of pyridoxine used and technique of administration

Administration of 350 mg/kg once per day for 3 to 4 days was used to produce a rapid onset of sensory deprivation. In pilot studies, values lower than 350 mg/kg (i.e. 100 to 250 mg/kg) failed to elicit motor deficits over a period of up to one month (unpublished observations). The dose level of 350 mg/kg was chosen as it corresponded with the dose levels used in rats and dogs that produced a slowly developed, mild syndrome. We wanted to reproduce this syndrome in the cat so that we could study the motor deficits in greater detail. However, while this dose level produced a mild motor syndrome in the rat and dog, it produced a severe motor syndrome, in most cases incapacitating, in the cat.

These earlier studies with rats and dogs administered the pyridoxine either orally (Schaeppi and Krinke, 1985; Antopol and Tarlov, 1985; Hoover and Carlton, 1981a, 1981b) or through interperitoneal injections (Krinke et al., 1985; Windebank et al., 1985; Xu et al., 1989). Pilot attempts to give the pyridoxine orally were unsuccessful. Therefore, i.p. injections were used and were successful in producing a severe sensory deprivation.

In fact the rapidity of the severe sensory deprivation was in all but one case remarkably fast (within 3-4 days), in contrast with the time course of sensory deprivation in rats and dogs with the same dose level (Hoover and Carlton, 1981a; Krinke et al., 1985). In all animals, with the exception of cat 11, the motor syndrome unfolded so quickly and to such an extent that they were unable to walk at the height of the motor syndrome, confirming the earlier reports of pyridoxine-induced loss of locomotion in other species (Krinke et al., 1985; Windebank et al., 1985). As a result, recordings of unsupported walking on the treadmill could not be made, and valuable information about the motor adaptation to sensory loss could not be acquired. With more experimentation of the dose level and time course of administration, production of a slower onset of sensory deprivation will allow for better recordings and higher resolution of the unfolding motor deficits and the subsequent motor adaptation to sensory loss.

4.2.4 Treadmill locomotion versus overground locomotion

With any recording paradigm, the aim should be to simulate the natural conditions as close as possible. Laboratory overground locomotion does mimic natural locomotion much more closely than treadmill locomotion. However, there are many advantages of treadmill locomotion that made it the practical choice for this study. Firstly, with treadmill locomotion, the speeds of walking could be controlled. Thus, data collected before pyridoxine treatment could be compared with data recorded after treatment at the same speeds. Secondly, the revolving treadmill floor served to motivate and/or initiate walking which was difficult to accomplish at the height of the motor syndrome for overground locomotion. Lastly, the spatial constraints of the treadmill allowed for high spatial resolution kinematic recordings of large numbers of step cycles since animals were

trained to walk at the middle of the treadmill. With the available kinematic recording technology, a very high number of overground passes in front of the cameras would be required to collect the same number of steps as in steady state treadmill locomotion for a much shorter period of time.

The drawback of treadmill locomotion is that constraints of speed and space lead to a stereotypical gait pattern that does not necessarily reflect the variety of movement seen in overground locomotion. This larger variety for overground locomotion in comparison to treadmill locomotion is really a result of different behavior, as animals walking overground tend to devote attention to various scents and objects and rarely walk in a straight line. Since the underlying features of natural walking (e.g. inter and intralimb coordination) were unchanged and because we were only interested in the development of locomotor deficits and not behavioral differences, treadmill locomotion was justified. In addition, for quantitative assessment of locomotion and locomotor deficits, a standardized paradigm (i.e. treadmill locomotion) was necessary.

4.2.5 Analysis of walking deficits

Studies that have taken kinematic recordings of locomotion have mainly used simple techniques of data analysis. These include illustration of joint angle displacement profiles (Smith, Hoy, Koshland, Phillips and Zernicke, 1985; Grillner and Zangger, 1984; Grillner and Rossignol, 1978; Goldberger, 1988a; Wetzel, Atwater, Wait and Stuart, 1976), cyclograms (Phillips, Roberts and Smith, 1983; Trank, Chen and Huang, 1996), stick diagrams (Forssberg et al., 1980a; Drew and Rossignol, 1987; Gorassini, Prochazka, Hiebert and Gauthier, 1994; Shen and Poppele, 1995) and/or measured the time required

to cross a walkway with varying degrees of difficulty (Goldberger, 1988b). These methods of analysis either illustrate qualitative differences or provide simple quantitative results. Together, these measures fail to elucidate the localization and extent of motor deficits. Even at the height of the motor syndrome, when all pyridoxine treated animals showed dramatic motor deficits in locomotion, simple analyses of treadmill locomotion using conventional kinematic measurements, i.e. 3-dimensional joint angle displacements and velocities, did not reveal the large scale deficits we observed (Bishop et al., 1996). This comes as no surprise since gait is a multivariate phenomenon involving orchestrated activation of numerous muscles and the coordinated motion of several joints. Hence, we decided to develop a relatively simple and readily interpretable measure of the more complex aspects of the locomotor pattern. The new method of locomotor analysis examined the length of the limb trajectories in 3-dimensional space and was developed based on path length analyses. Path length analysis has been previously used to study body sway while standing (Hughes, Duncan, Rose, Chandler and Studentski, 1996), mice swimming behavior in the Morris water maze task (D'Hooge, Nagels, Franck, Bakker, Reyniers, Storm, Kooy, Oostra, Willems and DeDeyn, 1997), and hand trajectories in goal-directed trajectories (Gilman, Carr and Hollenberg, 1976; Soechting, 1988; Sainburg et al., 1995).

Changes to the angular displacements and velocities of the major joints of a limb during locomotion may be small and insignificant when analyzed on their own. Paw trajectories during locomotion reflect the coordinated action of the multiple joints involved in producing the paw motion. Abnormal paw motion, as is the case after pyridoxine treatment, will then reflect the cumulative errors in that limb's joint angular

displacements. Therefore, a series of small errors in joint angles could result in a large error or abnormality in the motion of the paw. With path length analysis of the trunk and relative paw trajectories (see Methods 2.6.1.1), the abnormal trunk and paw motion was measured by calculating the increased path length of the trajectories between control and treatment recordings. This method of analysis provided another tool for illustrating the large magnitude of the differences in locomotion after pyridoxine treatment.

4.2.6 Species used

Cats, rather than smaller animals such as rats or mice, were used in this set of experiments. The larger size of cats permitted better spatial resolution of kinematic recordings of locomotion (i.e. of limb trajectories). Cats' larger size also facilitated the surgical microdissection and single afferent recordings during the electrophysiological experiments. Extensive studies of cat treadmill and fictive locomotion and electrophysiology have provided valuable information about cat locomotion kinematics and single unit electrophysiology, and techniques of analysis for comparison with the pyridoxine treated data (Goldberger, 1988a and 1988b; Giuliani and Smith. 1985 and 1987; Goodwin et al., 1972).

4.3 Critical discussion of kinematic data

4.3.1 Landing from short falls

Kinematic recordings of landing from short falls were used as a test of rapid corrective behavioural responses. All treated animals showed significant increases in yield upon landing (Figure 29). However, kinematic recordings of landing sequences provide no direct information regarding the underlying neural mechanisms involved in normal landing

responses. In addition, the abnormality of landing responses at the height of the motor syndrome cannot provide information regarding the neurological deficits. On kinematic evidence alone, failure of anticipatory reactions cannot be ruled out without at least EMG recordings of the postural muscles involved, nor can failure of short-latency segmental reflex responses be automatically inferred. However, the latter was to be expected, if pyridoxine intoxication indeed caused a selective large fibre sensory neuropathy that would compromise feedback from muscle spindle afferents and hence the segmental stretch reflex. In fact, the time course of the loss of normal function was identical for the tendon tap reflex responses and the landing from short falls.

That all treated animals showed such a dramatic increase in yield upon landing was somewhat surprising considering the results of earlier studies. Melvill-Jones and Watt (1971) observed a burst of EMG activity in the gastrocnemius of man at about 75 ms after the onset of an unexpected fall. This finding of pre-innervation was later confirmed in the upper limb postural muscles by Dietz and Noth (1978). Both groups believed that this activity originated in the otilith apparatus as a result of the downward acceleration in falling. In addition, Melvill-Jones and Watt (1971) showed that there was an increase in gastrocnemius EMG activity prior to ground contact when walking down stairs. They suggested that deceleration upon landing is brought about by a preprogrammed response rather than by activity resulting reflexly from any mechanical stretch upon landing. Therefore, since the vestibular afferents were intact, as shown by qualitative assessments, the pyridoxine treated cats should have been prepared for the landing with a high degree of contraction in the postural muscles. Since EMG were not recorded from the postural muscles, a quantitative statement about the animals' muscle activity can not be made.

However, at the height of the motor syndrome, the rapidity in which these animals fell directly to the floor after foot contact of the treadmill would indicate that this otilith induced preparation was not sufficient in compensating for the inertial load upon foot contact.

The first experiments with landing from short falls in cats illustrated that there were in fact two segments of increased EMG activity in the postural muscles (Prochazka, Schofield, Westerman and Ziccone, 1977). In accord with the human landing responses, the first EMG burst preceded the landing. But the second EMG burst occurred at a latency of 8 ms after foot contact and was sustained for a period of up to 100 ms. During this time, spindle primary afferents were excited to very high firing rates (Prochazka, Westerman and Ziccone, 1977). A proper reflex response was thought to occur since 90 ms remained after the monosynaptic stretch reflex for the resulting force production, adequate time for a reflex activity. It is perhaps this second EMG burst, that is dependent upon sensory feedback, that may determine the success of the landing from falls. Since pyridoxine treated animals have been shown to suffer dramatic losses of large sensory afferents, then dramatic increases in yield and falling on their trunks should be expected.

The variability in the yield upon landing between animals is due mainly to the different animal sizes. In addition, during control recordings there was also variance, qualitatively, in the amount of limb flexion during the fall. Because of this, some animals may have made foot contact with both limbs already flexed; and if these flexed limbs were stiff, then the amount of yield measured would have been minimized. As stated in the results,

all animals except cat 11 were unable to walk at the height of the motor syndrome. The treated animals that were unable to walk were also unable to compensate for the inertial loading and landed on their trunks. The variability in yield within an animal's recordings at the height of the motor syndrome results from the differences in trunk rotation upon landing.

4.3.2 Frontal plane abnormalities in gait

After pyridoxine treatment, the most striking deficit in locomotion was seen visually as abnormal trunk and paw motion in the frontal plane. This abnormal locomotion has been classified as ataxic and is characteristic of pyridoxine intoxication (Krinke et al., 1985; Windebank et al., 1985; Albin et al., 1987; Antopol and Tarlov, 1985; Xu et al., 1989; Schaumburg et al., 1983; Hoover and Carlton, 1981a; Schaeppi and Krinke, 1982) and human sensory neuropathy (Lajoie et al., 1996; Rothwell et al., 1982). However, in these earlier studies of pyridoxine induced deafferentation and of sensory neuropathies the ataxic gait was not quantified. In our pyridoxine treated animals, gait abnormalities were shown quantitatively by the increased path lengths in the frontal plane, crouching and plantigrade walking strategies, and qualitatively by the fact that the animals were falling to their sides during treadmill and overground walking.

For most of the kinematic measurements, cats 6 and 7 failed to show significant differences between control and after pyridoxine treatment recordings. These quantitative results were in contrast to the qualitative fact that they both showed equally severe motor deficits as all other animals. At the height of the motor syndrome, cats 6 and 7 were unable to walk. The reason for this discrepancy was the poor temporal resolution of the

kinematic recordings of locomotion. Unfortunately, not enough recordings were made for cat 6 and recordings at the height of the motor syndrome for cat 7 were troubled by technical errors. Therefore, for both animals, the kinematic analyses used the kinematic recording session preceding the height of the motor syndrome when only mild motor symptoms were apparent. Judging from the video recordings, these sequences of walking did show differences in limb trajectories but not as severe as those observed at the height of the motor syndrome. This problem again reflects the rapidity of the development of motor deficits and the problem of temporal resolution of kinematic recordings.

The fact that the most severe walking deficits were seen in the frontal plane is most likely due to the straightforward anatomical and mechanical characteristics of the cat. The cat's quadrupedal configuration is characterized by a large separation between forelimbs and hindlimbs (longitudinal axis) and a narrow separation between left and right contralateral limbs (transverse axis). Thus, the base of support, the area under all limbs in contact with the floor, is a narrow rectangule. The cat's anatomy and corresponding rectangular base of support provides greater stability along the longitudinal axis of the cat because the center of gravity would have to be shifted a long way in this direction before it was moved outside of the base of support and resulted in imbalance. The resulting imbalance would make the cat fall over its front or behind. This type of fall was never observed.

In contrast, the center of gravity does not have to be displaced far in the transverse direction before it is moved outside of the base of support, resulting in a sideways fall. All treated animals did show this type of fall on numerous occasions at the height of the

motor syndrome. For a given error in limb placement along the transverse axis, the potential for shifting the center of gravity outside of the base of support was therefore larger than for a similar degree of inappropriate limb placement along the longitudinal axis.

When the cats are walking, signals about the center of gravity position and limb positions are continually monitored. To ensure maintained balance, corrections of limb placement are often made on line or in subsequent steps. Since limb afferents were severely compromised, the limb position, feedback on velocity and acceleration profiles of the limb and interaction forces (Gordon et al., 1995; Sainburg et al., 1993) were not known. Ghez et al. (1990) and Sainburg et al. (1993, 1995) have shown that deafferented patients are incapable of producing smooth planar reversal motions, as seen for instance in normal bread-cutting actions, because of their failure to control interaction torques and coordinate the reversals of individual shoulder and elbow joint movements, so as to achieve the synchrony normally present. The proper positioning of a limb before foot contact in cat locomotion requires knowledge about the limb during the preceding stance and swing phases. This information is not present for the deafferented animal. Thus, improper limb placement occurs, resulting in a change in the size, shape and location of the base of support which may shift the centre of gravity outside of the base of support. This was evident by the occasions on which treated animals fell to their sides or to the treadmill floor. If the center of gravity moves to the edge of the base of support, postural reflexes will direct subsequent limb movements to compensate for this abnormality. In fact this was also observed in instances where limb placement errors were less severe. In these cases of stumbling, the corrective limb placements were accurate enough and did not

result in falls. However, these corrective limb placements were often inaccurate with overadduction and overabduction and lead to further imbalance.

To prevent this whole scenario, the deafferented cats learned to accommodate their lack of balance by lowering their center of gravity closer to the treadmill floor by adopting a more crouched posture, as well as tending to display a broader stance or base of support. These two interventions act to diminish the chances of center of gravity shifting outside the base of support.

4.3.3 Crouched trunk posture and plantigrade walking strategy

Walking is a highly practised activity that is often considered to be automatically controlled. Nevertheless, continuous monitoring of sensory inputs of various origin is required to preserve the postural balance and to achieve appropriate adaptation to the ever-changing environment. Cats normally walk with limbs in a relatively extended position and by making foot contact in a digitigrade fashion (ankles and wrists never touching the ground). After pyridoxine treatment, cats walked with all limbs flexed and in a crouched style (Figures 14, 15 and 16). Also, the foot contact and kinematics of foot movement resembled plantigrade locomotion as the wrist and ankle joints often made contact with the floor (Figures 14, 15 and 16). Antopol and Tarlov (1985) also observed increased flexion of the limbs in rats that were treated with pyridoxine, while Lajoie et al. (1996) observed an enlarged base of support and increased limb flexion in a sensory neuropathy patient. Both phenomena, crouched gait and plantigrade walking strategy, have the effect of lowering the center of gravity and thereby increasing stability.

In cat 13, pyridoxine treatment appeared not to affect the height of gait or degree of plantigrade walking strategy. During the control recordings, this animal showed a much greater than average extent of flexion in its limbs during treadmill locomotion. This was due to this animal's behavioural idiosyncrasy, as it was comparatively more timid and extremely curious about the various scents detected on the treadmill floor. As the motor deficits unfolded, this animal maintained a low profile of gait, but the limb excursions became increasingly abnormal.

4.3.4 Disruption of interlimb coordination

As stated before, limb excursions after pyridoxine treatment were abnormal in all three axes of movement. Along the longitudinal axis, abnormalities were manifest as shorter or longer than average steps for steady state treadmill locomotion. These shorter or longer than average steps were often isolated to the forelimb or the hindlimb. As a result we observed occasions of disrupted interlimb coordination in all treated animals, as did Windebank et al. (1985). However, only two animals displayed significant amounts of disrupted interlimb coordination.

Related observations of loss of phase coordination between ipsilateral and contralateral hindlimb movements was previously reported by Giuliani and Smith (1987), Wetzel et al., (1976) and Bem, Gorska, Majezynski and Zmyslowski (1995). In Giuliani and Smith (1987), stepping movements were elicited reflexly in spinalized animals that were held in the air. Following unilateral deafferentation by segmental dorsal rhizotomy, the coordination between hindlimbs, which normally expresses itself in regularly alternating movements, was no longer present. In this air-stepping paradigm, locomotor movements do not have to support body weight and therefore, are less restrained mechanically. This

in turn might favor independent operation of the CPGs that control individual limbs. After lesioning the ventral quadrants and the dorsolateral funiculi at the 10th thoracic (T_{10}) vertebra in cats, Bem et al. (1995) observed that the operated animals changed the ipsilateral coupling of forelimbs and hindlimbs. In an intact cat, the hindlimb movements precede the forelimb movements. In contrast, the operated animals showed forelimb movement in advance of hindlimb movement. Yet the step cycle durations remained unchanged after the spinal lesions. Since the spinal cord transections were extensive and at the level of T_{10}, this form of decoupling may not be such a surprise. Yet, both studies (Bem et al., 1995; Giuliani and Smith, 1987) suggest that sensory feedback from the moving limbs may contribute significantly to normal interlimb coordination. The fact that after pyridoxine treatment animals showed gait patterns where forelimb and hindlimb step cycles acted independently of each other, supports such an implication.

These findings are of interest in the light of the current theories of interlimb coordination, which mostly envisage some network of coupled oscillators (representing limb or joint CPGs), where typically coupling is thought to be mediated by segmental and propriospinal interneuronal systems (Grillner and Wallen, 1985; Grillner, Wallen and Brodin, 1991; Grillner and Matsushima, 1991; Baev, Esipenko and Shimansky, 1991). The signals carried by these coupling neurons are phasically modulated, reflecting the rhythmic output of the source oscillator (Grillner et al., 1995). They can be viewed as internal feedback signals. On this interpretation, internal coupling feedback signals may have similar temporal profiles and carry similar rhythmic information as external feedback signals from the length and force sensors of moving limbs. During locomotion, these proprioceptive feedback signals are well known to be modulated with an approximately

fixed phase relationship to the locomotor cycle (Prochazka, Trend, Hulliger and Vincent, 1989; Procazka, 1996). It is therefore conceivable that, with appropriate propriospinal projections, such rhythmic proprioceptive feedback from one limb might contribute to the physiological coordination of rhythmic activation, or entrainment, between the parent limb and the other limbs of the system. Thus internal feedback coupling would be supplemented by external feedback coupling. Functionally, such external feedback modulation of interlimb coordination could serve the purpose of adapting interlimb coordination to the conditions of the environment in which locomotion takes place.

At the height of the motor syndrome, treated animals experienced extreme difficulty in walking. Their fastest speed of locomotion was 0.2 m/s. However, before pyridoxine treatment, animals had considerable difficulty walking in a steady state fashion (at a constant position on the treadmill) at the slow 0.2 m/s treadmill speed. Paradoxically, the limb trajectories along the length of the treadmill were more variable before rather than after pyridoxine treatment. In addition, at the height of the motor syndrome, the number of recorded 4 s step sequences was much smaller than the number of control step sequences recorded because of the shortened time window for maximum syndrome recordings (see later in Discussion 4.7). Therefore, even though all animals showed disrupted forelimb-hindlimb coordination, the variability of the control recordings and the smaller number of recorded step cycles at the height of the motor syndrome prevented significant results of disrupted interlimb coordination to be demonstrated statistically in the other treated animals.

4.4 Critical discussion of electrophysiological data

Terminal acute electrophysiological experiments showed that the early components of orthodromic sensory compound potentials in dorsal roots were abolished. This was true for compound potentials elicited electrically from both skin and muscle nerves. Thus, the motor syndromes of these treated animals cannot be attributed to the loss of a single sensory modality, since feedback from several types of receptors, including muscle spindles, Golgi tendon organs and low-threshold cutaneous mechanoreceptors must have been reduced, and in some cases (e.g. spindle primary afferents) probably entirely abolished. It turned out that in all treated animals, the neuropathy abolished all sensory feedback in axons conducting at or above 70 m/s and, in all but one case, in axons conducting above 60 m/s. Thus, the single afferent unit recordings revealed a complete deficit of group I afferent responses and a significant deficit of fast conducting group II muscle afferent responses, while slowly conducting group II and group III fibres were responsive and readily isolated. These findings support those of Schaeppi and Krinke (1985) who also observed electrophysiologically a selective sparing of sensory evoked potentials in group III sensory neurones but not group I and II afferents after pyridoxine administration. Moreover, there appears to be a correlation between the clinical severity of the syndrome and the extent of deafferentation: the animals with the most striking motor dysfunction, qualitatively, were also those with the most pronounced sensory loss.

4.4.1 Tendon tap reflexes

Tendon tap reflex responses were recorded to monitor the Ia (primary muscle spindle) function throughout the time course of motor deficit development. The tendon tap reflex is thought to be mediated by Ia afferents which synapse monosynaptically with alpha motoneurones that activate the agonist muscle to contract in a corrective manner

(Matthews, 1972). Absence of reflex responses was observed in all treated animals at the height of the syndrome, and were not present for the duration of the testing period.

The absence of a tendon reflex may be due to several factors such as, 1) failure of encoding the mechanical (stretch) signal to an electrical signal in the form of an action potential at the level of the muscle spindle receptors during the dynamic stretch of the gastrocnemius muscle, 2) impaired conduction of action potentials along the sensory nerve to the spinal cord, 3) impaired propagation of signal across the sensory neurone and alpha motoneurone synapse, 4) impaired conduction of an action potential along the motor axon, or 5) impairment of neuromuscular transmission. However, at the height of the motor syndrome, when tendon tap reflex responses could not be elicited, vigorous withdrawal reflexes were easily produced. In addition, while monitoring tendon tap reflex responses during standing, the background tonic EMG activity after pyridoxine treatment was identical in magnitude with that of control responses. These observations indicated that the motoneurone action potential conduction and production of muscle activity was unaffected. Therefore, the abolished tendon reflex was indicative of a sensory nerve defect, and the exact localization of the damage could be better elucidated in the terminal experiments.

As stated before, all animals experienced a total loss of tendon tap responses while relaxed and standing. The loss of tendon tap reflexes in these animals confirms the findings in other pyridoxine-induced deafferentation studies (man: Albin et al., 1987; Schaumburg et al., 1983; McClachlan and Brown, 1995; dog: Schaeppi and Krinke,1985) and studies in patients with sensory neuropathy patients of unknown etiology (Gordon et

al., 1995). However, abolition of reflex responses might simply reflect changes in motoneurone excitability. The recordings of Figure 3 demonstrate that this was unlikely to be the case. During standing, the ankle extensor muscles were tonically active under control conditions. Even in superimposed raw EMG records, as shown in Figure 3.A.2, short-latency reflex responses were readily identified. That motoneurone excitability mattered was emphasized by the appreciable increase in reflex response magnitude that was observed during standing (Figure 3.B.2) compared with the relaxed condition (Figure 3.B.1) at the early stages of the sensorimotor impairment. However, even in the presence of background activation, tendon tap reflexes were abolished by the time the motor syndrome was fully developed (Figure 3.C).

The time at which tendon tap reflexes were abolished coincided with the height of the motor symptoms (inability to walk, see Figure 4.A). For the remainder of the recording protocol the tendon taps were not elicited in any animal, even though all but one animal in the extended recording protocol (3 weeks) did recover locomotor function and ability (not illustrated). These results indicate that locomotor recovery can occur in the absence of primary muscle spindle feedback.

4.4.2 Tetanic force

The first diagnostic test conducted in the terminal experiment was the measurement of force in a maximal tetanic contraction. This was examined to assure that motor output and muscle contractility was not affected by the pyridoxine treatment. As seen in Figure 5, the force productions of the pyridoxine treated animals did not differ from those recorded from the control animals. This could not be predicted a priori since excess

pyridoxine is stored in the liver and muscle tissue. Therefore, excesses of circulating pyridoxine could prevent proper muscular contraction. In addition, other studies have observed pronounced weakness after pyridoxine treatment (Antopol and Tarlov, 1985; Schaeppi and Krinke, 1982; Dalton and Dalton, 1987). However, in Dalton and Dalton (1987) muscle weakness was qualified as the difficulty in walking, lifting and climbing stairs which were all hampered by stiffness, and this stiffness was associated with a higher degree of cocontraction, not weakness of the muscles. The weakness of muscle activity was not observed in the pyridoxine treated animals reported here (see Results 3.3.1 and 3.3.2) and other studies (Schaumburg et al., 1983; Xu et al., 1989), nor in sensory neuropathy patients (Rothwell et al., 1982; Sainburg et al., 1993).

4.4.3 Compound evoked potentials

In support of the tetanic force recordings and literature stating that motoneurones are unaffected by pyridoxine treatment, compound potentials for the ventral root recordings of antidromic potentials for treated animals showed no difference in conduction velocity or wave amplitude from the control measurements (c.f. Figures 8.A and 9.A; Windebank et al., 1985; Schaumburg et al., 1983). In contrast, dorsal root recordings of both predominantly motor (MG) and cutaneous (SP) sensory nerve electrical stimulation demonstrated complete absence of evoked potentials after pyridoxine treatment in this study (Figures 9.B and 9.C). This loss of orthodromic compound potentials has been reported in other pyridoxine neurotoxicity studies as well (Windebank et al., 1985; Albin et al., 1987; Berger et al., 1992; Schaumburg et al., 1983). At stimulus intensities that in control animals elicit clear orthodromic responses, 1 x T_{α}, no responses were found in any of the treated animals. Even at 20 x T_{α}, only minimal responses were elicited

in the animal that experienced the lowest degree of sensory neuropathy as indicated by the single unit isolation measurements (see later in Discussion 4.4.5). Together, these observations highlight the selectivity of pyridoxine intoxication for sensory and not motor nerves.

However, compound evoked potentials reflect the ensemble activity of the nerves studied. Therefore, compound evoked potentials reflect the activity of the nerve as a whole and does not demonstrate the extent to which afferents of different sizes are affected by the pyridoxine treatment. To properly elucidate whether individual neurones were affected by the pyridoxine treatment, a thorough examination of single sensory and motor neurones in the MG nerve would have to be conducted. This method of single unit isolation was carried out, but only for the dorsal roots because the CEP results indicated that the functional deficits were in the nerves of the dorsal root and not the ventral root. Extensive single efferent unit isolation of the ventral roots was not performed because of the added time demanded for this procedure. Therefore, the extent of motoneurone damage from pyridoxine intoxication was not further electrophysiologically investigated with single unit recordings. The results from the ventral root CEP combined with the EMG recordings during the tendon tap reflex monitoring and the tetanic force measurements strongly suggest that alpha motoneurones are unaffected. What cannot be ruled out with equal certainty is if there was any functional impairment of the δ motoneurones.

Small spurious components were consistently observed at short-latency and low stimulation intensity in the compound responses elicited by MG, but not SP nerve

stimulation. These components were not of sensory origin, but instead were due to cross-talk of large ventral root potentials. The main arguments to support this interpretation were that, 1) these stray components had the same thresholds and latencies as the alpha compound potentials in the ventral root; 2) they were only seen in responses to MG muscle nerve stimulation, but not in responses to stimulation of the purely cutaneous distal portion of the SP nerve; 3) very similar responses (in terms of latency and threshold) were encountered in practically all single unit recordings, which ruled out that these small compound responses were due to a small number of rapidly conducting sensory afferents; 4) these responses occurred in the latency and threshold range of group I afferents and they were present in intoxicated animals where screening of the dorsal roots failed to reveal a single group I sensory afferent; 5) the magnitude of these spurious responses was readily manipulated by changing the position of the reference electrode relative to the active electrode in the monopolar differential recording arrangement; and 6) the magnitude of these responses were not influenced by the increase in stimulation intensity.

4.4.4 Compound responses to stretch

All treated animals demonstrated vibration responses to sinusiodal stretch at 25 and 100 Hz, but the responses were dramatically reduced to a size 1/20 that of control vibration responses. Sensory neuropathy patients of unknown etiology have also been reported to demonstrate decreased response to muscle vibration (Sainburg et al., 1993) and neuropathies caused by overdoses of pyridoxine (Albin et al., 1987; Schaumburg et al., 1983). However, in one treated animal we observed a relatively large vibration response but were unable to isolate any group I afferents during single afferent unit isolation for

this animal. This larger response in comparison to the other treated animals is thought to reflect the difference in the extent of sensory deprivation as a greater number of group II afferents were readily isolated in this animal during the single afferent unit isolation (Figure 13.B.4). Since small responses were elicited for both vibration frequencies (25 and 100 Hz), the absence of a significant vibration response was not due to a frequency dependent conduction velocity block. In contrast with earlier vibration studies where vibration responses were tightly related to the phase of the stretch (Goodwin et al., 1972), our control and treated animals all displayed broad responses that showed a more variable relation with the stretch phase. In fact, vibration responses spanned almost 180 ° of the sinusoidal stretch.

Vibration responses have traditionally been used to selectively activate Ia afferents because of their high sensitivity to vibration (Brown, Engberg and Matthews, 1967). In contrast, no primary muscle spindle afferents were isolated during single unit recordings even though vibration responses of much smaller magnitude could be elicited. However, both GTO and secondary muscle spindles do show some sensitivity to vibration (Goodwin et al., 1972) and are presumably responsible for the vibration responses seen in pyridoxine treated animals. Therefore, group II afferents may also be sensitive to vibration but their responses are only uncovered in the absence of group I afferents.

In addition to the vibration response measurements, neurogram responses to trapezoidal stretch at a frequency of 0.125 Hz were also recorded. This stretch elicits responses from both primary and secondary muscle spindles and Golgi tendon organs, but is dominated by the primary muscle spindle responses. In control animals, primary muscle

spindles demonstrate their greater dynamic sensitivity to stretch at the onset and termination of the mechanical stretch by showing a large amplitude, dynamic overshoot during and at the end of the ramp stretch and a clear deceleration reponse at the end of the ramp stretch (Figure 12; Matthews, 1972). These dynamic characteristics are strongly reduced in the treated animals, thus indicating the total abolition of primary muscle spindles. The remaining response to this stretch, albeit quite small in magnitude, indicated that a number of mechanosensitive receptors, secondary muscle spindles and Golgi tendon organs, were unaffected by the pyridoxine treatment.

4.4.5 Single afferent unit isolation

Isolation of single muscle afferents demonstrated the functional impairment of large fast conducting axons after pyridoxine treatment. The smaller axons of group III and IV were unaffected and, superficially, even appeared to be increased in number after pyridoxine. However, these smaller and slower conducting axons were relatively more difficult to isolate in the control animals. In control animals, there is a large population of group I afferents that are much larger in diameter than the other groups of afferents. This characteristic made the isolation of these larger axons easier because they were easier to dissect free from the surrounding neurones and because they produced large responses to stretch which made them readily identifiable. This introduced a size bias of single unit isolation in the control animals, and a greater proportion of large fast conducting axons were isolated and recorded from in comparison to the lesser number of small, slowly conducting axons (Figure 13.A). This recording phenomenon is ironic because there are many more group III and IV afferents than group I and II in the medial gastrocnemius nerve.

The conduction velocity spectra of pyridoxine treated animals shows a total absence of afferents with conduction velocities greater than 70 m/s. In addition, the recorded number of smaller axons appears to be unchanged, if not increased in comparison with the control animals (c.f. Figures 13.B.1 with 13.B.2-4). However, this may not be true; in fact the smaller afferents could also be affected by the pyridoxine treatment. Nevertheless, paw pinch responses and reactions to noxious stimulation were qualitatively normal throughout the testing period even though tendon tap reflex reponses were abolished. The selection bias introduced during the control recordings does not provide an accurate guideline for the quantities or proportions of group III and IV afferents in a control animal and should be interpreted with caution.

Was the absence of rapidly conducting low-threshold sensory afferents simply due to a systematic increase in threshold and reduction of axonal conduction velocity, as might perhaps be observed following mild demyelination of large sensory afferents? It seems unlikely that this could be the exclusive or dominant cause of the complete absence of group Ia and the extreme scarcity of Ib afferents, for two main reasons. Firstly, neither in the ensemble neurogram recordings nor in the single unit recordings was there any indication of responses that showed any of the characteristic features of spindle primary (Ia) afferents. Secondly, although this is as of yet only based on a preliminary analysis, the histological evidence strongly indicates that only the larger diameter axons degenerated.

4.5 Role of sensory feedback - internal representation of the body

Pyridoxine treated animals showed dramatic leg swings in both the vertical and transverse direction (as verified by video recordings). However, upon close scrutiny of the video tapes it can be seen that the step cycles with large amplitude deviations in either direction were preceded by large shifts of the animals' trunk (center of gravity). Clinically, the vestibular systems of the pyridoxine treated animals were unaffected (see Results 3.1). Therefore, the vestibular system could have signalled to higher motor centres to compensate for these shifts, but the resulting compensatory limb placements were probably grossly underestimated or overestimated in direction and/or magnitude. Since the higher motor centres program for a compensatory movement and direct a limb to a position that would counter balance the center of gravity shift, the pyridoxine treated animals' internal body representation must be inadequate or inaccurate. Thus, the animals probably neither know entirely where its limbs are, to what degree of flexion and extension the joints are at, and can not accommodate passive interactive forces (Sainburg et al., 1993; Gordon et al., 1995). Therefore, compensatory trajectories of the limbs are more likely to be inappropriately scaled or directed and the normal corrections of these movements are absent due to the pyridoxine-induced deafferentation.

It has been believed that one major role of muscle afferents, muscle spindles and Golgi tendon organs, in addition to the visual system, was to update the central nervous system with information about the dynamic features of an internal model of the motor machinery and of the body space (Bard, Fleury, Teasedale, Paillard and Nougier, 1995; Lajoie et al., 1996; Sainburg et al., 1995; Ghez et al., 1990; Gordon et al., 1995). This egocentric frame of reference is used to coordinate the motor commands for spatially oriented movements, so that one could touch their nose in the dark by using the internal signals

from arm and head proprioceptors. A cat must have an internal model of its limbs, in particular the hindlimbs, which heavily relies on non-visual feedback. The accurate performance of multijoint movements requires the nervous system to transform the spatial coordinates of the target, represented in extrinsic coordinates, into a complex set of commands specified in terms of an intrinsic coordinate system based on the known biomechanics of the limb (Atkeson, 1989). Therefore, the accurate positioning of the limbs during a step cycle may depend upon the accurate internal representation of the limb before and during swing phase. If the limb position and inertial properties are incorrectly evaluated, the subsequent swing phase and foot placement could be incorrectly programmed and lead to abnormal excursion of the limb. This may take the form of overadduction or overabduction, and/or shorter or longer steps. This was observed for the pyridoxine treated animals and confirms the observations of Bard et al. (1995), Sainburg et al. (1993, 1995) and Gordon et al. (1995) that deafferented patients are unable to calibrate the initial position of the limb, resulting in large spatial errors in goal-directed movements without vision. At the height of the motor syndrome, the pyridoxine treated animals displayed abnormal limb excursions that resulted in trunk instability.

4.6 Topography of neuropathy

Studies of human neuropathy patients consistently report that the lower limbs are more affected than the upper limbs, and that neck proprioception is unchanged (Rothwell et al., 1982; Forget and Lamarre, 1987; Sanes et al., 1985). This gradient effect was thought to be due to the different susceptibility to injury of nerves with different lengths, as longer axons are thought to be more prone to injury than the shorter axons. In this case,

the longer axons of afferents innervating the lower segments of the forelimb and hindlimb would have greater susceptibility to the pyridoxine neurotoxic effects than the shorter axons of afferents innervating the neck musculature.

Recording the kinematics of ipsilateral forelimb and hindlimb allowed us to investigate whether there was a difference in pyridoxine effects between these limbs. Both the forelimb and hindlimb showed equal extents of motor dsyfunction as their combined path length increases in the frontal plane between control and treatment recordings were identical in amplitude and resulted in a strong positive correlation (Figure 23). Therefore, treated animals that experienced large abnormalities in the hindlimb excursions also experienced large abnormalities in the forelimb excursions. In addition, both limbs showed similar degrees of plantigrade rather than digitigrade locomotion. The electrophysiology of the treated hindlimbs showed pronounced sensory deprivation. However, the electrophysiology of the treated forelimbs was not investigated. Therefore, on kinematic results alone, the motor deficits experienced by the hindlimb were identical to those for the forelimb.

Qualitatively, the control of neck and head movements appeared unchanged after pyridoxine treatment. At the height of the motor syndrome, treated animals maintained good head control and were fully capable of directing their attention to distractions and maintaining quiet head posture during these bouts of curiosity. In addition, righting responses and vestibular roll tests were both qualitatively unchanged, indicating that the shorter axons of afferents innervating the neck musculature was unaffected.

Certain neck pathologies result in a loss of neck proprioception. This loss of neck proprioception has been shown to elicit motor deficits in body orientation, locomotion and coordination, and gait becomes virtually impossible (Bard et al., 1995; Carpenter, Fabrega and Glinsmann, 1959). That neck proprioception plays a major role in motor control of locomotion is pointed out by Mittlestaedt (1986) and Berthoz (1991) who proposed that the head is used as the internal egocentric frame of reference to which trunk and body segment positions are referred via neck afferent information. This may allow the head segment, which is visually anchored on stable environmental landmarks, to steer the locomotion of the body, even after deafferentation. The importance of neck afference is supported by the complete inability of a deafferented patient, devoid of neck proprioception, to walk unassisted (Lajoie et al., 1996). At the height of the motor syndrome, all but one pyridoxine treated animal were unable to walk, implying that neck proprioception could have been severely impaired. However, clinical assessment of vestibular reflexes appeared normal and those animals with the extended recording protocol recovered locomotion. Together, these results indicate that neck afferents were not affected by the pyridoxine treatment. Yet, more direct evidence clearly is required.

The argument that neck afferents are too short to be affected by pyridoxine treatment may not necessarily be true. In a collaborating laboratory, McPherson (personal communication, 1998) has performed a pilot study looking at the effects of pyridoxine on balance and postural control. McPherson found that at the height of the motor syndrome the trunk or axial musculature produces abnormal EMG activity in response to external perturbations. This result indicated that muscle afferents that regulate trunk musculature activity may be affected by the pyridoxine treatment. Trunk muscle afferents

are equally short as those found innervating neck musculature. Therefore, short axons may be affected by pyridoxine treatment as well as the longer afferents innervating limb musculature. It is difficult to properly assess whether the trunk muscle afferents were affected in our treated animals. Yet the treated animals did show abnormal trunk excursions in all three axes of movement. Whether abnormalities of trunk motion were the cause or effect of improper limb positioning is not known and can not be inferred.

In addition, the possible extent of neck afferent deprivation after pyridoxine treatment might be confounded by a dilution effect. Some deep neck muscles are innervated by 500 spindles/gram (Abrahams and Richmond, 1988) whereas the lateral gastrocnemius has only 5 spindles/gram (Chin, Cope and Pang, 1962). The control of head movement is undoubtedly complex as the neck must provide a stable platform for the visual system, and because the head is used in quadrupedals for a variety of tasks - eating, grooming, and manipulating objects. Pyridoxine might affect the shorter proprioceptors of the neck to an equal extent as it does the longer trunk and limb muscle afferents. Yet, the overall percentage of afferents affected might be quite small in comparison to the percentage of limb afferents effected because of the much greater density of afferents innervating the neck musculature. The large number of unaffected afferents may be able to maintain control of the neck muscles with little functional impairment.

No electrophysiological tests were performed on the trunk or neck muscle afferents. However, preliminary NMR spectroscopy analysis shows similar abnormalities in longer, distal (superficial peroneal) nerves and shorter proximal (semi-membranosus) nerves (not illustrated).

4.7 Correlation of sensory and motor deficits

Attempts were made to correlate the motor deficits observed at the height of the motor syndrome with the sensory deprivation observed at the terminal electrophysiological experiment. Qualitatively, the animals that experienced the least severe motor deficits were also the animals that demonstrated the least severe sensory deficits. For example, cat 11, the only animal that could continue walking throughout the recording program, showed the least deficits in fall responses and the greatest recovery of locomotion and fall response. This cat also showed the largest vibration and neurogram response, a compound potential in the cutaneous nerve recording, and had the single afferent conduction velocity spectra with the largest number of recorded group II afferents. Cat 12, the most severely compromised animal displayed the greatest motor deficits with the least amount of locomotor recovery and showed the smallest vibration response, and the slowest maximum single afferent conduction velocity. Yet, we were not able to show a strong correlation between the motor deficits and the sensory deficits, and there are two main reasons why. First, at the height of the motor syndrome, all animals but one were incapable of walking and the kinematic recordings of treadmill locomotion made just before the maximum syndrome was reached underestimated the severity of the motor deficits. Second, the time difference between the height of the motor syndrome and the terminal electrophysiological experiment did not permit recordings of the motor and sensory deficits at the same stage of testing. Therefore, the true underlying central and/or sensory functional deficits at the height of the motor syndrome were not known (see Discussion 4.2.1).

With regards to the first reason, the largest problem incurred in the recording of the animals at the height of the motor syndrome was the rapidity of the onset of the incapacitating syndrome. A difference of a couple of hours was the time required for a recordable ataxic gait to become non-recordable as the animals could no longer walk. Knowledge of where we were in the time course of unfolding motor deficits was not available and prevented high temporal resolution of recordings of the motor deficits. Nevertheless, with all animals demonstrating severe ataxia and subsequent loss of locomotion at different times, meant that on the morning of day 4 (i.e. average day of last locomotion recording) the recordings could illustrate different levels of locomotor abnormality between all cats. Then in the subsequent recording session in the afternoon, the animals might be unable to walk. Thus, a large variability was present between the treated animals regarding the extent of motor deficits in the last recorded walking sequences. The fact that all animals but one were unable to walk at the height of the motor syndrome indicates the dramatic severity of the pyridoxine treatment. However, since the animals were unable to walk, recordings of treadmill locomotion were not possible. At this stage, the animals' motor control was severely impaired, so testing of any motor tasks simpler than walking would most likely have obtained the same results as did the fall responses.

One way of obtaining higher resolution recordings of the animals at the height of the motor syndrome would be to increase the recording frequency as soon as the first symptoms of ataxic gait are observed. Instead of recording treadmill locomotion twice per day with 12 hours in between recordings, once the first symptoms of ataxic gait are observed, increase the frequency of the recordings to every other hour. This was not

possible within our experimental protocol because of four reasons. Firstly, the recording facilities were shared with other laboratories and hourly recordings would mean that other parties would not have been able to utilize the facilities for many weeks. Secondly, there were insufficient personnel in the lab to support recordings of this nature. Thirdly, at many times throughout the recording schedule, more than one animal was being treated and recorded. The time required to set up the recording equipment and prepare the animal and then record treadmill walking, landing from short falls and tendon tap reflex responses was over two hours in duration on its own. Therefore recording from more than one animal at the same time did not make such arrangements feasible. Fourthly, high numbers of recording sessions would depend on maintained motivation of the animals. However, our experience shows that regardless of the form of encouragement, animals eventually lose motivation to cooperate and walk on a treadmill.

4.8 The enigma of selective sensory neuropathy

Overdoses of pyridoxine have been shown to produce severe sensory neuropathies in the rat, dog and human, and now in the cat. However, the exact mechanism of the pyridoxine neurotoxic effect and the reason for its selectivity for large-fibre sensory neurones is not known.

Pyridoxine neurotoxicity may be due to selective accumulation of pyridoxic acid or another metabolite in peripheral sensory neurones where it may inhibit multiple pyridoxal phosphate-dependent enzymes by competitive displacement of the active coenzyme, PLP. This could happen if the renal transport mechanisms for pyridoxine metabolites (e.g. pyridoxic acid) were saturated with excessive amounts of pyridoxine and result in

increased blood concentrations. However, metabolite tissue concentrations in the CNS need not follow suit, if its uptake across the blood brain barrier is regulated by similar homeostatic mechanisms as for pyridoxine (Dakshinamurti, 1982). In contrast, such metabolites might accumulate in peripheral sensory neurones: not being protected by homeostatic uptake mechanisms, their extracellular milieu would follow the plasma concentration increase. It is then conceivable that, for very high intracellular concentrations, pyridoxic acid or a related metabolite could non-specifically displace pyridoxal phosphate from pyridoxal phosphate-dependent apoenzymes, forming inactive products (Meister, 1990). This mechanism would affect several pyridoxal phosphate dependent enzymes. This hypothesis would account for the depression in intermediary metabolism that was observed in our pilot experiments (Sutherland, Hulliger, Tyson and Bishop, unpublished observations).

The selectivity of pyridoxine neurotoxicity may be due to the protection of the CNS cells (including motoneurone soma) by the blood-brain-barrier (Albin et al., 1987). This could work only if uptake of toxins were restricted to cell bodies, as the axons of sensory and motoneurones are intermingled in peripheral nerves. An alternative mechanism may be that the sensory endings, perhaps due to their specific ion channel architecture (which renders them susceptible to sensory stimuli), also are more susceptible to toxin infiltration which may alter neuronal metabolism so that the neuron cannot support the axon and degeneration occurs (Windebank et al., 1985). If this is true, than perhaps there is also a dose dependent selectivity for sensory modality or axon size, as the pyridoxine treated animals showed severe degeneration of the group I and II afferents but not the group III and IV afferents.

The notion of an exclusively peripheral sensory neuropathy cannot be accepted uncritically: the severity of the motor deficits, their similarity with cerebellar syndromes and neck pathologies, and the possibilities of some functional impairment in the CNS that is not accompanied by histologically evident degeneration, all suggest that the primary neurotoxic process need not be restricted to the peripheral nervous system (see Figure 30). Thus the possibility of additional direct CNS impairment has to be examined with sufficiently sensitive methods.

This laboratory has considered investigating the direct CNS impairment from pyridoxine intoxication by using functional electrophysiological methods, including chronic recordings from motor centers, evoked sensory potentials, and stimulation of descending motor pathways, in an alternative study but dismissed these options. Results from these tests would be predictably ambiguous because practically all major motor structures of the CNS (motor cortex, basal ganglia, brainstem, cerebellum and spinal cord) receive powerful sensory input from the periphery. Since after pyridoxine intoxication inputs from large afferent axons are no longer functioning, the operation of numerous CNS circuits is bound to be impaired, most likely depressed, even in the absence of direct central neurotoxicity.

4.9 Histology

As other studies have shown, pyridoxine treatment induces sensory deafferentation by way of axonal degeneration (Windebank et al., 1985; Schaeppi and Krinke, 1982, 1985; Xu et al., 1989; Schaumburg et al., 1983; Hoover and Carlton, 1981a, 1981b). Preliminary

Figure 31. Photomicrographs of the cerebellum of a normal cat. A-C: Nissl stained picture showing the repeating molecular layer (ml), Purkinje cell layer (pcl), granular layer (gl) and white matter (wm). D: *Anti-zebrin II* immunoperoxidase stained picture illustrates the Purkinje cell dendritic arbors in the molecular layer and axonal projections down through the granular layer to the white matter. Scale bars: A, 500 micrometer; B, 100 micrometer; C, 50 micrometer; D, 100 micrometer.

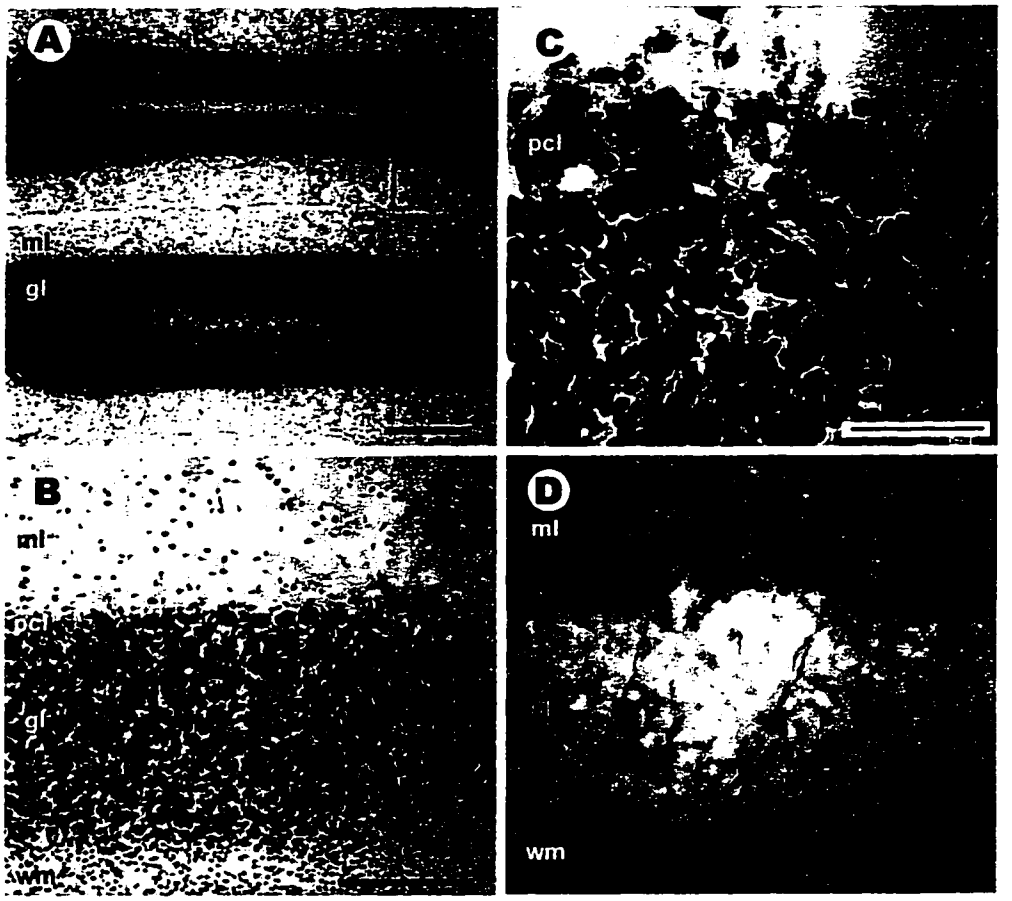
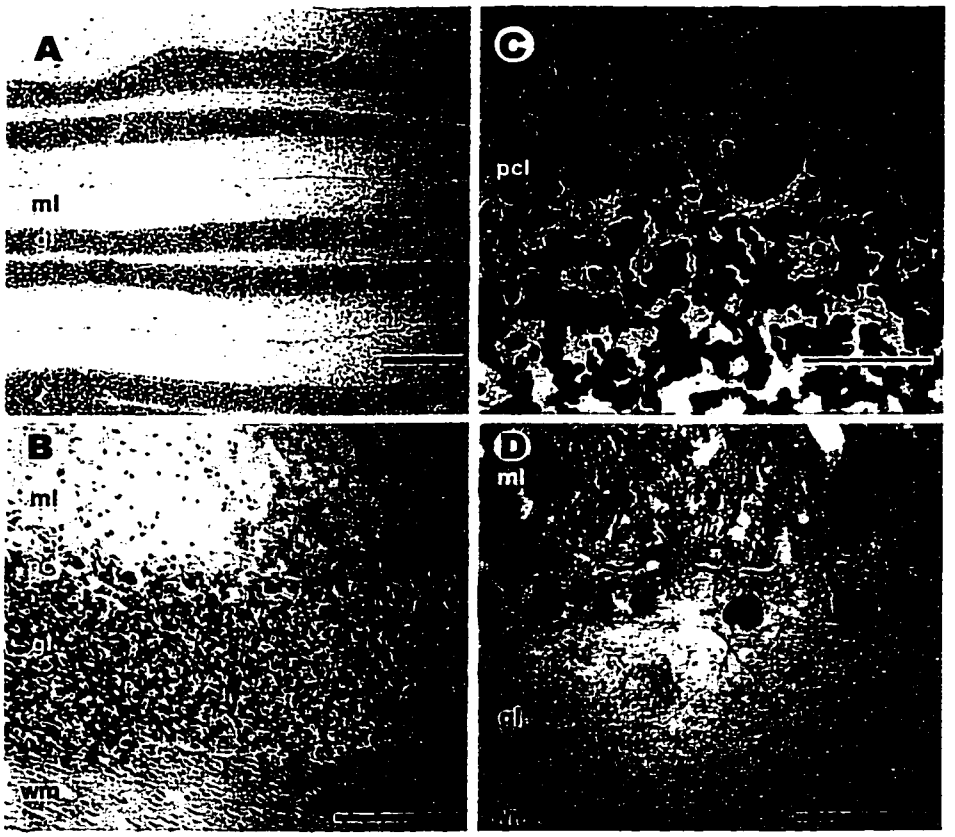

A
ml
gl
C
pcl
B
ml
pcl
gl
wm
D
ml
wm

Figure 32. Photomicrographs of the cerebellum of a pyridoxine treated cat. A-C: Nissl stained picture showing a normal repeating molecular layer (ml), Purkinje cell layer (pcl), granular layer (gl) and white matter (wm). No lesions are observed and normal cell numbers and distributions are seen. D: *Anti-zebrin II* immunoperoxidase stained picture illustrates normal Purkinje cell dendritic arbors in the molecular layer and axonal projections down through the granular layer to the white matter. Scale bars: A, 500 micrometer; B, 100 micrometer; C, 50 micrometer; D, 100 micrometer.


A
ml
gl
B
ml
gl
wm
C
pcl
D
ml
gl

histopathology of the peripheral nerves from treated animals indicates that large diameter fibres have experienced axonal degeneration while the CNS, frontal cortex and cerebellum, are unaffected. The fact that the cerebellum of pyridoxine treated animals appears morphologically unchanged supports the hypothesis that the motor deficits, similar in appearance to cerebellar ataxia, are not due to semi-chronic cerebellar damage (cf. control cerebellum Figure 31 and pyridoxine cerebellum Figure 32). However, as shown in Figure 30, the absence of morphological alterations in the cerebellum do not exclude the potential for transient reversible functional deficits at the time when the maximum motor syndrome was manifest (see earlier in Discussion 4.2.1). A full scale histological assessment of the pyridoxine treated animals will be conducted in the near future, but was not within the scope of this Masters project.

4.10 Future considerations and implications for science

The variation amongst animals as to the extent of pyridoxine selective sensory deprivation was largely by chance, since most animals received either 3 or 4 injections of the same amount of pyridoxine per kg body weight. Given the intraperitoneal route of administration, such variability is not surprising. However, the observation of a gradation of the severity of the motor syndrome suggests that it may be possible to standardize and control the extent of deafferentation by measuring blood and tissue concentrations of pyridoxine and its main metabolites and by adjusting daily dosages of pyridoxine (administered systemically) so as to achieve defined blood or tissue concentration standards. This technique could be used in an attempt to elicit selective group I fibre deafferentation and focus more specifically on sensorimotor deficits attributable to loss of group I propriocetive feedback.

A remarkable finding by Rothwell et al. (1982) when studying a patient with a sensory neuropathy was this patient's difficulty in learning a new motor behavior. Rothwell's patient was capable of driving his old car, even at night. When he purchased a new car, he found difficulty in driving during the day and an inability to drive in the dark. With this animal model of selective sensory neuropathy, the role sensory information plays in motor learning could be assessed in novel motor tasks, e.g. walking along a ladder or rotating beam.

One of the major hopes for this line of research is that we will be able to develop better ways to improve the capabilities of the neural systems left intact after nervous system injuries or lesions. Understanding the roles of specified sensory modalities, i.e. muscle afferents, in motor control and motor learning will provide a better understanding of their necessity. It will also provide us with a tool for investigating different rehabilitation approaches of cognitive strategies and supplementary sources of information to overcome the lack of proprioception in the control of coordinated actions.

Another project for future consideration is to elucidate the mechanism and reason for sensory selectivity of pyridoxine intoxication. Pyridoxine intoxication and megavitamin abuse are a growing concern, given the increasing appeal of complimentary medical therapies and megavitamin-oriented dietary habits in North America and Europe (Eisenberg, Kessler, Foster, Norlock, Calkins and Debanco, 1993; Abbott, 1997). In addition, pyridoxine has therapeutic use in such treatment as recurrent seizures in children (Jiao, Gao, Takuma, Wu, Liu, Zhang, Lieu, Ge, Chui, Cao, Bai and Liu, (1997): also see

Introduction 1.4). Yet, the pharmacokinetics of the therapeutic doses are not known (Zempleni, 1995). The mechanism and pharmacokinetics should be better understood to permit more accurate medical administration of the vitamin and to determine safety guidelines for its use.

4.11 Conclusion

In this study, pyridoxine intoxication was shown to produce severe and incapacitating motor deficits characterized by crouched gait, increased instability, abnormal limb excursions and disrupted interlimb coordination.

The pyridoxine induced deafferentation resulted in a rapid and non-invasive, selective large-fibre sensory neuropathy in the cat. Group I and rapidly conducting group II afferents were selectively impaired while motoneurones and muscle contractility were unaffected.

References

Abbott, A. (1997). Curbs on B_6 highlight dietary dilemnas. Nature, 389, 7.

Abrahams, V.C. and Richmond, F.J.R. (1988). Specialization of sensorimotor organization in the neck muscle system. In: Progress in Brain Research. Edited by Pompeiano, O. and Allum, J.H.J. New York, Elsevier, 125-135.

Albin, R.L., Albers, J.W., Greenberg, H.S., Townsend, J.B., Lynn, R.B., Burke, J.M. and Alessi, A.G. (1987). Acute sensory neuropathy-neuronopathy from pyridoxine overdose. Neurology, 37, 1729-1732.

Andersson, O. and Grillner, S. (1983). Peripheral control of the cat's step cycle. II. Entrainment of the central pattern generators for locomotion by sinusiodal hip movements during "fictive locomotion." Acta Physiologica Scandinivica, 118, 229-239.

Antopol, W. and Tarlov, I.M. (1942). Experimental study of the effects produced by large doses of vitamin B_6. Journal of Neuropathology and Experimental Neurology, 1, 330-336.

Armstrong, D.M. and Drew, T. (1984a). Discharges of pyramidal tract and other motor cortical neurones during locomotion in the cat. Journal of Physiology, 346, 471-495.

Armstrong, D.M. and Drew, T. (1984b). Locomotor-related neuronal discharges in cat motor cortex compared with peripheral receptive fields and evoked movements. Journal of Physiology, 346, 497-517.

Atkeson, C.G. (1989). Learning arm kinematics and dynamics. Annual Review of Neuroscience, 12, 157-183.

Baev, K.V., Beresovdkii, V.K., Kebkalo, T.G. and Savoskina, L.A. (1988). Afferent and efferent connections of brainstem locomotor regions study by means of horseradish peroxidase transport tecnique. Neuroscience, 26, 871-892.

Baev, K.V., Esipenko, V.B. and Shimansky, Y.P. (1991). Afferent control of central pattern generators: experimental analysis of scratching in the decerebrate cat. Neuroscience, 40, 239-256.

Banks, R.W., Hulliger, M., Scheepstra, K.A., Otten, E. (1997). Pacemaker activity in a sensory ending with multiple encoding sites: the cat muscle spindle primary ending. Journal of Physiology, 498, 177-199.

Bard, C., Fleury, M., Teasedale, N., Paillard, J. and Nougier, V. (1995). Contribution of proprioception for calibrating and updating the motor space. Canadian Journal of Physiology and Pharmacology, 73, 246-254.

Bassler, K.H. (1988). Megavitamin therapy with pyridoxine. International Journal of Vitamin and Nutrition Research, 58, 105-118.

Bassler, U. (1983). Neural Basis of Elementary Behavior in Stick Insects. Studies of Brain Function. Berlin: Springer, 10, 169.

Bassler, U. (1993). The femur-tibia control system of stick insects - a model system for the study of the neural basis of joint control. Brain Research Reviews, 18, 207-226.

Baumann, T.K. and Hulliger, M. (1991). The dependence of the response of cat spindle Ia afferents to sinusoidal stretch on the velocity of concomitant movement. Journal of Physiology, 439, 325-350.

Bem, T., Gorska, T., Majezynski, H. and Zmyslowski, W. (1995). Different patterns of fore-hindlimb coordination during overground locomotion in cats with ventral and lateral spinal lesions. Experimental Brain Research, 104, 70-80.

Bendich, A. and Cohen, M. (1990). Vitamin B_6 safety issues. In: Vitamin B_6. Edited by Dakshinamurti, K. Annals of the New York Academy of Sciences, 585, 321-330.

Berger, A.R., Schaumburg, H.H., Schroeder, C., Apfel, S. and Reynolds, R. (1992). Dose response, coasting, and differential fiber vulnerability in human toxic

neuropathy: A prospective study of pyridoxine neurotoxicity. Neurology, 42, 1367-1370.

Berthoz, A. (1991). Reference frames for the perception and control of movement. In: Brain and Space. Edited by Paillard, J. Oxford University Press, Oxford. pp 88-111.

Bishop, G.M., Hulliger, M., Foweraker, J.P.A. and Davis, L. (1998). Acute motor deficits following pyridoxine-induced deafferentation in the cat. To be submitted to Journal of Neurophysiology.

Bishop, G.M., Mah, C., Hulliger, M. and Wojciechowski, A. (1996). Anew quantitative analysis of quadrupedal gait. Society for Neuroscience Abstracts Vol. 22, 804.5.

Brochu, G., Maler, L. and Hawkes, R.B. (1990). Zebrin II: a polypeptide antigen expressed selectively by Purkinje cells reveals compartments in rat and fish cerebellum. Journal of Comparative Neurology, 291, 538-552.

Brown, T.G. (1911). The intrinsic factors in the act of progression in the mammal. Proceedings of the Royal Society of London, 84, 308-319.

Brown, M.C., Engberg, I. and Matthews, P.B.C. (1967). The relative sensitivty to vibration of muscle receptors of the cat. Journal of Physiology, 192, 773-800.

Carpenter, M.B., Fabrega, H. and Glinsmann, W. (1959). Physiological deficits occurring with lesions of labyrinth and fastigial nuclei. Journal of Neurophysiology, 22, 222-234.

Chin, N.K., Cope, M. and Pang, M. (1962). Number and distribution of spindle capsules in seven hindlimb muscles of the cat. In: Symposium on Muscle Receptors. Edited by Barker, D. Hong Kong: Hong Kong University Press, 241-248.

Clark, F.J., Horch, K.W., Bach, S.M. and Larson, G.F. (1979). Contribution of cutaneous and joint receptors to static knee-position sense in man. Journal of Neurophysiology, 42, 877-888.

Cole, J.D. and Sedgwick, E.M. (1992). The perceptions of force and of movement in a man without large myelinated sensory afferents below the neck. Journal of Physiology, 449, 503-515.

Conway, B.A., Hultborn, H. and Kiehn, O. (1987). Proprioceptive input resets central locomotor rhythm in the spinal cat. Experimental Brain Research, 68, 643-656.

Cooke, J.D., Brown, S., Forget, R. and Lamarre, Y. (1985). Initial agonist burst duration changes with movement amplitude in a deafferented patient. Experimental Brain Research, 184-187.

Cox, S. H., Murray, A. and Boone, I.U. (1962). Metabolism of tritium-labelled pyridoxine in rats. Proceedings of the Society for Experimental Biology and Medicine, 109, 242-244.

Cross, M.J. and McCloskey, D.I. (1973). Position sense following surgical removal of joints in man. Brain Research, 35, 606-611.

Dalton, K. and Dalton, M.J.T. (1987). Characteristics of pyridoxine overdose neuropathy syndrome. Acta Neurologica Scandinivica, 76, 8-11.

Dakshinamurti, K. (1977). B vitamins and nervous system function. In: Nutrition and the Brain. Edited by Wurtman, R.J. and Wurtman, J.J. Raven Press, New Yok. Vol 1, pp. 251-318.

Dakshinamurti, K. (1982). Neurobiology of pyridoxine. Adavnces in Nutritional Research, 4, 143-179.

Dakshinamurti, K., Paulose, C.S., Visvanathan, M., Siow, Y.L. and Sharma, K. (1990). Neurobiology of pyridoxine. In: Vitamin B_6. Edited by Dakshinamurti, K. Annals of the New York Academy of Sciences, 585, 128-143.

D'Hooge, R., Nagels, G., Franck, F., Bakker, C.E., Reyniers, E., Storm, K., Kooy, R.F., Oostra, B.A., Willems, P.J. and DeDeyn, P.P. (1997). Mildly impaired water

maze performance in male Fmr1 knockout mice. Neuroscience, 76, 367-376.

Dietz, V. and Noth, J. (1978). Pre-innervation and stretch responses of triceps brachii in man falling with and without visual control. Brain Research, 142, 576-579.

Dietz, V., Schmidtbleicher, D. and Noth, J. (1979). Neuronal mechanisms of human locomotion. Journal of Neurophysiology, 42, 1212-1222.

Drew, T. and Rossignol, S. (1987). A kinematic and electromyographic study of cutaneous reflexes evoked from the forelimb of unrestrained walking cat. Journal of Neurophysiology, 57, 1160-1183.

Duysens, J. and Pearson, K.G. (1980). Inhibition of flexor burst generation by loading ankle extensor muscles in walking cats. Brain Research, 187, 321-333.

Ebadi, M. (1981). Regulation and function of pyridoxal phosphate in CNS. Neurochemistry International, 3, 181-206.

Eidelberg, E. and Yu, J. (1981). Effects of corticospinal lesions upon treadmill locomotion by cats. Experimental Brain Research, 43, 101-103.

Eisenberg, D.M., Kessler, R.C., Foster, C., Norlock, F.E. Calkins, D.R. and Delbanco, T.L. (1993). Unconventional medicine in the United States. Prevalence, costs, and patterns of use. The New England Journal of Medicine, 328, 246-252.

Eisenman, L.M. and Hawkes, R.B. (1993). Antigenic compartmentation in the mouse cerebellar cortex: zebrin and HNK-1 reveal a complex, overlapping molecular topography. Journal of Comparative Neurology, 335, 586-605.

Feldman, A.G. (1974). Change of muscle length as a consequence of a shift in equilibrium of muscle-load system. Biophysics, 19, 544-548.

Ferrel, W.R., Baxendale, R.H., Carnachan, C. and Hart, I.K. (1985). The influence of joint afferent discharge on locomotion, proprioception and activity in conscious cats. Brain Research, 347, 41-48.

Forget, R. and Lamarre, Y. (1987). Rapid elbow extension in the absence of proprioceptive and cutaneous feedback. Human Neurobiology, 6, 27-37.

Forssberg, H., Grillner, S. and Halbertsma, J. (1980a). The locomotion of the low spinal cat. I. Coordination within a hindlimb. Acta Physiologica Scandinivica, 108, 269-281.

Forssberg, H., Grillner, S., Halbertsma, J. and Rossignol, S. (1980b). The locomotion of the low spinal cat. II Interlimb coordination. Acta Physiologica Scandinivica, 108, 283-295.

Gandevia, S.C. and Burke, D. (1992). Does the nervous system depend on kinesthetic

information to control natural limb movements? Behavioral and Brain Sciences, 15, 614-632.

Gandevia, S.C. and McCloskey, D.I. (1976). Joint sense, muscle sense, and their combination as position sense, measured at the distal interphalangeal joint of the middle finger. Journal of Physiology, 260, 387-407.

Gandevia, S.C. and McCloskey, D.I. (1977). Changes in motor commands, as shown by changes in perceived heaviness, during partial curarisation and periperal anaestesia. Journal of Physiology, 272, 653-672.

Ghez, C., Gordon, J. and Ghilardi, M.F. (1995). Impairments of reaching movements in patients without proprioception. II. Effects of visual information on accuracy. Journal of Neurophysiology, 73, 361-372.

Ghez, C., Gordon, J., Ghilardi, M.F., Christakos, C.N. and Cooper, S.E. (1990). Roles of proprioceptive input in the programming of arm trajectories. Cold Spring Harbour Symposium of Quantitaive Biology, 55, 837-847.

Gilman, S., Carr, D. and Hollenberg, J. (1976). Kinematic effects of deafferentation and cerebellar ablation. Brain, 99, 311-330.

Giuliani, C.A. and Smith, J.L. (1985). Development and characteristics of airstepping in chronic spinal cats. Journal of Neuroscience, 5,1276-1282.

Giuliani, C.A. and Smith, J.L. (1987). Stepping behaviors in chronic spinal cats with one hindlimb deafferented. Journal of Neuroscience, 7, 2537-2546.

Goldberger, M.E. (1977). Locomotor recovery after unilateral hindlimb deafferentation in cats. Brain Research, 123, 59-74.

Goldberger, M.E. (1988a). Spared-root deafferentation of a cat's hindlimb: Hierarchical regulation of pathways mediating recovery of motor behavior. Experimental Brain Research, 73, 329-342.

Goldberger, M.E. (1988b). Partial and complete deafferentation of cat hindlimb: the contribution of behavioral substitution to recovery of motor function. Experimental Brain Research, 73, 343-353.

Goldberger, M.E. and Murray, M. (1980). Locomotor recovery after deafferentation on one side of the cat's trunk. Experimental Neurology, 67, 103-117.

Goodwin, G.M, McCloskey, D.I. and Matthews, P.B.C. (1972). The contribution of muscle afferents to kinaesthesia shown by vibration induced illusions of movement and by the effects of paralysing joint afferents. Brain, 95, 705-748.

Gorassini, M.A., Prochazka, A., Hiebert, G.W. and Gauthier, M.J.A. (1994). Corrective responses to loss of ground support during walking. I. Intact cats. Journal of

Neurophysiology, 71, 603-610.

Gordon, J., Ghilardi, M.F. and Ghez, C. (1995). Impairments of reaching movements in patients without proprioception. I. Spatial errors. Journal of Neurophysiology, 73, 347-360.

Gossard, J.P., Brownstone, R.M., Barajon, I. and Hultborn, H. (1994). Transmission in a locomotor-related group Ib pathway from hindlimb extensor muscles in the cat. Experimental Brain Research, 98, 213-228.

Grigg, P., Finerman, G.A. and Riley, L.H. (1973). Joint position sense after total hip replacement. Journal of Bone and Joint Surgery, 55, 1016-1025.

Grigg, P. (1975). Mechanical factors influencing response of joint afferent neurons from cat knee. Journal of Neurophysiology, 38, 1473-1484.

Grillner, S. (1975). Locomotion in vertebrates: central mechanisms and reflex interaction. Physiology Reviews, 55, 247-304.

Grillner, S., Deliagina, T., Ekeberg, O., el Manira, A., Hill, R.H., Lansner, A., Orlovsky, G.N. and Wallen, P. (1995). Neural networks that co-ordinate locomotion and body orientation in lamprey. Trends in Neuroscience, 18, 270-279.

Grillner, S. and Matsushima, T. (1991). The neural network underlying locomotion in lamprey - synaptic and cellular mecanisms. Neuron, 7, 1-15.

Grillner, S and Rossignol, S. (1978). On the initiation of the swing phase of locomotion in chronic spinal cats. Brain Research, 146, 269-277.

Grillner, S. and Wallen, P. (1985). Central pattern generators for locomotion, with special reference to vertebrates. Annual Review of Neuroscience, 8, 223-261.

Grillner, S., Wallen, P. and Brodin, L. (1991). Neuronal network generating locomotor behavior in lamprey: circuitry, transmitters, membrane properties, and simulation. Annual Review of Neuroscience, 14, 169-199.

Grillner, S. and Zangger, P. (1984). The effect of dorsal root transection on the efferent motor pattern in the cat's hindlimb during locomotion. Acta Physiologica Scandinivica, 120, 393-405.

Hering, H.E. (1897). Uber centripetale Ataxie beim Menschen und beim Affen. Neurologisches Zentralblatt, 16, 1077-1094.

Hiebert, G.W., Whelan, P.J., Procazka, A. and Pearson, K.G. (1996). Contribution of the hindlimb flexor muscle afferents to the timing of phase transitions in the cat step cycle. Journal of Neurophysiology, 75, 1126-1137.

Hoover, D.M. and Carlton, W.W. (1981a). The subacute neurotoxicity of excess pyridoxine HCl and clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) in Beagle dogs. I. Clinical Disease. Veterinary Pathology, 18, 745-756.

Hoover, D.M. and Carlton, W.W. (1981b). The subacute neurotoxicity of excess pyridoxine HCl and clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) in Beagle dogs. II. Pathology. Veterinary Pathology, 18, 757-768.

Horcholle-Bossavit, G., Jami, L., Petit, J., Vejsada, R. and Zytnicki, D. (1990). Ensemble discharge from Golgi tendon organs of cat peroneus tertius muscle. Journal of Neurophysiology, 64, 813-821.

Houk, J.C. (1979). Regulation of stiffness by skeletomotor reflexes. Annual Review of Physiology, 41, 91-114.

Hulliger, M., Bishop, G.M., Djupsobacka, M. and Zanussi, A. (1998). Large fibre sensory contributions to the control of locomotion studied by non-invasive deafferentation. Posture and Gait (in press).

Hulliger, M., Nordh, E., Thelin, A.E. and Vallbo, A.B. (1979). The responses of afferent fibres from the glabrous skin of the hand during voluntary finger movements in man. Journal of Physiology, 291, 233-249.

Hughes, M.A., Duncan, P.W., Rose, D.K., Chandler, J.M. and Studentski, S.A. (1996).

The relationship of postural sway to sensorimotor function, functional performance and disability in the elderly. Archives of Physical Medicine and Rehabilitation, 77, 567-572.

Iles, J.F., Stokes, M. and Young, A. (1990). Reflex actions of knee joint afferents during contraction of thehuman quadriceps. Clinical Physiology, 10, 489-500.

Ito, M. (1978). Recent advances in cerebellar physiology and pathology. Advances in Neurology, 21, 59-84.

Ito, M. (1979). Adaptive modification of the vestibulo-ocular reflex in rabbits affected by visual inputs and its possible neuronal mechanisms. Progress in Brain Research, 50, 757-761.

Jiao, F.Y., Gao, D.Y., Takuma, Y., Wu, S., Liu, Z.Y., Zhang, X.K., Lieu, N.S., Ge, Z.L., Chui, W., Cao, Y.M., Bai, A.N. and Liu, S.B. (1997). Randomized, controlled trial of high dose intravenous pyridoxine in the treatment of recurrent seizures in children. Pediatric Neurology, 17, 54-57.

Jones, E.G. (1972). The development of the 'muscular sense' concept during the Nineteenth Century and the work of H. Carlton Bastian. Journal of Historical Medicine and Allied Sciences, 27, 298-311.

Jordan, L.M., Pratt, C.A. and Menzies, J.E. (1979). Locomotion evoked by brainstem

stimulation: occurrence without phasic segmental afferent input. Brain Research, 177, 204-207.

Koshland, G.F. and Smith, J.L. (1989). Mutable and immutable features of paw-sake responses after hindlimb deafferentation in the cat. Journal of Neurophysiology, 62, 162-173.

Krinke, G., Naylor, D.C. and Skropil, V. (1985). Pyridoxine megavitaminosis: an analysis of the early changes induced with massive doses of vitamin B_6 in rat primary sensory neurons. Journal of Neuropathology and Experimental Neurology, 3, 83-87.

Lajoie, Y., Teasdale, N., Cole, J.D., Burnett, M., Bard, C., Fleury, M., Forget, R., Paillard, J. and Lamarre, Y. (1996). Gait of a deafferented subject without large myelinated sensory fibres below the neck. Neurology, 47, 109-115.

Liddel, E.G. and Pillips, C.G. (1944). Pyramidal section in the cat. Brain, 67, 1-9.

Lassek, A.M. (1953). Inactivation of voluntary motor function following rhizotomy. Journal of Neuropathology and Experimental Neurology, 3, 83-87.

Matthews, P.B.C. (1972). Mammalian muscle receptors and their central action. Arnold, London.

Matthews, P.B.C. and Simmonds, A. (1974). Sensations of finger movement elicited by pulling upon flexor tendons in man. Journal of Physiology, 239, 27-28P.

McClachlan, R.S. and Brown, W.F. (1995). Pyridoxine dependent epilepsy with iatrogenic sensory neuronopathy. Canadian Journal of Neurological Sciences, 22, 50-51.

Meister, A. (1990). On the transamination of enzymes. In: Vitamin B_6. Edited by Dakshinamurti, K. Annals of the New York Academy of Sciences, 585, 13-31.

Melvill-Jones, G. and Watt, D.G.D. (1971). Observations on the control of stepping and hopping movements in man. Journal of Physiology, 219, 709-727.

Merrill, A.H. and Henderson, J.M. (1990). Vitamin B_6 metabolism in the human liver. In: Vitamin B_6. Edited by Dakshinamurti, K. Annals of the New York Academy of Sciences, 585, 110-117.

Merton, P.A. (1953). Speculations on the servo control of movement. In: The Spinal Cord. Edited by Wolstenholme, G.E.W. Churchill, London. PP. 247-255.

Mittlestaedt, H. (1986). The subjective vertical as a function of visual and extra-retinal cues. Acta Psychologica, 63, 63-85.

Mott, F.W. and C.S. Sherrington. (1895). Experiments upon the influence of sensory

nerves upon movement and nutrition of the limbs. In: Proceedings of the Royal Society of London, 57, 481-488.

Munk, H. (1909). Uber die folgen des sensibilitatsverlustes der extremitat fur deren motilitat. Kap III. In: Uber die Funktionen von Hirn und Ruckenmark, Gesammelte Mitteilungen, Hirschwald, Berlin, 247-285.

Pearson, K.G. (1995). Proprioceptive regulation of locomotion. Current Opinion in Neurobiology, 5, 786-791.

Pearson, K.G. and Collins, D.F. (1993). Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. Journal of Neurophysiology, 70, 1009-1017.

Pearson, K.G., Ramirez, J.M. and Jiang, W. (1992). Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscle in the spinal cats. Experimental Brain Research, 90, 557-566.

Phillips, S.J., Roberts, E.M. and Huang, T.C. (1983). Quantification of intersegmental reactions during rapid swing movement. Journal of Biomechanics, 16, 411-418.

Polit, A. and Bizzi, E. (1979). Caracteristics of motor programs underlying arm movements in monkey. Journal of Neurophysiology, 42, 183-194.

Prochazka, A. (1996). Proprioceptive feedback and movement regulation. In: Handbook of Physiology. Section 12. Exercise: Regulation and Integration of Multiple Systems. Edited by Rowell, L. and Shepard, J. Oxford University Press, New York. pp 89-127.

Prochazka, A., Schofield, P., Westerman, R.A. and Ziccone, S.P. (1977). Reflexes in the cat ankle muscles after landing from falls. Journal of Physiology, 272, 705-719.

Prochazka, A., Trend, P., Hulliger, M. and Vincent, S. (1989). Ensemble proprioceptive activity in the cat step cycle: towards a representative look-up chart. Progress in Brain Research, 80, 61-74.

Prochazka, A., Westerman, R.A. and Ziccone, S.P. (1977). Ia afferent activity during a variety of voluntary movements in the cat. Journal of Physiology, 268, 423-448.

Rose, J.E. and Mountcastle, V.B. (1959). Touch and kinesthesis. In: Handbook of Physiology. Section I. Neurophysiology. Edited by Field, J. American Physiological Society, Washington D.C. pp387-429.

Rossignol, S. and Dubuc, R. (1994). Spinal pattern generation. Current Opinion in Neurobiology, 4, 894-902.

Rothwell, J.C., Traub, M.M., Day, B.L., Obeso, J.A., Thomas, P.K. and Marsden, C.D. (1982). Manual motor performance in a deafferented man. Brain, 105, 515-542.

Russell, D.F. and Zajac, F.E. (1979). Effects of stimulating Deiter's nucleus and medial longitudinal fasiculus on the timing of fictive locomotor rhythm induced in cats by DOPA. Brain Research, 17, 588-592.

Sanes, J.N., Mauritz, K.-H., Dalakas, M.C. and Evarts, E.V. (1985). Motor control in humans with large-fibre sensory neuropathy. Human Neurobiology, 4, 101-114.

Sainburg, R.L., Ghilardi, M.F., Poizner, H. and Ghez, C. (1995). Control of limb dynamics in normal subjects and patients without proprioception. Journal of Neurophysiology, 73, 820-835.

Sainburg, R.L., Poizner, H. and Ghez, C. (1993). Loss of proprioception produces deficits in interjoint coordination. Journal of Neurophysiology, 70, 2136-2147.

Schaumburg, H.H. and Berger, A. (1988). Pyridoxine neurotoxicity. In: Clinical and Physiological Applications ofVitamin B-6. Edited by Liss, A.R. pp. 403-413.

Schaumburg, H.H., Kaplan, J., Windebank, A., Vick, N., Rasmus, S., Pleasure, D. and Brown, M.J. (1983). Sensory neuropathy from pyridoxine abuse. A new

megavitamin syndrome. New England Journal of Medicine, 309, 445-448.

Schaeppi, U. and Krinke, G. (1982). Pyridoxine neuropathy: correlation of functional tests and neuropathology in beagle dogs treated with large doses of vitamin B_6. Agents and Actions, 12, 575-582.

Schaeppi, U. and Krinke, G. (1985). Differentail vulnerability of 3 rapidly conducting somatosensory pathways in the dog with vitamin B_6 neuropathy. Agents and Actions, 16, 567-579.

Sherrington, C.S. (1900). The muscular sense. In: Textbook of Physiology. Edited by Schafer, E.A. Pentland, London. 2, 1002-1025.

Shen, L. and Poppele, R.E. (1995). Kinematic analysis of cat hindlimb stepping. Journal of Neurophysiology, 74, 2266-2280.

Shik, M.L. and Olovsky, G.N. (1976). Neurophysiology of locomotor automatism. Physiological Reviews, 56, 465-501.

Shik, M.L., Severin, F.V. and Orlovsky, G.N. (1966). Control of walking and running by means of electrical stimulation of the mid-brain. Biophysics, 11, 756-765.

Shimamura, M. and Kogure, I. (1983). Discharge patterns of reticulospinal neurons corresponding with quadrupedal leg movements in Thalamic cat. Brain

Research, 260, 27-34.

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

Snell, E.E. (1990). Vitamin B_6 and decarboxylation of histidine. In: Vitamin B_6. Edited by Dakshinamurti, K. Annals of the New York Academy of Sciences, 585, 1-12.

Soechting, J.F. (1988). Effect of load perturbations on EMG activity and trajectories of pointing movements. Brain Research, 88, 390-396.

Taub, E. (1976). Movement in nonhuman primates deprived of somatosensory feedback. Exercise and Sports Sciences Reviews, 4, 335-374.

Trank, T.V., Chen, C. and Smith, J.L. (1996). Forms of forward quadrupedal locomotion. I. A comparison of posture, hindlimb kinematics, and motor patterns of normal and crouched walking. Journal of Neurophysiology, 76, 2316-2326.

Twitcell, T.E. (1954). Sensory factors in purposive movement. Journal of Neurophysiology, 17, 239-252.

Vilensky, J.A. and Patrick, C. (1984). Inter and intratrial variation in cat locomotor behavior. Physiology and Behavior, 33, 733-743.

Von Holst, E. (1954). Relations between the central nervous system and the peripheral organs. British Journal of Animal Behavior, 2, 89-94.

Wetzel, M.C., Atwater, A.E., Wait, J.V. and Stuart, D.G. (1976). Kinematics of locomotion by cats with a single hindlimb deafferented. Journal of Neurophysiology, 39, 667-678.

Whelan, P.J., Hiebert, G.W. and Pearson, K.G. (1995). Stimulation of the group I extensor afferents prolongs the stance pase in walking cats. Experimental Brain Research, 103, 20-30.

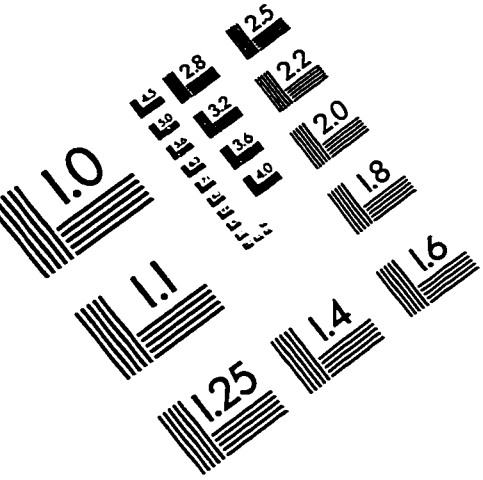
Woolson, R.F. (1987). Statistical methods for the analysis of biomedical data. John Wiley and Sons, New York. pp. 145-203.

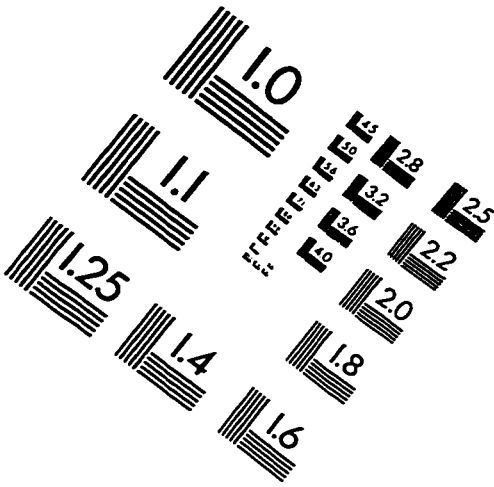
Windebank, A.J., Low, P.A., Blexrud, M.D., Schmelzer, J.D. and Scaumburg, H.H. (1985). Pyridoxine neuropathy in rats: specific degeneration of sensory axons. Neurology, 35, 1617-1622.

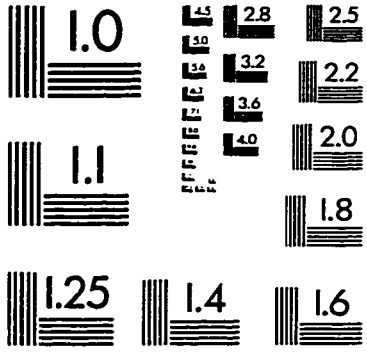
Xu, Y., Sladky, J.T. and Brown, M.J. (1989). Dose-dependent expression of neuronopathy after experimental pyridoxine intoxication. Neurology, 39, 1077-1083.

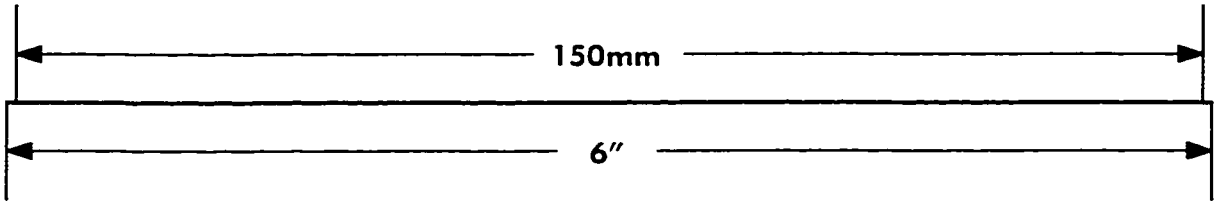
Zempleni, J. (1995). Parmacokinetics of vitamin B_6 supplements in humans. Journal of American College of Nutrition, 14, 579-586.

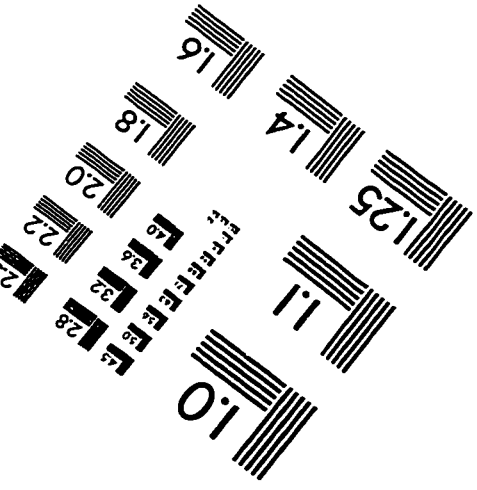
IMAGE EVALUATION
TEST TARGET (QA-3)

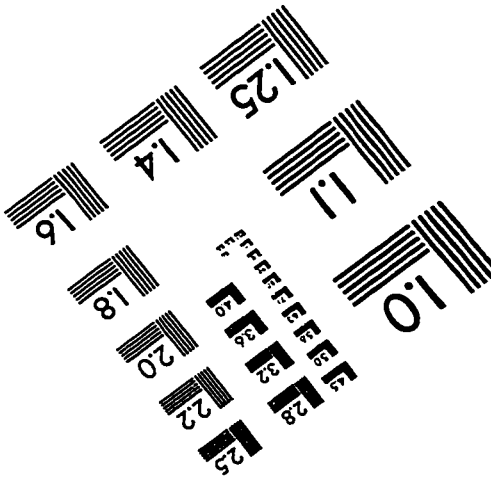












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989