LOCOMOTOR PLASTICITY IN THE CHICK

by

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ABSTRACT

This thesis examines the behavioural plasticity of bipedal locomotion in the chick, both during normal development and after spinal cord injury. The locomotor characteristics of chicks were quantified as animals moved overground unrestrained. Kinematic data were recorded on videotape, while kinetic data were collected from a force platform built into the walkway. All measurements were made over a range of velocities and at regular intervals throughout the first 2 weeks posthatching. The results show that, although chicks can innately run as well as adults, they need to learn to walk in a mature manner over the first week posthatching. This disparity may arise from the distinct actions of the legs in these two behaviours, and the requirement for longer durations of single leg support during walking. In a separate group of animals, locomotor abilities were quantified as above prior to hemisection of the left thoracic spinal cord, and thereafter at regular intervals for 2 weeks. Chicks were also videotaped while they swam in a tank of water. In one group, phasic cutaneous stimulation was provided during swimming trials. Twenty-four to 48 hours after hemisection, chicks moved overground with a distinctly asymmetric gait. Range of joint motion of the left leg (ipsilateral to the hemisection) was reduced. Over the two week recovery period, left leg joint angles during walking recovered to normal pre-operative values, but did not return to pre-operative values during swimming trials. However, when chicks were provided with phasic cutaneous stimulation during swimming trials for 14 days, they showed improvements in leg motion which were retained even when the cutaneous stimulation was not provided. Possible neurophysiological mechanisms include reflexive activation of limb extensors in response to cutaneous stimulation, and subsequent strengthening of reflex pathways after repeated training with stimulation. It is proposed that, because the locomotor neural circuitry has completed development well before hatching in
chicks, changes in locomotor behaviour posthatching or after spinal injury necessarily arise from plasticity of existing neural circuits rather than growth/regeneration of new projections. Chicks may therefore be a useful model for examination of neuroanatomical plasticity responsible for locomotor development and recovery after spinal injury.
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Chapter One:

Introduction

This thesis examines the plasticity of locomotor behaviour in the posthatching chick, both during normal development and after spinal cord injury. This chapter will initially review what is known about the central nervous system circuits that control vertebrate locomotion. The ontogeny of these circuits will then be described and correlated with the development of locomotor behaviour in the neonatal animal. Subsequently, the functional consequences of spinal cord injury will be examined, with emphasis on the importance of anatomical and behavioural plasticity for the recovery of locomotor functions in the absence of significant axonal regeneration. The use of the bird as a model for recovery of locomotion after spinal cord injury will also be discussed, including the features of bipedal locomotion which are common to birds and humans.

A. Neural control of locomotion

It has been well established in vertebrates that the essential neural circuits capable of generating basic locomotor movements are contained completely within the spinal cord (see Grillner, 1975, Grillner and Wallen, 1985). Animals whose limbs have been deafferented and have undergone a complete spinal cord transection are still capable of generating stepping activity (Grillner and Zangger, 1979, 1984). The neural circuits responsible for this activity are often referred to as central pattern generators (CPG) for locomotion, and consist of populations of spinal interneurons which are interconnected so as to generate rhythmic oscillating activity (Armstrong, 1986). Connections between the locomotor CPG's and motoneurons produce alternating stepping movements in the limbs (Forssberg, 1983).

In the intact animal, locomotor CPG's do not act alone to produce locomotion. There are two
major sources of input to spinal cord circuits: 1) segmental afferent input providing information
from limb structures and 2) descending inputs arising from rostral spinal and supraspinal centres
(Grillner, 1981). Segmental afferent input arises from two major sources: 1) proprioceptive
information from muscle and tendon receptors, which detect muscle force and length changes for
example, and 2) exteroceptive information, from receptors in the skin which transduce a variety of
modalities including pressure, pain and temperature sensations.

Afferent input from the limbs plays an important role during locomotion, reinforcing and
stabilizing the locomotor pattern (Grillner and Zangger, 1975; Grillner, 1975). Afferent feedback
may reinforce ongoing muscle activity during stepping (Pearson, 1993). For example, the stance-
related activity in ankle extensor muscles is increased by lengthening or by imposing an increased
load on the same muscles (Akazawa, et al., 1982; Dietz, et al., 1979). Proprioceptive feedback
from limb extensor muscles has also been shown to regulate the transitions between the phases of
the step cycle (Pearson, 1993). Extensor muscles are unloaded at the end of the stance phase and the
concomitant decrease in proprioceptive feedback provides a signal for the transition from stance to
swing (Duysens and Pearson, 1980; Whelan, et al., 1995).

Exteroceptive, or cutaneous, feedback is less well studied but is known to be important for
responses to perturbations of locomotion. For example, contact of the dorsum of the foot by an
obstacle during the swing phase of walking elicits an increased limb flexion response, such that the
object is overcome (Prochazka, et. al., 1978; Forssberg, 1979). This reflexive adjustment is greatly
reduced in animals in which the skin has been denervated (Prochazka, et. al., 1978). Afferent input
therefore also plays an important role in modulating the output of the CPG's in the event of
unexpected peripheral perturbations.

The second major source of input to the CPG's, from supraspinal centres, is necessary to
initiate voluntary locomotor activity, as well as to refine the basic output pattern (Shik and
Orlovsky, 1976, Wetzel and Stuart, 1976). There are many supraspinal regions which are known to be involved with the initiation and/or control of locomotion in vertebrates. These include the cerebral cortex, basal ganglia, and cerebellum as well as numerous brainstem regions such as the red nuclei, mesencephalic locomotor regions, vestibular nuclei, and nuclei in the pontine and medullary reticular formation (for review, see Armstrong, 1986). Neurons in the motor regions of the cerebral cortex project to the spinal cord directly (via the pyramidal tracts) and also indirectly via brainstem regions. Examination of locomotor behaviour after pyramidal tract or cortical lesions indicates that the motor cortex plays an important role in fine locomotor control, eg. animals are able to walk overground relatively normally but are unable to perform difficult tasks such as beam walking (Liddell and Phillips, 1944; Buxton and Goodman, 1967). The basal ganglia and cerebellum do not project directly to the spinal cord, but instead act to modulate cortical and brainstem output to the cord. Brainstem nuclei, notably the red nuclei, vestibular nuclei and nuclei within the reticular formation project to many levels of the spinal cord and are known to make direct synaptic connections with motoneurons and with interneurons of spinal locomotor CPG's (Armstrong, 1986; Orlovsky, 1972a,b; Hongo et al., 1969a,b). Thus the brainstem-spinal pathways can be seen as the final common pathway for control of locomotion in vertebrates.

The diffuse and parallel organization of motor control systems throughout the CNS, including the brainstem, makes it difficult to assign specific roles to different brainstem structures. However, studies involving lesioning, electrical stimulation or recording of discharges from several brainstem nuclei have shown that they appear to affect different aspects of the locomotor pattern. Neurons in the vestibular nuclei, for example, are facilitory to motoneurons serving extensor muscles of the limbs, and fire immediately prior to the onset of the stance phase, when most extensor muscles are active (Orlovsky, 1972a,b; Orlovsky and Pavlova, 1972). Rubrospinal neurons, conversely, have been shown to be facilitory to flexor motoneurons associated with distal limb muscles and are most
active during the swing phase of the step cycle (Orlovsky, 1972a,b,c). The firing of individual reticulospinal neurons during locomotion is correlated with the activity of particular groups of limb muscles (Drew, et al., 1986), and appears to regulate the amplitude of firing of both extensor and flexor muscles (Orlovsky, 1970). Nuclei of the pontine and medullary reticular formation are also important in the initiation of locomotor movements, receiving signals for this function from rostral locomotor regions which have no direct projections to the spinal cord (Steeves and Jordan, 1984; Armstrong, 1986). Brainstem nuclei are therefore essential in the initiation and ongoing control of functional locomotion in vertebrates.

B. Ontogeny of neural circuitry and locomotor behaviour in vertebrates

The spinal locomotor circuitry responsible for generating alternating limb activity in vertebrates is established early in development. For example, coordinated lumbosacral motor activity is present in the chick embryo in ovo prior to the 6th day of the 21 day developmental period (Sholomenko and O'Donovan, 1995). Afferent input from the limb also develops early, soon after the motor spinal circuitry (Windle and Orr, 1934; Narayanan, et al., 191 A; Oppenheim, 1972). This is evident in the fact that neonatal rats and cats are capable of producing coordinated stepping movements with partial weight support a few days after birth (Bekoff and Trainer, 1979; Bradley and Smith, 1988). Electromyographic (EMG) output of hindlimb muscles reveal an adult-like pattern of muscle coordination (Bradley and Smith, 1988). Even human infants are able to produce supported stepping movements within the first few days after birth (Forssberg, 1985). In addition, kittens and rats spinally transected as neonates are still capable of supporting their weight and producing rhythmic stepping with the hindlimbs (Weber and Stelzner, 1977; Bradley and Smith, 1988; Commissiong and Toffano, 1989; Howland, et al., 1995b). Therefore, basic features of the locomotor pattern such as alternating limb action and weight support appear to depend largely upon
early developing spinal and segmental afferent pathways.

In spite of the early establishment of alternating limb motion and partial weight support, however, neonatal mammals are incapable of incorporating these features into overground locomotion until they are able to coordinate movements of the entire body (Howland, et al., 1995b). This ability relies upon supraspinal pathways responsible for balance and postural control, which are incompletely developed at birth (Cabana and Martin, 1981; Kudo, et al., 1993; Cassidy, et al., 1994). These pathways include the vestibulospinal, rubrospinal and corticospinal tracts. Some pathways, primarily the corticospinal tract, develop axonal projections postnatally (Kudo, et al., 1993). Thus, use of the limbs during overground locomotion does not begin until 11 days of age in rats and 2 to 3 weeks of age in kittens (Westergra and Gramsbergen, 1990; Peters, 1983; Howland, et al., 1995b). In humans, independent locomotion does not begin until approximately a year after birth (Forssberg, 1985).

It is important that most ontogenetic studies of locomotor behaviour to date have been carried out on altricial animals, where many fundamental aspects of neural development are still proceeding at the time of investigation. Few studies have examined the development of locomotion in precocial animals (eg. chickens). In contrast to altricial mammals, supraspinal projections are well developed prior to hatching in the chicken (Okado and Oppenheim, 1985). Chicks are able to walk within several hours of hatching and adult-like electromyographic (EMG) patterns can be recorded from leg muscles in the first 24 hours after hatching (Jacobson and Hollyday, 1982). However, EMG analysis provides information on only one aspect of locomotor behaviour, ie. the neuromuscular output. A detailed examination of locomotor behaviour using kinematic (ie. joint angles, step and stride timing measurements) and kinetic (ie. ground reaction force measurements) techniques could provide more information about the ontogeny of leg and body movements during overground locomotion. Chapter 2 of this thesis uses these techniques to demonstrate that the production of
mature gait is not necessarily innate in even highly precocial animals.

C. Axonal regeneration and functional recovery after spinal cord injury - plasticity and mechanisms of repair.

The importance of supraspinal input for the control of overground locomotion is most evident when the connections between spinal locomotor circuits and supraspinal pathways are interrupted as a result of spinal cord injury. A complete spinal cord transection removes the ability of the animal to move in a voluntary and controlled manner. There is considerable evidence that the adult central nervous system of higher vertebrates recovers poorly from damage (for review, see Eidelberg, 1981). Full functional regeneration after CNS injury requires several successive events (Guth et al., 1980). Neurons which have undergone axotomy must remain viable and be able to grow new axons in order to re-establish severed connections. Regenerating axons must be able to grow across the lesion site, elongate toward target cells and make functional synapses with appropriate neurons. The regenerated connections must then contribute to the recovery of behaviour which was lost when the injury occurred (Guth et al., 1980). The lack of normal functional recovery from CNS damage in higher vertebrates can be attributed in large part to marked absence of axonal regeneration in the CNS after damage. While damaged axons in the peripheral nervous system can regenerate over long distances, damaged axons within the central nervous system cease growing after 0.5 - 1.0 mm (eg. Schnell and Schwab, 1990). There is evidence to suggest that lack of axonal regeneration can be attributed to both the absence of an appropriate extracellular environment to support axonal elongation, as well as to the suppression of intrinsic growth programs in mature, well-differentiated neurons. For example, studies in which axotomized retinal ganglion cell axons are allowed to grow into a relatively permissive environment, such as a peripheral nerve bridge, show that some CNS neurons are capable of regenerating long axons in such a permissive environment (David et al.,

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However, the centrally-projecting axons from dorsal root ganglion (DRG) cells do not spontaneously regenerate into the favourable environment provided by peripheral nerve grafts after axotomy (Richardson and Issa, 1984). DRG axons will grow into the grafts only after induction of appropriate growth-associated programs, produced by damage to the peripheral axons of the same DRG cells (Richardson and Issa, 1984; Richardson and Verge, 1987). Thus, attempts to induce axonal regeneration in the mature CNS need to address both the intrinsic ability of mature neurons to grow new axons as well as intervention to provide a more favourable CNS environment. The difficulty of combining both of these approaches is illustrated by the fact that only a small number of axons have ever been induced to regenerate in the adult vertebrate CNS (David et al., 1981; Schnell and Schwab, 1990).

The lack of axonal regeneration, and thus the inability to restore normal anatomical connections after a complete spinal cord injury, is the main basis for the permanent loss of motor and sensory function which occurs caudal to the level of the injury. Of course, the extent of functional loss depends largely on the severity of the lesion. Many higher vertebrates show a marked capacity for recovery of function, including locomotion, after partial spinal cord injury. In neonatal and adult cats and rats, locomotor function recovers almost completely after a spinal cord hemisection (Eidelberg, 1981, Eidelberg, et al., 1986, Bregman and Goldberger, 1982, 1983a,b). The neuroanatomical basis for much of the recovery which occurs after partial spinal injury arises from three sources: 1) maintenance of synaptic input from undamaged axons, 2) new connections made by late-growing axons in neonatal animals, and 3) sprouting from undamaged axons and presumably the reorganization of intrinsic spinal cord circuitry. The maintenance of undamaged synaptic inputs, especially supraspinal and propriospinal inputs, is of primary importance in recovery of function after partial injury. This is illustrated by experiments involving bilateral hemisections; when a second contralateral hemisection is performed, after recovery from and rostral
to the first, there is complete and permanent loss of locomotor function (Kato, et al., 1984; Little, et al., 1988). Undamaged segmental afferent projections have also been shown to be necessary for functional recovery after spinal cord hemisection. When cats undergo spinal hemisection after recovery from complete or partial lumbosacral deafferentiation, normal motor patterns return only in those animals with at least one spared dorsal root (Goldberger, 1988a, b).

The second anatomical mechanism underlying functional recovery after hemisection is new outgrowth of descending axons in developing animals. It is generally accepted that spinal cord lesions which occur early in development, ie. embryonic or early post-natal, result in less severe deficits than adult injuries. Neonatal cats and rats show better recovery of locomotor and reflex behaviour after spinal cord hemisection than adult animals (Prendergast and Shusterman, 1982; Bregman and Goldberger, 1982, 1983a and b). This phenomenon, known as the infant lesion effect, is due in part to the outgrowth of late-developing supraspinal projections. After partial spinal injury in the neonatal rat, axons from the sensorimotor cortex grow through the undamaged cord after the injury (Schreyer and Jones, 1983; Bernstein and Stelzner, 1984). These late-growing tracts also form functional connections after neonatal spinal injury - the contact placing reflex, which relies specifically upon input from the cerebral cortex, is present after recovery from spinal hemisection in neonatal animals but not in adult animals (Robinson and Goldberger, 1986).

The third possible mechanism underlying functional recovery after partial spinal injury is the reorganization of spinal cord circuitry. Strengthening of previously latent or silent synapses, in combination with the establishment of new synaptic connections through sprouting of undamaged axons, may restore or replace the functional output of the spinal cord and thus contribute to recovery. Plastic changes in synaptic connections have been shown to occur in several systems after spinal cord injury. After cervical spinal hemisection in the rat, recovery of diaphragmatic function occurs via unmasking of synapses between brainstem neurons and phrenic motoneurons which were
functionally ineffective before injury (Goshgarian, *et al*., 1989, 1993; Moreno, *et al*., 1992). In the cat, segmental afferent axons have been shown to sprout within the spinal cord after thoracic hemisection and/or partial limb deafferentation (Murray and Goldberger, 1974; Goldberger and Murray, 1988; Helgren and Goldberger, 1993). Presumably, this afferent sprouting leads to the formation of new synapses on neurons which were partially denervated after the hemisection. Functionally, increased input from segmental afferents may underlie the increased motoneuronal excitability which occurs after thoracic hemisection in the cat (Mendell, 1984). In addition, intraspinal sprouting of afferent dorsal root axons has been found to occur to a greater extent in neonatal rats compared to adult animals (Hulsebosch and Coggeshall, 1983), and may be another mechanism mediating the infant lesion effect.

Interestingly, reorganization of spinal circuitry also underlies much of the functional recovery that occurs even in animals which are capable of true axonal regeneration. Although regenerating axons in the lamprey spinal cord show remarkable specificity in their directional growth and synaptic connections, the pattern of synaptic connections which existed before transection is not duplicated exactly (Cohen *et al*., 1988). Similarly, regenerated descending projections in the spinal cord of the goldfish show significant synaptic reorganization, in that new descending axons synapse on interneurons rather than directly on motoneurons as they did prior to injury (Bernstein and Gelderd, 1973). Thus, behavioural recovery in these animals is presumably achieved through axonal regeneration in combination with reorganization of neural circuitry.

Neuroanatomical plasticity, in the form of reorganization of existing circuitry, therefore has potential for mediating functional recovery after adult spinal cord injury. Methods which promote plastic changes in appropriate spinal cord circuits would have extremely important consequences for spinal cord-injured patients. Chapter 4 of this thesis demonstrates that manipulation of specific types of segmental afferent input can lead to improved motor recovery after partial spinal injury.
Chapter 5 outlines the possible neuroanatomical processes mediating this recovery and discusses methods currently employed to promote functional locomotor recovery after injury in humans.

D. Bipedal locomotion and the use of birds as models for human locomotion

This thesis examines bipedal locomotion during normal development and after spinal cord injury. This differs from most studies, which analyze the locomotion of quadrupeds such as cats or rats, with the justification that mammalian systems are most relevant for applications to human locomotion. Neurologically, however, the anatomy, physiology and pharmacology of brainstem regions involved in the initiation and control of avian locomotion are analogous to those of all other vertebrates, including mammals (Steeves, et al., 1987, Sholomenko et al., 1991a,b,c). The anatomy of brainstem locomotor nuclei and the funicular pathways of their descending connections are essentially the same in birds as in mammals (Webster and Steeves, 1988, 1991). Transection of these pathways in the spinal cord of adult birds produces locomotor deficits analogous to those produced in adult mammals (Sholomenko and Steeves, 1987).

The advantage of examining locomotion in a bipedal animal becomes especially important after spinal cord injury. The locomotor movements adopted after partial spinal injury will not only reflect the direct consequences of the injury, ie. paresis, hyperreflexia, but will represent the adjustments necessary to produce effective bipedal locomotion. These adjustments, and the means available to the animal to produce locomotion, differ significantly between bipedal animals and quadrupeds. The postural requirements for maintenance of upright stance and distribution of weight between two limbs are not addressed with quadrupedal spinal cord injury models. After a partial thoracic spinal cord injury, quadrupeds are able to compensate for dysfunction of a single limb or of two limbs by distributing their weight amongst the four limbs and coordinating forelimbs and hindlimbs in a complex manner (Silver, et al., 1983; Merkens and Schamhardt, 1988; Gorska, et al., 1993; Bem,
et al., 1995; Howland, et al., 1995b). Birds, in contrast, are subject to the same constraints as humans and in this way serve as a useful bipedal model for compensatory mechanisms available after spinal cord injury. Chapter 3 of this thesis demonstrates that birds make many of the same postural adjustments as humans to compensate for hemiplegia.

Comparative studies provide further support for the fundamental similarities between locomotor constraints of birds and humans. The common requirements of moving overground with two limbs has produced similar features in the locomotor patterns in both groups. The common features are those relating to the overall energetics of overground locomotion and to more general kinematic aspects of limb movements. For example, the cost of transport is similar for humans and equivalently sized birds (Fedak, et. al., 1974, 1979). Humans and birds also display consistent patterns of stride length, stride frequency and step length with changes in speed and gait (Gatesy and Biewener, 1991). Therefore, the investigation of locomotor development and adaptations after spinal injury in a non-human biped can provide insight into comparable aspects of human bipedalism.

This thesis examines the behavioural plasticity of bipedal locomotion during development and after spinal cord injury. Chapter 2 will examine the normal posthatching development of locomotion in a precocial biped. Chapter 3 describes the locomotor adaptations that result from hemiplegia due to spinal cord hemisection in young chicks. Chapter 4 investigates the role that afferent feedback plays in plasticity of motor recovery after spinal hemisection. The results of these studies are discussed in Chapter 5 in the context of neuroanatomical mechanisms that may underlie the behavioural plasticity found in posthatching chicks. I also discuss the potential role of sensorimotor stimulation in exploiting the plastic potential of spinal circuits to enhance locomotor recovery after spinal cord injury.
Chapter Two:  
Developmental plasticity of bipedal locomotion -  
a kinetic and kinematic analysis

Introduction

All terrestrial walking vertebrates, including humans, use different gaits to locomote at different speeds. It has been shown that animals change gaits in order to minimize energy requirements (Hoyt and Taylor, 1981). Studies which evaluate the mechanical work of overground locomotion have defined walking and running in terms of energy exchange (Cavagna, et al., 1977, Heglund, et al., 1982). During locomotion at a constant average speed, the body's centre of mass rises and falls, decelerating and accelerating with each step (Heglund, et al., 1982). Walking gaits are energetically efficient because there is an alternating transfer between the potential and kinetic energy of the body within each stride. During walking, the leg acts as a solid strut, so that the body’s centre of mass rises over the leg to reach a maximum in the middle of the stance phase, while the opposite leg is swinging forward (Figure 2.1a). Potential energy of the body is therefore greatest during midstance. However, the forward velocity of the body is lowest at this point and therefore kinetic energy is at a minimum. As the body moves ahead of the leg during the latter half of the stance phase, the centre of mass falls but forward velocity increases. Consequently, during bipedal locomotion, for example, when both limbs are equally weightbearing (ie. beginning of the stance phase of the opposite leg), the centre of mass is lowest and forward velocity is highest. At this point, potential energy has been converted to forward kinetic energy. As the body rises onto the opposite leg, potential energy is again recovered from kinetic energy as the body slows down. These oscillating exchanges between potential and kinetic energy thus minimize the energy that needs to be expended to move at a walk.
In contrast, during running, the body's centre of mass is lowest at midstance (Figure 2.1a) and highest during the suspension (airborne) phase of the stride, when forward velocity is also greatest. Potential and kinetic energy simultaneously increase and decrease within each running stride and thus exchange between potential and kinetic states is not possible. Instead of oscillating exchanges between potential and kinetic energy, running involves exchanges between elastic and kinetic energy. The leg acts as a spring during running, absorbing kinetic energy as elastic energy in muscles and tendons during the first half of the stance phase and releasing the stored elastic energy as kinetic energy during the latter half of stance (Cavagna et al., 1977).

What remains unclear is whether the oscillating transfer between potential and kinetic energy during walking is strictly an innate, biomechanical consequence of leg movement during walking or is a form of walking which requires practice. Recent studies on load bearing in humans suggest that, with experience, some individuals can effectively exploit this energy exchange such that an increased load imposes little additional work. Through a more effective exchange of kinetic and potential energy during walking, African women experienced in carrying head loads can carry up to 70% of their bodyweight much more economically than untrained subjects (Heglund, et al., 1995). Thus, it would appear that this method of energy exchange may be a learned adaptation rather than a fixed or innate characteristic of walking.

This question can be investigated by examining the locomotion of animals in which apparently little locomotor development occurs. Precocial animals such as the chicken are able to locomote within hours of hatching. While the in ovo development of locomotor systems in the chick embryo have been well studied (Hamburger, et al., 1965; Hamburger and Oppenheim, 1967; Bekoff, 1976; Landmesser and O'Donovan, 1984), there are no studies which have investigated the early ontogeny of the biomechanics of overground locomotion by the hatchling chick. Birds are the only vertebrates in which bipedalism is the sole form of terrestrial locomotion; chicks are therefore useful models for
the biomechanical study of human locomotion. As bipeds, both birds and humans are subject to similar postural constraints during locomotion. For example, bipeds must necessarily undergo a period of single limb support while walking and running, requiring sufficient balance and muscular strength. This study has used kinetic and kinematic techniques to demonstrate that, although young chicks are innately able to run as well as adults, they must learn to walk in the controlled and efficient manner of an adult.

**Methods**

Fertilized eggs were incubated at 37.5 degrees Celsius and rotated four times per day. After hatching, chicks were moved to brooders equipped with food and water *ad lib*. All animals were cared for according to standards outlined by the Canadian Council on Animal Care. A total of 28 chicks were used for this study.

Kinetic and kinematic data were collected from chicks as they walked overground. Chicks were encouraged to move down a 50 cm straight path, restrained by an opaque wall on the right and a clear plexiglass wall on the left. The width of the path was adjusted to prevent the chick from touching the sides of the hallway. The ground surface consisted of balsa wood covered with a fine nylon mesh to increase traction. After each pass, the chicks were returned to the beginning of the path for a subsequent trial. Chicks underwent 15-20 trials over a 10 - 30 min period each day.

*Kinetic measurements:* Kinetic (ground reaction force) data were collected from a force platform (10 cm long and 8 cm wide) built in the middle of the path and level with the surface. The force platform was modified from a basic design by Full and Tu (1990) and consisted of foil strain gauges cemented onto modified brass box beams. Output from the platform consisted of force measurements in three orthogonal directions - vertical, horizontal fore-aft, and horizontal medial-lateral. Only output from the vertical and fore-aft directions were used in this study, as horizontal
medial-lateral forces were generally small (<5-8% of vertical force) and displayed a large amount of interindividual variation. Output from the platform was linear over a range of forces from 0.1N to 4N and the plate had a resonant frequency of 240 Hz. Cross-talk between vertically- and horizontally-directed gauges was less than 5%. Data collection was triggered by the chick's body interrupting an infra-red beam situated 3 cm in front of the force platform. A digital LED time display was also triggered by interruption of the infra-red beam. The time clock was stopped by another infra-red beam, located 3 cm after the force platform. The time displayed after each pass was used to calculate average velocity across the platform. Output from the force platform was amplified, analog-to-digital converted (RC Electronics, Inc.) and collected on a PC. Data from each pass was considered acceptable if: 1) the chick maintained a constant velocity over the force platform, and 2) initial foot placement on the platform was complete, ie. the first foot to touch the platform was located completely on the platform.

Kinematic measurements: To facilitate visualization of the leg joints, the down on the left leg was trimmed, and markers were placed on the skin to mark the location of the ischium, the hip joint, the knee joint, the ankle joint, the distal tarso-metatarsal bone, and the toe of the third digit. Chicks were videotaped from the left side as they moved past the camera. The camera was located 1.5 metres from the platform and positioned perpendicular to the direction of the chick's movement, with the camera lens level with the force platform.

Kinetic analysis: Ground reaction force records were analyzed using custom written software according to the method of Cavagna (1975). Briefly, the data in millivolts was converted to Newtons x (kg bodymass)$^{-1}$. After subtracting acceleration due to body mass (ie. 9.8 Newtons kg$^{-1}$) from the vertical acceleration, acceleration in both the vertical and horizontal fore-aft directions was integrated over time to yield vertical and fore-aft velocity as a function of time (Figure 2.1). Vertical velocity was then integrated over time to produce vertical displacement as a function of time (see
Figure 2.1). Constants for integrations of both vertical acceleration and vertical velocity were assumed to be zero - this assumption was valid because the integration was performed over an integral number of steps. The integration constant for the fore-aft acceleration was the average velocity for each run, which was obtained from the time clock on the videotape. Kinetic energy of the centre of mass was calculated over time as \( \frac{1}{2} m v^2 \), where \( m \) is body mass in kilograms and \( v \) is velocity in the fore-aft direction. Kinetic energy changes represent velocity changes only in the fore-aft direction because the actual distances moved during one stride in the vertical direction (approx. 1-2 mm) are small compared to those in the horizontal direction (approx. 150-175 mm). Hence, kinetic energy changes in the vertical direction are negligible (ie. less than 5%) compared to those in the fore-aft direction. Potential energy changes were calculated over time as \( mgh \), where \( g \) is the gravitational acceleration constant (9.8 m s\(^{-2}\)) and \( h \) is the change in vertical displacement of the centre of mass. Total energy as a function of time was calculated by summing potential and kinetic energy over time. Mass-specific force and energy were calculated by dividing whole body values by body mass in kilograms. To determine quantitatively the amount of energy conserved by transfer between kinetic and potential energy, the following equation was used:

\[
\text{percent energy recovery} = 100 \times \frac{(\Sigma + \Delta E_H) + (\Sigma + \Delta E_V) - (\Sigma + \Delta E_{TOT})}{(\Sigma + \Delta E_H) + (\Sigma + \Delta E_V)}
\]

where \( (\Sigma + \Delta E_H) \) is the sum of the positive increments of the horizontal kinetic energy, \( (\Sigma + \Delta E_V) \) is the sum of the positive increments of vertical potential energy, and \( (\Sigma + \Delta E_{TOT}) \) is the sum of the positive increments in total energy (Heglund, et al. 1982).

Kinematic data analysis: Single frame analysis was carried out on the videotapes at 60 frames per second\(^1\) ('V' Professional Imaging, Digital Optics, Inc.). Stride and step parameters collected from video included onset time of stance phase for right and left legs (onset of foot contact), onset time
for swing phase (end of foot contact) and the onset time of subsequent stance phases for each limb. The position of the joint markers were digitized manually for each frame over at least one complete stride for each trial. Stride measurements and joint angles were calculated using custom written software. For all stride measurements, at least 5 samples per chick were collected from each of data acquisition day P1, 3, 5, 9 and 14. Stride length was calculated as the horizontal distance between the position of the metatarsophalangeal joint at each onset of consecutive stance phases. Stride duration was the time between the onset of consecutive stance phases. Velocity, calculated as stride length divided by stride duration was found to differ by at most 5 - 8 % from that determined from the LED timer; consequently, the latter method was used to calculate velocity throughout this thesis.

In order to allow comparison between chicks of different sizes, velocity and stride length were normalized to dimensionless variables using the method of Gatesy and Biewener (1991). Stride length was divided by hip height (h) and velocity was divided by \( (gh)^{0.5} \), where \( g \) is the gravitational acceleration constant (9.8 m s\(^{-2}\)). Animals run in a dynamically similar fashion at similar normalized velocities (Alexander and Jayes, 1983). Reference to these variables in the remainder of this thesis refer to these normalized values.

For the remaining stride variables, duty factor was defined as the duration of the stance phase divided by the total stride duration. Single support time for each limb was defined as the time each limb was weightbearing while the opposite limb was not weightbearing (in the swing phase). Single support time was divided by stance duration to obtain the single support proportion of stance. Least squares regression was used to determine the relationships between duty factor and velocity, as well as single support proportion of stance versus velocity. Slopes were compared using analysis of variance (SigmaStat, Jandel Scientific).

To compare joint angle motion throughout the step cycle, polynomial regression (SigmaPlot, Jandel Scientific) was used to calculate a representative curve for each age group. Each plot
consists of joint angle data from a number of animals (one stride per individual) moving at the same velocity. In each case, a 6th order polynomial produced the best fit to each set of data for hip, knee and ankle joint angles ($R^2 > 0.80$). Maximum joint angles at initial foot contact and stride length of P1-2 and P14 chicks were compared using Students t-test (SigmaStat, Jandel Scientific). Average measurements for each chick were calculated from at least 5 samples for each day of examination.

**Results**

*Ground reaction forces and energy exchange during walking*

Ground reaction force patterns produced by P1-2 and P14 chickens at a walk and run were generally similar to those produced by other birds and humans (Heglund *et al.*, 1982; Willems, *et al.*, 1995, Figures 2.1, 2.2 and 2.5). At a walk (Figures 2.1, 2.2), the body centre of mass rose over the leg placed on the ground, and thus potential energy reached the greatest value during the middle of the stance phase. This produced a reduction in the upward vertical movement of the centre of mass at midstance and thus a decrease in the vertical force exerted on the ground. This is illustrated by the characteristic dip in the vertical force record which occurred midstance during a walking step (Figure 2.2). Both the P1-2 chicks and P14 chicks produced a similar pattern of vertical force, although there was a marked difference between the two in the amplitude of force oscillations within each step, especially at the beginning and end of each stance phase. In the P1-2 chicks, there was a smaller upward deflection of the force values, indicating less movement of the body downwards, at the beginning and end of each stance phase compared to P14 chicks. This corresponds to a point in the stride when the body was supported by both legs. P1-2 chicks also display a smaller downward deflection of the force record, indicating less upward movement of the body, in midstance compared to P14 chicks, corresponding to the time when the body was only supported by one leg.
Figure 2.1

Diagrammatic representation of the analysis of ground reaction force records used to determine velocity and displacement of the body's centre of mass in the vertical and horizontal (fore-aft) directions. The stick figures (a) demonstrate leg positions and the path of the centre of mass at the beginning, middle and end of the stance phase as the chick moves from left to right. For both walking (left column) and running (right column), oscillations in vertical force (b), as a function of time, were integrated successively to yield oscillations in vertical velocity (c) and vertical displacement (d) over time. Oscillations in horizontal (fore-aft) force (e), as a function of time, were integrated to yield oscillations in fore-aft velocity (f). Thick lines represent the portion of the traces generated during a single step. Note that while oscillations in fore-aft force (e) and velocity (f) are similar for walking and running over a single step, oscillations in vertical force (b), velocity (c) and displacement (d) differ between running and walking. Also note that the vertical displacement trace (d) mirrors the path of the body’s centre of mass illustrated in (a).
a. WALK

path of body centre of mass

b. Vertical force

bodyweight

c. Vertical velocity

0.0

d. Vertical displacement

e. Fore-aft force

0.0

f. Fore-aft velocity

-20-
Figure 2.2

Ground reaction forces for a single walking step plotted against the proportion of stance duration for Pl-2 chicks and P14 chicks. Chicks were moving at a normalized velocity of 0.38. Forces have also been normalized for body mass, and body weight has been subtracted from vertical force such that 0 N/kg represents body weight. Solid lines represent a 6th order regression through data for all animals (n = 11 for Pl-2, n = 14 for P14). Dotted lines represent 99% confidence limits.
P1-2 WALKING

P14 WALKING

Force (N/kg)
vertical
fore-aft

0.0 0.2 0.4 0.6 0.8 1.0
proportion of stance phase

0.0 0.2 0.4 0.6 0.8 1.0
proportion of stance phase
The pattern of fore-aft forces were similar for both P1-2 and P14 chicks, and the same as those produced by other vertebrates (Heglund et al., 1982 and Figures 2.1, 2.2). During the first half of the stance phase, decelerative forces (negative force values) were produced by the ground on the body, when the limb was ahead of the body centre of mass. During the latter half of the stance phase, accelerative forces (positive force values) were produced as the body pushed ahead of the limb.

The consequences of the vertical force differences between P1-2 and P14 walking animals become clearer after force records were integrated to evaluate the energy changes occurring within each step (Figure 2.3). In P14 animals, the total energy changes during each stride were reduced because potential and kinetic energy changes are out of phase and of the same magnitude (Figure 2.3e). However, in a P1 animal, the total energy (Figure 2.3e) was dominated by changes in horizontal kinetic energy because of the small changes in potential energy within the same stride (Figure 2.3c). The difference between P1 and P14 walking animals are more clearly apparent when potential energy was plotted against kinetic energy for a single stride (Figure 2.4). Theoretically, a slope of -1 (origin 0,0 on the Figure 2.3 plot) would indicate a walking stride where there is a 100% exchange between kinetic and potential energy; the total energetic output would be zero for such a stride. Data from a single P14 walking stride approximated an efficient exchange (thin dotted loop in upper half of Figure 2.4); whereas, data from a P1 animal (thick solid line in upper half of Figure 2.4) has a slope of 0 indicating that there was little exchange between potential and kinetic energy.
Figure 2.3

Representative ground reaction force records and corresponding potential, kinetic and total energy estimates for a single walking step (normalized velocity of 0.38) from P1 and P14 chicks, as a function of time. Force records (a, b) are normalized for body mass, but energy traces (c, d, e) are not. The same scale is used for both P1 and P14 animals. Note the difference in the change of potential energy (c) between P1 and P14. The efficient alternation between potential (c) and kinetic (d) energy in the P14 chick serves to reduce the change in total energy (e). However, energy conversion is not as efficient for the P1 chick, as evidenced by the lack of contribution of potential energy (c) to total energy (e).
Figure 2.4

Potential energy as a function of kinetic energy, for single steps taken from Figures 2.3 and 2.6. Both potential and kinetic energy have been normalized for body weight and initial values have been set to zero to facilitate comparison between P1 and P14 animals. The data falls only in the left quadrants because kinetic energy (horizontal axis) decreases in the first half of each stance phase and subsequently increases during the latter half of the stance phase for both walking and running. However, walking data falls in the upper left quadrant because potential energy first rises, then falls during each walking stance phase. Running data falls in the lower left quadrant because potential energy first decreases and then increases during each stance phase of running, paralleling the changes in kinetic energy. Note that, while the data of P1 and P14 running chicks do not differ in slope, data from a P14 walking step approximates a slope of -1 (100% exchange), whereas data from the P1 chick has a slope that approximates 0. The difference between P1 and P14 walking steps is due to the lack of a significant change in potential energy during walking by the P1 chick.
In contrast to walking, where the body's centre of mass was highest during midstance, the centre of mass was lowest at midstance during running. This produced a single peak in the vertical force record (Figures 2.1, 2.5). During running, both P1-2 and P14 chicks produced quantitatively similar vertical force patterns. Likewise, fore-aft forces followed the same pattern during running and walking and showed no difference between P1-2 and P14.

Integration of force traces of a single running stride from a P1 and a P14 chick revealed no differences between these two age groups (Figure 2.6). Because the centre of mass was lowest at midstance during running, potential energy was also lowest at this point (Figure 2.6c). Midstance also coincided with a minimum for kinetic energy (Figure 2.6d). Because these two forms of energy are in phase, there is no possibility for exchange, and total body energy reflects the summation of the two (Figure 2.6e). Thus, a plot of potential versus kinetic energy for a running stride (Figure 2.4) produced a positive slope (in the lower negative quadrant of Figure 2.4). Once again, there was no difference in slope between the running stride of a P1 and P14 chick (Figure 2.4).

Analysis of a number of walking strides from both age groups showed that younger animals were not as effective in recovering energy from the alternation between potential and kinetic forms as were older animals (Figure 2.7). Figure 2.7 also shows that little to no energy is recovered through an exchange between potential and kinetic states during running (normalized velocity over 0.8). During walking (normalized velocities less than 0.6), older animals were able to recover on average 60% of total energy through this exchange, whereas young chicks recovered approximately one-third of this value.
Figure 2.5

Ground reaction forces for a single running step plotted against the proportion of stance duration for P1-2 and P14 chicks. Chicks were moving at a normalized velocity of 0.9. Forces have also been normalized for body mass, and body weight has been subtracted from vertical force such that 0 N/kg represents body weight. Solid lines represent a 6th order regression through data for all animals (n = 13 for P1-2, n = 15 for P14). Dotted lines represent 99% confidence limits.
P1-2 RUNNING

P14 RUNNING

Force (N/kg)

vertical

fore-aft

proportion of stance phase

proportion of stance phase
Figure 2.6

Representative ground reaction force records and corresponding potential, kinetic and total energy estimates for a single running step (normalized velocity of 0.9) from P1 and P14 chicks, as a function of time. Force records \((a, b)\) are normalized to body mass, but energy traces \((c, d, e)\) are not. The same scale is used for both P1 and P14 animals. Note that, unlike Figure 3, potential \((c)\) and kinetic \((d)\) energy are in phase and are of the same order of magnitude and contribute similarly to total energy \((e)\).
P1 RUNNING

P14 RUNNING

<table>
<thead>
<tr>
<th>Force (N/kg)</th>
<th>time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>vertical</td>
<td>0.18 0.22 0.26 0.30</td>
</tr>
<tr>
<td>fore-aft</td>
<td></td>
</tr>
<tr>
<td>potential</td>
<td>0.0000 0.0010 0.0020 0.0030</td>
</tr>
<tr>
<td>Energy (J)</td>
<td>0.0080 0.0060 0.0040 0.0020</td>
</tr>
<tr>
<td>kinetic</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>0.0180 0.0140 0.0100 0.0060</td>
</tr>
</tbody>
</table>
Figure 2.7

Percent energy recovery as a function of normalized velocity for 3 - 5 steps from 12 chicks at P1 (filled triangles) and 3 - 5 steps from 8 chicks at P14 (open squares). Percent recovery is a measure of the total body energy recovered through exchange between potential and kinetic energy. Percent recovery is highest during walking by P14 chicks, lower during walking by P1 chicks and lowest during running by both groups of animals. Vertical dotted lines indicate the range of velocity over which the transition between running and walking occurs.
relative velocity

% energy recovery

transition

WALK

RUN

△ P1-2

□ P14

0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4

relative velocity

-34-
Kinematic changes during the first 2 weeks post-hatching

Analysis of stride lengths and joint angle changes indicated one mechanism by which P1-2 animals may reduce the vertical movement of the body. P1-2 walking chicks had significantly shorter stride lengths (normalized velocity = 0.38) than did P14 chicks (Figure 2.8). When stride length was measured at normalized running speeds (normalized velocity = 0.9), there were no differences between P1-2 and P14 chicks (Figure 2.8). Stride length determines, in part, the degree to which the centre of mass is lowered between the two legs, so that shorter stride lengths in P1-2 chicks may contribute to a reduction in the sinking of the centre of mass between legs.

During walking in both P1-2 and P14 chicks, the joint angle changes I observed were the same as those found by other investigators (Figure 2.9 and Johnston and Bekoff, 1992; Jacobson and Hollyday, 1982). The normal stride cycle can be divided into a stance phase when the limb is in contact with the ground and a swing phase when the limb is off the ground (Fig. 2.9). At the onset of the stance phase, the ankle flexes due to limb loading and reverts to extension by midstance. The avian knee begins to flex immediately prior to the onset of the stance phase and continues to flex throughout stance. The onset of the swing phase coincides with the onset of ankle flexion. Both knee and ankle flex as the limb is lifted from the ground and then both extend as the limb is moved rostrally. The ankle thus flexes and extends twice during each step cycle, similar to the pattern observed in mammals (Goslow, et. al., 1973). The knee in birds flexes and extends only once during each step cycle. This is in contrast to mammals where the knee joint flexes and extends during both the swing and stance phases (Goslow, et. al., 1973). Similar to mammals, the hip joint extends during the stance phase and flexes during the swing (Fig. 2A, 3A). In contrast to mammals, however, the hip joint moves very little in birds. It also moves very little relative to the amount of movement at the avian knee and ankle joints.
Figure 2.8

Average stride length during walking and running for P1 (filled bars) and P14 chicks (open bars).

Walking: P1-2, n=7; P14, n=5. Running: P1-2, n=5; P14, n=6. The asterisk indicates that the walking stride length of P1-2 chicks is significantly shorter than P14 animals, p<0.001.
Figure 2.9

Joint angles of the left leg during walking (normalized velocity = 0.27) versus the proportion of the step cycle, for P1-2 chicks (n = 9), and P14 chicks (n = 7). Bars indicate duration of stance phase for left leg. Solid lines represent a 6th order regression through data for all animals. Thin dotted lines represent 99% confidence limits. The asterisks indicate that knee and ankle angles are significantly smaller, at the onset of the stance phase, for P1-2 chicks when compared to P14 (p < 0.01).
Figure 2.10

Joint angles of the left leg during running (normalized velocity = 0.82) versus the proportion of the step cycle, for P1-2 chicks (n = 6), and P14 chicks (n = 7). Bars indicate duration of stance phase for left leg. Solid lines represent a 6th order regression through data for all animals. Thin dotted lines represent 99% confidence limits. There are no significant differences in the joint angle motion of P1-2 and P14 chicks during running.
Examination of the joint angle changes during walking at both ages showed that P1-2 chicks did not extend their knee and ankle at the beginning of the stance phase as much as P14 chicks (Figure 2.9). As extension of the knee is primarily responsible for the forward movement of the foot in birds (Jacobson and Hollyday, 1982), young birds did not move the foot as far forward during the swing phase. This naturally resulted in a shortened walking stride length. Differences in knee and ankle movement between P1-2 and P14 animals might also be expected to be accompanied by differences in hip movement. The body centre of mass is positioned over the knee and in front of the hip joint in birds, unlike in humans where both the hip and knee are beneath the centre of mass during stance (Jacobson and Hollyday, 1982). Small changes in hip angle in birds will alter the position of the knee joint under the centre of mass and therefore may have significant effects on balance. However, I found no statistically significant differences in the action of the hip between P1-2 and P14 animals. In addition, examination of joint angle changes during running by P1-2 and P14 chicks (Figure 2.10) revealed no significant differences for hip, knee or ankle at any stage of the stride.

One remaining question was why young birds took shorter steps than older birds while walking, but not while running. A possible explanation may be that young birds have not acquired sufficient balance to support the body on one leg while moving the opposite leg fully through the swing phase. Lack of muscular strength might also be considered as a cause for shorter strides except for the fact that running, which young chicks do well, requires more muscular output than walking. Young birds shortened the swing phase by placing the leg on the ground sooner, prior to full knee and ankle extension. Examination of the duration of foot contact with the ground during walking and running supported this suggestion. Duty factor (ratio of the stance phase to total stride duration) in P1 and P3 chicks was significantly more dependent on velocity than for P14 chicks (Figure 2.11). At lower velocities, each limb
Figure 2.11

Duty factor (duration of the stance phase divided by stride duration) as a function of normalized velocity for chicks at P1, P3, P5 and P14. Solid line represents a 1st order regression. Note that regression slopes for P1 and P3 chicks are significantly greater than for P5 and P14 (ANOVA of slopes: F = 7.57, p < 0.001). This indicates that duty factors of walking P1 and P3 chicks are greater than those of P5 and P14 walking chicks, but duty factor values do not differ with age during running. For all regressions, p < 0.05.
Normalized velocity vs. duty factor for different conditions:

- **P1**: 
  - Duty factor: 0.8 to 0.0
  - Velocity: 1.0 to 0.0
  - Slope: $m = -0.292$

- **P3**: 
  - Duty factor: 0.8 to 0.0
  - Velocity: 1.0 to 0.0
  - Slope: $m = -0.250$

- **P5**: 
  - Duty factor: 0.8 to 0.0
  - Velocity: 1.0 to 0.0
  - Slope: $m = -0.128$

- **P14**: 
  - Duty factor: 0.8 to 0.0
  - Velocity: 1.0 to 0.0
  - Slope: $m = -0.077$
Figure 2.12

Single support proportion of stance duration as a function of normalized velocity for chicks at P1, P3, P5 and P14. Solid line represents a 1st order regression. Note that slopes for P1 and P3 chicks are significantly greater than for P5 and P14 chicks. (ANOVA of slopes, F = 4.55, p < 0.01). This indicates that P1 and P3 chicks spend less time supported by one leg while walking when compared to P5 and P14 chicks. However, single leg support durations are similar during running at all ages. For all regressions, p < 0.01.
maintained contact with the ground for a longer proportion of the stride duration when compared to older animals. For example, at a relative velocity of 0.2, the duty factor for a P1 chick was approximately 0.7 (ie. 70% of the stride duration), whereas that for a P14 chick was 0.6 (60% of the stride duration). Figure 2.11 also shows that there was a gradual change within the first week of life before attaining the P14 pattern. Thus the relationship between duty factor and velocity for P5 chicks was intermediate between that for P1 and P14. The relationship between duty factor and velocity of P9 chicks (data not shown) showed no difference from that for P14 chicks.

A more detailed examination of the characteristics of the stance phase of each limb revealed that walking birds less than 5 days old spend significantly less time supported by a single leg than do older birds. When the duration of single leg support as a proportion of the stance phase of each leg was plotted against velocity, P1 and P3 birds had much shorter durations of single leg support during walking (Figure 2.12). For example, at a normalized velocity of 0.2, P1 chicks spent approximately 125 msec (40% of the stance duration) supported by a single leg, whereas P14 animals maintained single leg support for 200 to 300 msec (60% of the stance duration). The relationship between single leg support duration and velocity for P5 (and for P9, data not shown) chicks does not differ significantly from that for P14 birds. Single leg support durations at a run are similar for all age groups (approximately 100 ms or 90-100% of the stance duration).

Discussion

In summary, the data suggest that young birds (≤ P5) are less competent than older birds in maintaining single leg support while walking and take shorter steps to compensate. Young birds also walk with smaller vertical oscillations of the center of mass and consequently undergo smaller potential energy changes within each step. P14 chicks are able to conserve energy within each walking stride by exploiting the exchange between potential and kinetic energy, but younger chicks
are unable to conserve energy in this manner and thus walk less efficiently.

The fact that young chicks can run in an adult-like manner, but walk inefficiently, may be due to different actions of the limb in these two behaviours. During walking in adult animals, the leg is used as a rigid strut and the body rises and falls over each leg in succession (Heglund, et al., 1982). However, young chicks do not appear to effectively use the leg in this manner. I have shown that P1-2 chicks walk with shorter stride lengths than P14 chicks. If the leg was acting as a rigid strut in young animals, shorter stride lengths would cause a reduction in the vertical oscillations of the centre of mass and also a proportionate reduction in kinetic energy oscillations in the fore-aft direction. Thus, the magnitude of potential and kinetic energy oscillations within each step would be reduced but efficient exchange between the two forms of energy would be maintained. For example, a plot of potential energy as a function of kinetic energy (Figure 2.4) would show a slope comparable to that of walking P14 animals, although the line would be shorter. In contrast, the measured data from P1-2 animals display an entirely different slope in Figure 2.4, because young chicks walk in a manner that causes potential energy oscillations to be disproportionately small compared to oscillations in fore-aft kinetic energy. Therefore, young chicks do not appear to innately use their leg as a rigid strut, but learn to do so during the first week after hatching.

In contrast to walking, chicks appear to have an innate ability to run in an adult-like manner. The leg acts like a spring during running, storing elastic energy in the first half of stance and releasing the elastic energy as kinetic energy in the latter half of stance (Cavagna, et al., 1977). The rapid loading of the leg upon foot contact increases muscle tone by activating stretch reflexes, including monosynaptic stretch reflexes (Griffith, 1991). This stiffens the leg spring and allows efficient storage of kinetic energy as elastic energy in tendons and muscle. Monosynaptic stretch reflexes are thought to play less of a role during walking, in part because the time course of the walking stance phase is much longer than the duration of the monosynaptic reflex. Instead,
polysynaptic reflexes, involving type Ib, II and cutaneous afferents, may contribute to maintaining appropriate muscle stiffness during stance (Aniss, et al., 1992, Duysens, 1993, Pearson and Collins, 1993, Gossard, et al., 1994). Interestingly, it has been shown that while polysynaptic reflexes are reduced or absent in young children, monosynaptic reflexes are present and may even be hyperactive compared to adult reflexes (Dietz, 1987, Evans, et al., 1990, Vecchierini-Blineau and Guihneuc, 1982, 1981). Thus, the development of mature walking by chicks may depend, in part, upon the differential maturation of reflex pathways. Further studies will be required to examine developmental changes in reflex strength and/or timing and their potential effects during the ontogeny of bipedal locomotion.

The effect of size on bipedal locomotion

Gatesy and Biewener (1991) compared the locomotion of adult bipeds of varying sizes and found that small birds, such as the quail, walk with higher duty factors than humans and large birds, such as ostriches. Higher duty factors mean that small birds maintain contact with the ground for a longer proportion of the total stride duration. However, in contrast to the present developmental study, higher duty factors in small adult birds are the result of increased limb compliance. Small bipeds consistently have a crouched leg posture, where resting leg joint angles are smaller than those of large birds and humans. A similar effect can be imitated by humans walking or running with intentionally bent knees (referred to as 'Groucho running', McMahon et al., 1987). This increase in limb compliance results in a reduction of the vertical force when compared to a more normal upright gait at the same speed. Essentially, vertical movement of the centre of mass in Groucho running is reduced by carrying the entire body closer to the ground throughout the stride. Importantly, Groucho running also results in longer relative stride lengths when compared to upright human locomotion.
The present study showed that, in contrast with the results of adult bipeds, young animals initially utilize a walking gait where increased duty factors and decreased vertical forces are accompanied by a decrease in stride length. Comparisons of joint angles (Figure 2.9) also showed that Pl-2 chicks do not maintain a more crouched limb posture throughout the stride than P14 birds. Instead of carrying the body closer to the ground and taking longer stride lengths, as would be expected if young chicks were increasing limb compliance, Pl-2 chicks increase their stability by decreasing stride length.

Comparisons with the development of human locomotion

In children, independent locomotion begins to develop at approximately one year of age. The initial period of digitigrade walking is followed by a gradual transformation, over the following 1 to 2 years, to a more adult-like plantigrade pattern (Forssberg, 1985). Subsequent improvements in stability occur until at least 7 years of age (Sutherland, et al., 1988). Many of the changes seen during the first few weeks of a chicken’s life are comparable to the locomotor changes in children from 1 to 7 years. Children show an decrease in duty factor of 0.67 to 0.62 during development from 1 to 7 years (Sutherland, et al., 1988). The proportion of the stride cycle occupied by single leg support increases from 0.32 to 0.38 over the same time period. More importantly, alterations in the vertical forces generated by children show similar changes during development as those observed in the present study. Young toddlers, 1 - 4 years, show smaller peaks and less of a trough in the vertical force traces than 6-7 year olds. As in the present study, the reduction in vertical movement of a young child’s centre of mass results in a less efficient energy transfer during walking.

There are obviously some differences between avian and human locomotion which relate to differences in leg morphology and the production of plantigrade gait in humans. However, leg
morphology does not influence the general characteristics of ground-reaction force patterns, as these are common to the patterns produced by a variety of terrestrial vertebrates (Cavagna, et al., 1977).

Furthermore, plantigrade gait development in humans is normally restricted to the first 2 years of life, whereas developmental changes in duty factor and single leg support durations occur over a period of 6-7 years. Hence comparisons in the development of bipedal locomotion between birds and humans may be insightful.

It appears, then, that many of the changes which occur during human locomotor development also occur, albeit over a much shorter time period, during the early post-hatching development of chicks. These changes relate to common constraints inherent to bipedal locomotion, including the necessity for a period of stable single leg support. This constraint is most obvious during walking, where the action of the leg and the durations of single leg support differ from running gaits. Thus, contrary to conventional wisdom, perhaps we run before we walk!
Chapter Three:  
Asymmetric bipedal hopping -
an adaptation for hemiplegia in the chick

Introduction

The previous chapter demonstrated that posthatching chicks are capable of locomotor plasticity during normal development. This plasticity was evident after kinetic and kinematic analysis of unrestrained locomotion over a range of velocities. The present study employs the same gait analysis techniques to examine the effect of a spinal cord injury on locomotor behaviour.

Gait analysis has been used widely for examination of pathological locomotion in humans (Winter, 1985, 1993). Both kinetic and kinematic techniques have provided a detailed description of many abnormal locomotor patterns, including those resulting from hemiplegia (Finch and Barbeau, 1986; Olney, et al., 1994). One of the major goals of human gait analysis is to identify the cause or causes of the abnormal pattern so that more effective therapy can be directed towards restoration of lost function (Finch and Barbeau, 1986; Olney, et al., 1994). Identification of the cause of the abnormality is necessary because conditions such as hemiplegia in humans arise from several different pathological processes, including stroke, cerebral palsy or spinal cord injury (Olney, et al., 1986, 1987; Tesio, et al., 1985). Hence, in any group of patients there often exists a diverse and variable range of locomotor abilities. In contrast, the present study examines the locomotor abilities of a group of animals with the same deficit, ie. hemiplegia due to spinal cord hemisection. This allows me to draw conclusions regarding the common features of gait in spinal cord hemisected chicks and to discuss the adaptive strategies chosen by a bipedal animal when locomotor function of one leg is compromised. The gait adopted by hemisected chicks is compared to human hemiplegic gait and the asymmetric gaits of other animals.
The present study also differs from human gait studies in that I examine the locomotion of a group of hemisected animals over a range of velocities. In clinical gait analysis, the locomotion of each individual is examined at a single speed, usually the speed at which the patient moves most comfortably. This is because walking speed is often used as an indicator of overall gait performance, ie. to indicate the severity of the deficit and/or to monitor recovery (Olney, et al., 1994). In the present study, examination of the locomotion of hemisected chicks over a range of velocities allows me to distinguish between features of their locomotion which change with velocity and those which are less mutable. These results are discussed with respect to the implications for the flexibility of the locomotor system and constraints of bipedal locomotion.

Methods

Locomotor data were collected from 23 experimental (hemisected) and 10 sham-operated animals one day pre-operative and at 1, 2, 3, 5, 7, 9, 12 and 14 days post-operative. In order to examine the gait adaptations that chicks make to compensate for hemiplegia, this chapter compares pre-operative locomotor data with data collected 2 days post-operative, when most chicks were able to locomote. Chapter 4 will examine locomotor data throughout the 14 day recovery period. Kinetic and kinematic measurements during unrestrained overground locomotion were made according to the methods outlined in Chapter 2. For 8 experimental animals, videotape records were made from both the right and left sides as the animals moved past the camera, for each day of data collection pre- and post-operative.

Surgery: Three-day-old hatchling chicks were anaesthetized with 50 mg/kg of ketamine (Ketalean, MTC Pharmaceuticals) and 10 mg/kg of xylazine hydrochloride (Rompun, Chemagro Ltd.) injected intramuscularly. Chicks were maintained under surgical anaesthesia, defined by lack of withdrawal to noxious stimuli (wing pinch), for the duration of the operation. Under aseptic
conditions, a skin incision was made over the lumbar and lower thoracic spinal column and muscles overlying the column were bluntly dissected. A bilateral laminectomy was performed on thoracic column segments 5-6. Hemostasis was achieved using small pledgets of gelfoam soaked in epsilon aminocaproic acid (Amicar, Lederle). The left lateral cord was severed at the sixth thoracic segment using a fine dissecting knife. A pin was inserted vertically at the midline of the cord to the bottom of the vertebral canal and passed laterally to ensure completeness of the hemisection. The skin was sutured over the laminectomy site and the chick was returned to the brooder to allow recovery from the anaesthetic. Sham-operated chicks underwent the same procedure except that no damage was done to the spinal cord.

**Neuroanatomical studies:** After behavioural testing was completed 14 days post-operative, chicks were anaesthetized with ketamine and xylazine as above and 1-2 ul of rhodamine-conjugated dextran amine (25% in 5% Triton-X) (Molecular Probes, Inc.) was injected into the lumbar cord. Chicks were allowed to recover and 48 hours after injection were overdosed with sodium pentobarbital and perfused intracardially with 20-30 mls of 10% heparinized saline (37 C) followed by cold 4% paraformaldehyde. The spinal cord and brain were postfixed for 24 hours in 4% paraformaldehyde and then placed in 30% sucrose in 0.1 M phosphate buffered saline. After 24 hr, brains and spinal cords were cut transversely into 30 um sections on a cryostat and mounted on slides. Brain sections were examined using a Zeiss Axioptep epifluorescence microscope equipped with standard filter blocks. Spinal cord sections at the site of hemisection were stained with thionin and examined with standard light microscopy.

**Kinetic and kinematic data analysis:** Ground reaction forces were analyzed according to the methods outlined in Chapter 2, with the following exception. The kinetic energy of the centre of mass for hemisected animals includes both the horizontal kinetic energy and the vertical kinetic energy. This differs from the calculation of kinetic energy for control animals, which includes only
horizontal kinetic energy, as vertical kinetic energy is negligible in normal animals. As shown in Fig. 3.4, this difference is because the vertical motion of the centre of mass is relatively large in hemisected animals and minimal in control animals (see also Chapter 2, methods). Thus vertical kinetic energy contributes significantly to the total kinetic energy of the body in hemisected animals, in contrast to control animals, and therefore needs to be included in the calculation of total kinetic energy.

Kinematic variables were obtained from single frame analysis of videotape as previously described. In addition to the variable defined in Chapter 2, the following measurements were used in this study:

1. Stride frequency, the number of strides per second, is the inverse of stride duration.
2. Swing duration is the time within each stride that the leg is non-weightbearing.
3. Double leg support time is the time during which both legs are weightbearing simultaneously.
   Double leg support times are distinguished as to whether the transfer of weight occurred from right to left leg or from left to right leg. Negative values of double support time indicates the duration of time that neither leg is weightbearing, i.e. the animal was jumping from one leg to the other.
4. Right and left temporal phase delay were calculated as the time during the stride cycle of one leg when the contralateral leg first contacts the ground, and presented as a proportion of the total stride time.
5. Step length was calculated as the horizontal distance between right and left metatarsophalangeal joints (located in the middle of the foot) when both legs were weightbearing. Right step length was the distance between the two feet when the right foot was in front, left step length was the distance between feet when the left foot was in front.
6. Relative left step length is the left step length divided by total stride length.
Statistical analysis was carried out according to methods outlined in Chapter 2. Briefly, least
squares regression was used to analyze the relationship between kinematic variables and stride
duration or velocity. Analysis of variance was used to compare the slopes of regression lines.

Results

At least 80% of the left half of the spinal cord was severed in all animals used in this study
(Figure 3.1). In those chicks in which 20% of the hemicord remained intact, the only spinal
pathways partially preserved were found within the dorsal columns (Figure 3.1B). In all cases, the
dorsolateral, lateral and ventral funiculi were completely disrupted. This is comparable to the degree
of damage caused by hemisection in other studies (Helgren and Goldberger, 1993). Further
confirmation of disruption of brainstem-spinal pathways was obtained by examining the retrograde
labelling of brainstem-spinal cells (Figure 3.2). As in other vertebrates, the rubrospinal tract of
birds is known to travel in the dorsolateral funiculus and to project unilaterally to the contralateral
lumbar cord (Webster and Steeves, 1988). For each chick used in this study, fluorescent dye
injected bilaterally into the lumbar cord only labelled rubrospinal neuron cell bodies ipsilateral to
the hemisection (Figure 3.2), confirming that the hemisection had disrupted the left hemicord, and
that there was no regeneration of brainstem-spinal projections. There were no consistent behavioural
differences detectable between animals regardless of whether the hemisection severed 80 or 100% of
the left thoracic cord.
Figure 3.1

Photomicrographs of cross sections of the left thoracic hemisection site from two animals used in this study. A) shows a complete hemisection while arrow in B) shows that portions of the left dorsal columns were undamaged in some animals. Bar length = 500 microns.
Figure 3.2

Transverse section at the level of the midbrain in a chick subjected to left thoracic hemisection 2 weeks previously. Rhodamine-labelled neurons are present in the left red nucleus (undamaged by left hemisection) (A), while no labelled neurons are present in the right red nucleus (axotomized by left hemisection) (B). Thus, axotomized rubrospinal neurons do not regenerate after left thoracic hemisection. Bar length = 50 microns.
Effect of hemisection on walking behaviour

Twenty-four to 48 hours after surgery, the hemisected animals could stand and supported most of their weight on the right leg (contralateral to the hemisection). The left leg was held abducted and positioned caudal to the body when standing. Inability to flex the left leg resulted in failure of the chick to right itself when lying on the left side. Ten chicks could stand and walk 24 hours post-operative, 6 walked after 48 hours and the remaining 7 chicks could walk within 72 hours. The data in this study was obtained from the 16 animals which were walking by 48 hours post-operative. All chicks moved overground with a visibly asymmetric gait. In brief, chicks would step from the left leg onto the right leg normally, but hopped from the right limb onto the left limb. Closer examination revealed that the swing phase of the left leg was shortened because the leg failed to move rostrally during the latter part of the swing phase (Fig. 3.3B). The left limb was thus placed beneath the body center of mass at the beginning of stance, rather than ahead of the center of mass as in unoperated control or sham-operated animals.

Effect of hemisection on ground reaction forces

The ground reaction force patterns of hemisected chicks showed gross similarities to the patterns produced by unoperated control animals, with notable exception that both right and left legs of hemisected animals were required to simulate the pattern of a single leg in walking control animals (Fig. 3.4). In the control pattern, two vertical force peaks occur during the stance phase of each leg. Each of these peaks occur concurrently with successive peaks in fore-aft force, first in the decelerative direction and then in the accelerative direction (Fig. 3.4). In hemisected animals, a distinctive feature of the force patterns was that all decelerative fore-aft force was produced by the right leg (contralateral to the hemisection), while accelerative fore-aft force was produce mainly by the left leg (ipsilateral to the hemisection) (Fig. 3.4). The peak of decelerative force produced by the right leg in hemisected animals coincides with a peak in
Figure 3.3

Limb positions of chick walking 1 day prior to left thoracic cord hemisection (A), 1 day after hemisection (B). From left to right, each figure illustrates 1) the end of stance phase for left leg, 2) mid swing of left leg, 3) beginning of stance phase for left leg, and 4) mid stance for left limb. In (B), the left leg is not moved rostrally during the swing phase which results in placement of the leg directly beneath the body instead of in front of the body (compare A and B).
vertical force. This vertical peak in early stance of the right leg is significantly higher than peak vertical force for control animals. Vertical force generally showed a second peak during right leg stance which was significantly smaller than the first. During this second peak, little to no fore-aft force was produced. Transfer of weight from right to left leg in hemisected animals occurred with a hop, as demonstrated by the large decrease in vertical force between stance phases of the right and left legs (Fig. 3.4). A single vertical force peak was then produced during the stance phase of the left foot in hemisected animals, in contrast to the two vertical force peaks of control animals. This single vertical peak occurred concurrently with the single peak of accelerative fore-aft force. Subsequent transfer of weight from the left to right leg was always accompanied by a period of dual weight support by both right and left legs in hemisected chicks.

Energetic analysis of hemisected gait

Integration of ground reaction force traces demonstrates that the gait adopted by hemisected animals roughly approximates the pattern of energy oscillations occurring in a normal walking gait (Fig. 3.5 c and d). In short, kinetic and potential energy oscillations cycle out of phase with each other in both control and hemisected animals. However, kinetic and potential energy oscillations undergo a complete cycle twice per stride in control animals but only once per stride in hemisected animals. In control animals, potential energy reaches a maximum in midstance of each leg, when kinetic energy is at a minimum (Fig 3.5c and d, see also Chap. 2, Figure 2.3c and d). In the hemisected animal, potential energy reaches a maximum during the transition from right to left leg support (Fig. 3.5c). The body centre of mass in hemisected animals is highest at this point because the animal hops from the right to the left leg. Kinetic energy in the hemisected animal decreases to a minimum early in right stance and remains at a minimum until left leg stance (Fig. 3.5d). During left leg stance, kinetic energy begins to increase, mainly due to the accelerative fore-aft force produced at this time, while potential energy is decreasing (Fig. 3.5c and d).
Figure 3.4

Representative ground reaction forces for one complete stride (2 steps) plotted against time for control chick (P10) and for 2 complete strides (4 steps) for hemisected chick, 2 days post-operatively. Normalized velocity for control chick = 0.42, for hemisected chick = 0.38. Solid bars represent stance duration for right (R) and left (L) legs. Forces have also been normalized for body mass, and body weight has been subtracted from vertical force such that 0 N/kg represents body weight. Note the similar pattern of the fore-aft force when 2 steps of control walking are compared to 4 steps of hemisected gait.
CONTROL

HEMISECTED
2 DAYS POST-OP

Force (N/kg)

vertical

0.10 0.20 0.30 0.40 0.50 0.60

0.32 0.44 0.56 0.68 0.80 0.92

time (seconds)
time (seconds)

fore-aft

0.10 0.20 0.30 0.40 0.50 0.60

0.32 0.44 0.56 0.68 0.80 0.92

time (seconds)
Closer examination of kinetic and potential energy oscillations in hemisected animals reveals several important differences between the control and hemisected gait. Total energy changes during each control stride (Fig. 3.5e) were reduced because potential and kinetic energy changes not only oscillate out of phase but are also of similar magnitude. However, total energy changes within each hemisected stride (Fig. 3.5e) mainly reflect potential energy oscillations because these oscillations were disproportionately larger than changes in kinetic energy. This is also in contrast to the findings discussed in Chapter 2, where changes in potential energy were disproportionately smaller than kinetic energy changes in young chicks.

The differences in energy oscillations between control and hemisected animals is demonstrated more clearly when potential energy is plotted as a function of kinetic energy (Fig. 3.6). Data from a single walking step in control animals demonstrates the smooth alternation between potential and kinetic energy (Fig. 3.6, thin solid line). The body centre of mass rises and slows during the initial half of stance and then falls and speeds up in the latter half of stance. In contrast, energy data from the hemisected stride shows several characteristics which indicate less than optimal energy transfer (Fig. 3.6, thick solid line). In early right leg stance, both kinetic and potential energy are decreasing, because the centre of mass is moving downward while the forward motion of the body is abruptly slowed (Fig. 3.6, segment of thick line located below the horizontal axis). Energy cannot be exchanged during this segment because both forms of energy are decreasing simultaneously, as occurs in a normal running step (Fig. 3.6, dotted line and Fig. 2.3). Subsequently, potential energy increases while kinetic energy does not change (Fig. 3.6, vertical segment of thick line). This occurs as the centre of mass rises upward during right leg stance while forward velocity does not change. Similarly, energy exchange cannot occur during this segment of the stride because only potential energy is changing. At the end of right leg stance, the animal jumps from the right to the left leg, so
Figure 3.5

Representative ground reaction force records and corresponding potential, kinetic and total energy estimates for a single walking step (normalized velocity of 0.38) from control chick (P10) and hemisected chick (2 days after hemisection) as a function of time. Force records and energy traces are normalized for body mass. The same scale is used for both animals. Note that, in general, kinetic and potential energy are out of phase for both control and hemisected animals. In the control animal, the proportionate changes in potential (c) and kinetic (d) energy serves to reduce the change in total energy (e). However, energy conversion is not as efficient for the hemisected chick, as evidenced by the disproportionately large contribution of potential energy (c) to total energy (e).
CONTROL

HEMISECTED
2 DAYS POST-OP

Force (N/kg)

vertical

0 4 8

-4 -8

0.27 0.32 0.38 0.43 0.49

time (sec)

0.20 0.25 0.30 0.35 0.40

time (sec)

fore-aft

0 2 4 6

-2 -4

R L

Energy (J/kg)

potential

0.02 0.04 0.06 0.08 0.10

0.06 0.08 0.10

0.02 0.04 0.06 0.08

total

0.02 0.04 0.06 0.08 0.10

-0.02 0.02 0.04

-0.02 0.02 0.04
that the height of the centre of mass, and therefore potential energy, increases to a value greater than
that reached in control walking. The centre of mass then falls throughout left leg stance and forward
velocity increases. It is only during this latter portion of the stride that energy can be conserved by
conversion of potential energy to kinetic energy. However, energy conversion is not entirely smooth
because kinetic energy undergoes several small oscillations during the initial stages of left leg stance
(see also Fig. 3.5D). This represents the contribution of the vertical kinetic energy of the body. It is
here that the centre of mass undergoes the most dramatic vertical changes in motion, ie. the animal
is reaching the height of the jump and beginning to fall. Subsequently, it is only during mid to late
left stance that energy transfer from potential to kinetic occurs smoothly.

Consistent with the above results, analysis of a number of strides from hemisected animals
showed that they were not as effective in recovering energy through exchange between potential and
kinetic forms as were control animals (Fig. 3.7). Hemisected animals recovered less than 40% of the
energy, compared to 80% in P14 animals. Most of the recovery in hemisected animals likely
occurred during the stance phase of the left leg, as discussed above. However, the amount of energy
recovery during hemisected gait is comparable to that recovered by young (Pl-2) animals during
normal walking (Fig. 3.7 and Fig. 2.7).

Kinematic analysis of hemisected gait

Analysis of the relationships between stride length, stride frequency and velocity shows that
increases in velocity are attained by increases in both stride length and stride frequency. Compared
to control animals, hemisected animals use similar increases in stride length and frequency to
increase speed (Fig. 3.8, compare A with B, and C with D). The slopes of the regression lines in
Fig. 3.8A and B are not significantly different, nor are the regression slopes in C and D significantly
different. However, hemisected animals do use slightly shorter stride lengths overall when compared
to control animals (Fig 3.8A and B; y intercept = 9.76 for control, 6.61 for hemisected, p < 0.0001).
Figure 3.6

Potential energy as a function of kinetic energy, for a complete stride of a hemisected animals from Fig. 3.4, and for single steps of control animals taken from Figures 3.4 and 2.6. Both potential and kinetic energy have been normalized for body weight and initial values have been set to zero to facilitate comparison between hemisected and control animals. Note that the data of control walking animal shows efficient alternation between kinetic and potential energy, while both forms of energy increase and decrease simultaneously during control running. In contrast, data from the hemisected animal shows asynchronous changes in kinetic and potential energy throughout the stride (see text for details).
CONTROL - WALKING

CONTROL - RUNNING

HEMISECTED - HOPPING

kinetic energy (J/kg)

potential energy (J/kg)
Figure 3.7

Percent energy recovery as a function of normalized velocity for P1, P14 and hemisected animals, 2 days after hemisection. Percent recovery is a measure of the total body energy recovered through exchange between potential and kinetic energy. Percent energy recovery in hemisected animals is comparable to the amount of recovery in P1 walking chicks, but not as great as P14 animals. Vertical dotted lines indicate the range of velocity over which the transition between running and walking occurs in control animals.
Hemisected animals also use slightly higher stride frequencies at any single velocity compared to control animals (Fig. 3.8 C and D; y intercept = 1.30 for control, 2.01 for hemisected, p < 0.01).

Characterization of the degree of asymmetry in hemisected gait requires analysis of interlimb timing variables. Instead of a symmetrical 50:50 phase relationship between left and right foot contact (Fig. 3.9, control), hemisected animals use on average a 35:65 phase relationship which does not change with velocity (Fig. 3.9). Analysis of duty factor shows that the right leg spends proportionately more time in stance during hemisect gait than during control walking, especially at slow speeds (Fig. 3.10A and B, regression slope for data of right leg = -0.173 for control, -0.321 for hemisect, p<0.0001. Y-intercept = 0.673 for control, 0.755 for hemisect, p<0.001). Similar to control animals, the duty factor of the right leg decreases with increasing speed in hemisected animals. However, duty factor of the left leg during hemisect gait is approximately 50% of the cycle duration and changes very little with speed.

The differences between stance duration of the right and left legs of hemisected animals is illustrated clearly when stance and swing duration are plotted against stride duration (Fig. 3.11). In control animals, as in all terrestrial animals including humans, the stance duration of each limb increases with increasing stride duration, whereas the duration of the swing phase is more independent of stride duration. During hemisected gait, the stance and swing durations of the right leg show similar relationships with stride duration as control animals (slopes of the corresponding regression lines in Fig 3.11 A and C are not significantly different). For the left leg, however, both stance and swing duration increase at the same rate with increasing stride duration. This symmetrical relationship between stance and swing durations results in the relatively constant duty factor of 0.50 for the left leg seen in Fig. 3.10.
Figure 3.8

Stride length (A and B) and stride frequency (C and D) as a function of normalized velocity for P5 control chicks (A and C) and hemisected chicks, 2 days post-operative (B and D). Solid lines represent a 1st order regression for each set of data. Note that, while regression slopes in A and B are not different, Y-intercept in B is significantly smaller than in A (p < 0.0001). Similarly, regression slopes in C and D are not different, but Y-intercept in D is significantly greater than in C (p < 0.01). This indicates that hemisected chicks at 2 days post-operative use slightly shorter stride lengths and higher stride frequencies than do age-matched controls at equivalent velocities. For all regressions, p < 0.05.
CONTROL

HEMISECTED
2 DAYS POST-OP

A

y-int = 9.76

B

y-int = 6.61

C

y-int = 1.30

D

y-int = 2.02

stride length (cm)

stride frequency (s⁻¹)

normalized velocity

normalized velocity
Figure 3.9

Temporal phase delay as a function of normalized velocity for P5 control chicks and hemisected chicks, 2 days post-operative. Temporal phase delay is the proportion of the stride duration at which the stance onset of the right leg begins after the stance onset of the left leg. Note that, while the stance of the right leg occurs in the middle of the stride cycle in the control animal (0.5), right stance onset occurs approximately at 0.35 of the stride cycle for hemisected chicks. Phase delay does not change with velocity for both control and hemisected chicks.
Figure 3.10

Duty factor (duration of the stance phase divided by stride duration) as a function of normalized velocity for P5 control chicks and hemisected chicks, 2 days post-operative. Solid lines represent 1st order regression for each set of data. Note that the regression slope of the left leg of hemisected animals is significantly smaller than right or left leg in control animals (p < 0.01). The regression slope of the right leg in hemisected animals is also greater than for right or left legs of control animals (p < 0.001). Regression slopes for the right and left legs of control animals do not differ. This indicates that stance durations of the left leg are significantly shorter than normal, and stance durations of the right leg are greater than normal, especially at slower speeds. For all regressions, p < 0.05.
Figure 3.11

Durations of stance and swing phases as functions of stride duration, for left leg of P5 control animals (A), left leg of hemisected animals (B), and right leg of hemisected animals (C), 2 days post-operative. Solid lines represent 1st order regression through each set of data. For A and C, stance duration increases with increasing stride duration, while swing duration changes little with stride duration. The slopes of corresponding lines A and C are not significantly different. Note that, for the left leg of hemisected animals (B), both stance and swing duration change in a similar manner with stride duration, and regression slopes are significantly different from slopes in A and C (p < 0.001). For all regressions, p < 0.05.
Asymmetry in hemisected gait is also evident in the step lengths of hemisected animals. Hemisected animals take a large step with the right leg, such that the right leg is placed rostral to the body, whereas left leg is placed on the ground directly beneath the body. The step length of the right leg is therefore significantly larger than the left step length in hemisected animals and is also slightly larger than control step lengths during walking at equivalent speeds (Fig 3.12, y intercept = 5.04 for control, 6.17 for right hemisect, p = 0.0144). The left step length of hemisected animals is significantly shorter than control step lengths (p < 0.0001). Left step length also shows a different relationship with velocity than does right hemisected step length. Left hemisected step length increases faster with velocity (slope of regression line = 6.817 for left leg, 3.67 for right leg, p < 0.0001). This has the effect of reducing the differences between right and left step lengths in hemisected gait at higher velocities, and thus step lengths become more symmetrical at higher speeds (Fig. 3.13).

Effect of hemisection on joint angle motion

It is evident from the above kinetic and kinematic results that the right and left legs are used differently to accomplish overground locomotion after hemisection. Analysis of left joint angle motion shows a reduced range of motion at both the knee and ankle joints (Fig. 3.14B). The reduction in knee range was due to decreased knee extension during the latter part of swing and initial part of stance (Fig. 3.14B). The reduction in ankle movement was due to decreased flexion during swing (Fig. 3.14B). The action of the left ankle during the stance phase also changed. Instead of flexing with the onset of weightbearing, as occurs in control walking, the ankle angle was maintained at a constant angle or actively extended. This suggests excessive activation of ankle extensors upon foot contact, possibly due to hyperreflexia after loss of descending input.
Figure 3.12
Step length of right and left legs as a function of normalized velocity for P5 control and hemisected chicks, 2 days post-operative. Solid lines represent 1st order regression through each set of data. Note that the regression slope of the left leg of hemisected chicks is greater and the Y-intercept is significantly smaller than for control animals (p < 0.001). This indicates that the left step length is much shorter than control at low speeds, but increases more with increasing speeds. The regression slope for the right leg of hemisected chicks does not differ from control, although the Y-intercept is greater than control, indicating that the right step lengths of hemisected chicks are slightly larger than control at all speeds (p = 0.014). For all regressions, p < 0.05.
CONTROL  

HEMISECTED  
2 DAYS POST-OP

- Right leg  
- Left leg
Figure 3.13

Relative left step length (left step length divided by stride length) as a function of normalized velocity. Solid line represents 1st order regression. Control animals show symmetry between right and left step lengths at all speeds. Note that the relative left step length of hemisected animals increases with increasing speed, indicating that right and left step lengths become more symmetrical at higher speeds.
Figure 3.14

Leg joint angles during walking plotted against percent cycle duration for hip, knee and ankle one day prior to hemisection (A), and 2 days after left thoracic cord hemisection (B and C). Joint angles of the left leg are shown in (A and B) and joint angles of right leg are shown in (C). Bars indicate duration of stance phase. Solid lines represent 6th order regression line through data for all animals (n = 12 for control animals, n = 8 for hemisected animals) (see methods). Thin dotted lines represent 99% confidence limits.
Joint angle motion in the right limb (contralateral to the hemisection) also showed minor
cchanges when compared to control animals. Range of right knee motion was reduced, due to
decreased flexion of the knee in both stance and swing, although the pattern of knee joint motion is
maintained (Fig. 3.14C). Joint angle motion of the ankle was not significantly differed from control.
Sham-operated animals showed no locomotor deficits after surgery and, in all respects, were similar
to age-matched, unoperated animals. For both the right and left legs, hip motion of hemisected
animals was not significantly different from control.

Discussion

Comparisons with human hemiplegic gait

The asymmetric gait adopted by spinal cord hemisected chicks share some features with human
hemiplegic gait. Hemiplegics commonly walk with reduced stance time on the affected leg, and
increased stance time on the unaffected leg. They also show reduced stride length (Mizrahi, et al.,
1982), and reduced joint angle excursions on the affected leg (Olney, et al., 1994; Murray, 1967).
These features were all evident in the gait of hemisected chicks. Another common feature of human
hemiplegic gait is the reduced gait speed and reduced range of speeds used by the patient (Mizrahi,
et al., 1982; Olney, et al., 1994). This reduced gait speed has the effect of decreasing the kinetic
energy changes of the body centre of mass within each stride. At the same time, potential energy
changes are often larger than normal in some hemiplegic patients because the affected limb cannot
be flexed adequately during the swing phase. This necessitates movement of the body upwards, with
a hip hike or a hop, so that the affected limb can clear the ground (Tesio, et al., 1985; Olney, et al.,
1986). The result is disproportionately large potential energy changes compared to kinetic energy
changes, and thus smaller percentage energy recovery (Olney, et al., 1987). Hemisected chicks in
this study show the same disproportionately large potential energy changes (Fig. 3.6).
Detailed within-stride examination of energy oscillations of human hemiplegic gait also shows close similarities with the gait adopted by hemisected chicks. The propulsive force produced by the affected limb can be close to normal in hemiplegic patients (Tesio, et al., 1986; Carlsoo, et al., 1974). Humans hemiplegics tend to lean the trunk forward during the stance of the affected limb (Finch and Barbeau, 1986), similar to chicks falling onto the right limb during left limb stance. Like hemisected chicks, human hemiplegics saved more energy during the stance phase of the affected limb than during the stance phase of the unaffected limb (Tesio, et al., 1986 and Fig. 3.6). This pattern of energy exchange may be due to the inability of the affected limb to do muscular work (Tesio, et al., 1986). Therefore, a common locomotor strategy in hemiplegic bipeds, both bird and human, may be the production of a gait which allows mechanical energy exchange during the stance of the affected limb.

Comparisons with other assymmetrical gaits

The asymmetric gait of hemisected chicks also bears some resemblance to the hopping gait of birds in the family *Corvidae* (crows, ravens and magpies). These birds walk symmetrically at low speeds but hop at higher speeds (Hayes and Alexander, 1983). However, unlike the symmetrical or "in-phase" hopping of most birds (eg. sparrows, finches) the hopping of *Corvidae* has been described as "out-of-phase", in that one leg is placed ahead of the other and there is a delay in the timing of alternate foot contact (Hayes and Alexander, 1983). Thus, the hopping gait of *Corvidae* resembles the gait of hemisected chicks, although there are a few differences. The average phase delay for hopping *Corvidae* was 0.25, and there was a tendency for the delay to decrease with increasing velocity. In addition, stride length increased with velocity, as occurs in hemisected chicks, but stride frequency did not change significantly with velocity. In contrast, relative phase delay averaged 0.35 in hemisected chicks and did not change with velocity. In addition, both stride length and stride frequency increased with velocity in hemisected chicks. Comparisons between these two
studies should be made with caution, however. The number of animals in the Corvidae study was very small (n = 1 from each of 4 species) and there were also marked differences in velocity range over which these studies were undertaken. Species of Corvidae hop over a range of relative velocity from 0.9 to 2.5, whereas the velocity range in the present study was 0.2 to 1.0, a range over which crows would normally walk symmetrically rather than hop.

Humans can also hop asymmetrically, using a gait which has been described as a gallop (Whitall, 1989). Interestingly, the phase delay naturally adopted by children (3 to 10 years old) and adults moving at a gallop is 0.31, with a variation of less than 4%. This value is similar to the 0.35 delay used by hemisected chicks. The degree of asymmetry of step length during galloping in humans was approximately 0.40, which was also constant for both children and adults. Corresponding values for hemisected chicks ranged from 0.2 at slow speeds to 0.5 at higher speeds (Fig. 3.13). Galloping in humans has not been examined at different velocities, so it is unclear whether the phase delay or the degree of step length asymmetry are affected by galloping speed. Step length asymmetry did show greater variation (up to 8%) than phase delay in humans (Whitall, 1989) and it is possible that this reflects some variation with galloping speed.

The energy exchanges occurring during locomotion in hemisected chicks resemble the energy exchange during asymmetrical quadrupedal gaits such as galloping (Cavagna, et al., 1977). Galloping quadrupeds use the fore- and hindlimbs asynchronously. At slower galloping speeds, potential and kinetic energy exchanges occur at 2 stages in the stride (Cavagna, et al., 1977). During the stance phase of the hindlimbs, the animal falls forward onto the forelimbs, losing potential energy but gaining kinetic energy. Energy recovery also occurs during the stance phase of the forelimbs, when the speed of forward progression, and thus kinetic energy, decreases. At the same time, the body rises up over the forelimbs, so that potential energy is gained as kinetic energy is lost. During the remaining portion of the stride, the animal is airborne. Both kinetic and potential
energy are high at this point and efficient exchange is not possible.

Quadrupeds are able to recover up to 30% total energy with each galloping stride (Cavagna, et al., 1977). This is comparable to the recovery obtained during asymmetric hopping in hemisected chicks (Fig. 3.7). Most of the energy exchange during asymmetrical hopping in chicks occurs during the stance phase of the left foot, when the body is falling forward onto the right foot. This is analogous to the exchange occurring during hindlimb stance in quadrupeds. However, during the initial stance phase of the right leg, hemisected animals continue to fall while simultaneously producing a large braking force and thus slowing down. The concurrent decrease in both potential and kinetic energy at this point prevents efficient energy exchange. Even the subsequent gain of potential energy during the right stance phase (analogous to the gain in potential energy during forelimb stance in quadrupeds) is not associated with loss of kinetic energy because most of the decrease in kinetic energy has occurred early in right stance. The precise timing of oscillations in kinetic and potential energy during overground locomotion are therefore important in determining the amount of energy exchange occurring within each stride.

**Velocity-dependent changes in asymmetric bipedal hopping**

Gait speed is considered to be an important indicator of gait performance in the analysis of hemiplegic gait and is often monitored to evaluate recovery (Andriacchi, et al., 1977; Brandstater, et al., 1983). There is some controversy over the usefulness of gait speed as a diagnostic measurement however, because many temporal and distance measurements, including speed, are interdependent (Olney, et al., 1994). It is therefore difficult to determine whether differences in gait speed are the cause or the result of differences in certain timing and/or distance variables.

Analysis of the gait of hemisected chicks over a range of velocities shows that some features of the gait change with velocity and some features remain constant at all speeds. Stride length and stride frequency both increase with velocity in hemisected chicks, a relationship that is common to
both control and hemisected animals (see the following section, *Strategies for overground locomotion*...). In contrast, there are marked differences between control and hemisected animals when right and left legs are compared. Both temporal and distance variables are asymmetric in hemisected animals (Fig. 3.9, 3.13). However, as speed increases, distance measurements become more symmetric, while temporal asymmetries do not change. As hemisected chicks move faster, the asymmetry in step length decreases because the step length of the left leg increases faster than the step length of the right leg (Fig. 3.12). Throughout the range of speeds examined in this study, right step length contributes more to increases in speed than does left step length. However, at higher speeds, the step length of the right leg is limited because the animal maintains ground contact with the left leg as the right leg steps forward. To increase stride length further, the animal therefore needs to increase left step length. Observations of ground reaction force records and videotape shows that as hemisected chicks move at high speeds, they produce propulsive force with the right leg (in addition to decelerative force) and jump forward as well as upward during the transfer of weight from the right to the left leg. In this way, they are able to increase left step length and reduce step length asymmetry.

In contrast to step lengths, the asymmetry in right and left leg timing remains constant at all speeds (Fig. 3.9). Duty factor of the left leg also remains relatively constant as velocity increases, with the left leg spending equal amounts of time in stance and swing (Fig. 3.10, 3.11). This same 50:50 relationship between stance and swing duration also occurs during locomotor behaviours which involve reduced afferent input, such as swimming (Johnston and Bekoff, 1992), and during walking in chicks whose limbs have been deafferented (Bekoff, *et al.*, 1987). Interestingly, removal of descending input by spinal cord transection also results in more equal stance and swing durations, although to a lesser extent than deafferentation (Bekoff, *et al.*, 1989). It has been suggested that a 50:50 relationship between stance and swing represents a "basic" element of the
limb pattern generating circuit in the spinal cord (Bekoff, et al., 1987). This basic relationship is normally modified by both segmental afferent and descending inputs in the intact animal (see Fig. 3.11, control) but returns when these inputs are removed. However, it is unclear in the present study whether the 50:50 relationship between stance and swing durations of the affected left leg is due to the emergence of a basic spinal pattern or is a function of the minimum time that the affected leg must spend in stance in order to generate effective overground locomotion.

**Strategies for overground locomotion and the role of central pattern generation**

Chicks compensate for hemiplegia due to spinal cord hemisection by adopting a markedly asymmetrical gait which nevertheless maintains some kinetic and kinematic similarities with normal symmetrical locomotion. Those features of the gait which are maintained after hemisection are those which are required to achieve overground locomotion at a constant speed. For example, all forms of limbed terrestrial locomotion require accelerative force to be exerted against the ground at some point in the stride to provide propulsion in the forward direction. Accelerative force must alternate with decelerative force so that forward motion of the body is controlled and a constant speed is maintained. This force pattern, deceleration alternating with acceleration, occurs within each single hemisected stride as it does in each step of control locomotion (Fig. 3.4).

The pattern of vertical force exerted on the ground during terrestrial locomotion is also subject to certain limitations, namely that bodyweight of the animal is supported. Vertical force for any gait necessarily fluctuates around a constant value (equivalent to bodyweight) within each stride. However, the vertical force pattern does differ between gaits, as illustrated by the patterns obtained during running (Fig. 2.5) compared to walking (Fig. 2.3). Control and hemisected animals show gross similarities in the pattern of vertical force (Fig. 3.4). As a function of these general similarities, potential energy increases and decreases within each hemisect stride, comparable to each step in control animals (Fig. 3.5). However, vertical force patterns do differ more than the
fore-aft forces, both qualitatively and quantitatively, and a detailed analysis of potential energy changes reveals important differences from the control pattern (Fig. 3.7).

The requirements of overground locomotion also influence the manner in which stride length and frequency changed with velocity in hemisected and control animals (Fig. 3.8). Alterations in stride length and/or frequency are the only mechanisms available to change the speed of locomotion in all animals. Velocity-dependent increases in stride length and frequency often occur smoothly over gait transitions, eg. from walking to trotting to galloping in quadrupeds (Heglund and Taylor, 1988). This indicates that the pattern of interlimb coordination in quadrupeds does not necessarily affect these parameters. Similarly, it appears that marked differences in interlimb coordination between control and hemisected chicks do not affect the relationship between stride length or frequency and velocity.

Posthatching chicks can therefore produce effective overground locomotion in spite of partial disruption of CNS connections. The neural circuitry controlling locomotion in chicks is obviously highly flexible, capable of altering interlimb coordination during locomotion while maintaining adequate propulsion and weight support. The flexibility of spinal cord output is also demonstrated by the fact that chicks are capable of a variety of motor behaviours that are not normally expressed (Bekoff, 1992). For example, posthatching chicks returned to a hatching position inside an artificial egg will undergo the entire process of hatching, including synchronous right and left leg motion (Bekoff and Kauer, 1984). Chicks will swim if placed in water (Johnston and Bekoff, 1992; Chapter 4), and will locomote by hopping with both legs in synchrony if alternating leg action is prevented. It has been suggested that elements of the same pattern generating circuits are utilized in each of these behaviours, with differences arising due to alterations in peripheral feedback (Bekoff, 1992). The present study demonstrates that the neural circuitry controlling locomotor behaviour can also effectively reconfigure motor output to adapt to centrally imposed restrictions such as
partial spinal injury.
Chapter Four:

Locomotor plasticity after spinal injury -
improved recovery with cutaneous stimulation

Introduction

To produce functional locomotion in vertebrates, spinal locomotor circuits require input from two major sources: supraspinal input, such as that from brainstem nuclei, and afferent feedback from the limbs (Grillner, 1975; Grillner and Wallen, 1985; Armstrong, 1986). As brainstem-spinal connections are essential for the initiation and ongoing control of locomotion, disruption of these descending projections after a spinal cord injury will necessarily alter the ability of the animal to move in a voluntary and controlled manner. However, even in the case of a complete spinal cord transection, where the sole source of input to locomotor circuits is restricted to afferent input from the limbs, self-supporting walking can still be generated on a moving treadmill in both cats (Barbeau and Rossignol, 1987; Lovely et al., 1990) and chicks (Bekoff, et. al., 1987). This illustrates the important role of segmental afferent feedback in refining and controlling limb movements in the absence of supraspinal inputs. Even after partial spinal injuries such as a hemisection, where some supraspinal connections remain intact, segmental afferent input plays an important role in recovery of function (Goldberger, 1988; Goldberger and Murray, 1988). For example, increased terminal sprouting of primary afferent pathways occurs after spinal cord hemisection in the adult cat, and this sprouting is correlated with recovery of reflex control and precise limb placement during locomotion (Helgren and Goldberger, 1993). In addition, artificial cutaneomuscular stimulation can partially restore normal reflex modulation in spastic spinal cord-injured patients (Fung and Barbeau, 1994).

Afferent feedback from a limb consists of three general types: 1) input which arises from
receptors in muscle and tendon, i.e. proprioceptive, 2) input which arises from joint receptors, 3) input which arises from cutaneous receptors, i.e. exteroceptive. All three of these inputs may function to maintain general excitability in spinal cord circuits (Grillner, 1975) but they also appear to have important phasic actions during locomotion. Proprioceptive feedback from limb extensor muscles is increased during the stance phase of the step cycle when the limb is bearing weight. The roles of proprioceptive feedback are generally considered to be temporal, i.e. regulation of muscle activation and transitions between the phases of the step cycle, and reinforcement of ongoing muscle activity (Pearson, 1993). It has been suggested that proprioceptive feedback during limb loading is largely responsible for the recovery of treadmill stepping in chronic spinal cord transected cats (Edgerton, et. al., 1992). Input from joint afferents is thought to entrain the locomotor rhythm in spinal cats, in that extension at the hip has been shown to signal the transition from stance to swing phases during treadmill locomotion (Grillner and Rossignol, 1978, Anderson and Grillner, 1983), although more recent studies suggest that muscular afferents rather than joint afferents are responsible for locomotor entrainment during fictive locomotion (Kriellaars, et. al., 1994). The function of exteroceptive (cutaneous) feedback, in the absence of limb loading, is not as well investigated. Cutaneous feedback is known to be important for responses to perturbations of locomotion. For example, contact of the dorsum of the foot by an obstacle during the swing phase of walking elicits an increased limb flexion response, such that the object is avoided (Prochazka, et. al., 1978; Forssberg, 1979). This reflexive adjustment is greatly reduced in animals in which the skin has been functionally denervated (Prochazka, et. al., 1978). However, it has been difficult to investigate the role of cutaneous feedback in the absence of proprioceptive input during locomotor recovery after a spinal cord injury.
This study addresses the role of afferent feedback, specifically cutaneous feedback, in recovery of function after partial spinal injury by examining the recovery of two behaviours, walking and swimming. Although the importance of recording and measuring behavioural and reflex function after spinal cord injury has been previously emphasized (Kunkel-Bagden, et. al., 1993), there have been no studies which examined the detailed kinematics of more than one locomotor behaviour in the same animal. Both walking and swimming are naturally occurring behaviours of chicks. The kinematics of normal swimming have been previously documented in the chick (Johnston and Bekoff, 1992). Walking and swimming are similar behaviours in that they both consist of alternating leg movements, but differ significantly in the type and degree of afferent feedback experienced. During walking, both cutaneous and proprioceptive feedback are increased phasically during the step cycle; ground contact during stance activates cutaneous receptors while proprioceptive input increases when muscle and tendons are stretched during weightbearing. This is in sharp contrast to swimming where the animal experiences reduced phasic proprioceptive and cutaneous feedback. By studying the recovery of these two behaviours after a spinal cord injury, and by manipulating the amount of cutaneous input experienced by the limb during swimming, I have determined that increased phasic cutaneous input can improve limb motion during locomotor recovery.

Methods

Data were collected from 23 experimental (hemisected) and 10 sham-operated animals one day pre-operative and at 1, 2, 3, 5, 7, 9, 12 and 14 days post-operative. Both swimming and overground data were collected for 28 animals (20 experimental and 8 shams). In 11 animals (9 experimental, 2 shams), swimming data was collected both with and without phasic cutaneous input.
Surgery and data collection: Surgery and kinematic data collection for overground locomotion was carried out as outlined in Chapters 2 and 3. Chicks were also trained to swim the length of a tank of water (20°C). Chicks were gently held by the tail so as to maintain their position perpendicular to the camera, which was positioned to the left of the animal. To provide phasic cutaneous input during swimming, 0.5 ml Eppendorf tubes (approximately 3 cm in length, 0.5 cm diameter) were filled with water to make them neutrally buoyant and each was attached with a piece of thread of equal length to a grid at the bottom of the tank. Approximately 80 tubes were used to cover a horizontal area of 20 x 15 cm. The depth of the tubes within the water column was adjusted so that they would only contact the ventral surface of the foot when the limb was partially extended. The neutrally buoyant tubes provided little to no resistance to limb extension or support of the body.

Kinematic data analysis: Joint angle data was collected from videotape as outlined in Chapter 2. To compare joint angle motion throughout the step cycle at each stage of recovery, representative curves were calculated as outlined in Chapter 2, using single samples from each animal at the same cycle duration (250-300 msec duration for walking, 200-250 msec duration for swimming). Data presented in Figure 4.2 A and B is same in Figure 3.13 A and B except that percent cycle duration begins with the swing phase in order to facilitate comparisons with swimming data in Figures 4.7 and 4.9. Range of angle motion as well as maximum and minimum angles measured at specific times throughout the study were compared using repeated measures analysis of variance (ANOVA) (SigmaStat, Jandel Scientific). Bonferroni t-test was used to detected differences between means. Average measurements for each chick were calculated from at least 10 samples for each day of examination.
Results

After hemisection, the limited motion of the left leg, discussed in Chapter 3, was associated with a reduced range of motion at both the knee and ankle joints (Fig. 4.2, and Fig. 4.4, \( F=12.3, p<0.001 \) for knee, \( F=9.65, p<0.001 \) for ankle). The reduction in knee range was due to decreased knee extension (ie. reduced maximum knee angle) during the latter part of swing (Fig. 4.2B and Fig. 4.5A; \( F=6.74, p<0.01 \)); whereas the reduction in ankle movement was due to decreased flexion (ie. reduced minimum ankle angle) during swing (Fig. 4.2B and Fig. 4.5B; \( F=8.25, p<0.001 \)). The temporal coordination between knee and ankle motion also changed. As shown in a plot of knee angle against ankle angle, the knee and ankle extended and flexed synchronously after hemisection (Fig. 4.3C). Normally, knee and ankle demonstrate an asynchronous relationship throughout the limb cycle, where knee action precedes ankle movement (Fig. 4.3, A and B).

Over the two week recovery period, 23 out of 31 hemisected animals recovered overground locomotion. Of the 8 animals which did not walk, three became very weak several days after the surgery and were killed humanely, and five never recovered the use of the left leg. Examination of the spinal cord in the latter 5 animals did not reveal any consistent differences in the extent of the lesion. Recovery of walking followed a predictable time course, and all animals, with the exceptions noted above, recovered within a few days of each other. As recovery progressed, each chick began to place the left leg slightly ahead of the body during the swing phase. This ability was temporally correlated with the return to the normal range of motion at both knee and ankle joints (Fig. 4.2C, Fig. 4.4). In addition, knee extension and ankle flexion during swing returned to normal values (Figs. 4.5). Timing between knee and ankle motion also returned to a normal asynchronous pattern by 14 days post-operative (Fig. 4.3D). In summary, at 14 days post-operative, the walking abilities of hemisected animals could not be distinguished from those of age-matched, sham-operated animals (Figure 4.1C).
Figure 4.1

Limb positions of chick walking 1 day prior to left thoracic cord hemisection (A), 1 day after hemisection (B) and 14 days after hemisection (C). See Figure 3.2 for explanation of each limb position. In (C), after 14 days, the left leg once again contacts the ground rostral to the body and the limb positions throughout the stride cycle are the same as those in (A).
Figure 4.2.

Joint angles of left leg during walking plotted against normalized cycle duration for hip, knee and ankle one day prior to hemisection (A), 1 day after left thoracic cord hemisection (B) and 14 days post-operative (C). Bars indicate duration of stance phase for left leg. Solid lines represent 6th order regression line through data for all animals (n=12). Thin dotted lines represent 99% confidence limits.
Figure 4.3

Knee angle vs ankle angle during walking one day pre-operative (B), one day post-operative (C) and 14 days post-operative (D). The diagram in A was generated from the data in B indicating the stance phase (small crescent shaped segment of the loop) and swing phase (large crescent shaped segment of the loop), as well as the approximate limb positions during the step cycle. Data in B, C and D are from 6-8 step cycles recorded from the same animal.
Figure 4.4

Range of knee (A) and ankle motion (B) of left leg during walking, swimming without cutaneous stimulation, and swimming with phasic cutaneous stimulation at different times during recovery from hemisection. Error bars represent standard error. Asterisks indicate values which are significantly different from pre-operative values (p < 0.05).
A
RANGE OF KNEE MOTION

B
RANGE OF ANKLE MOTION

walking

swimming

swimming + phasic cutaneous stimulation
Figure 4.5

Maximum knee angle (A) and minimum ankle angle (B) excursions of left leg during walking, swimming without cutaneous stimulation, and swimming with phasic cutaneous stimulation at different times during recovery from hemisection. Error bars represent standard error. Asterisks indicate values which are significantly different from pre-operative values (p < 0.05).
A  MAXIMUM KNEE EXTENSION

B  MINIMUM ANKLE FLEXION

walking

swimming

swimming + phasic cutaneous stimulation
Recovery of swimming behaviour

During normal swimming, the joint angle motion that I observed was the same as that found by Johnston and Bekoff (1992). As in walking, most of the limb movement occurs at the knee and ankle (Fig. 4.6A, 4.7A). The onset of the cycle in Figure 4.6A and 4.7A begins with the onset of ankle flexion. This correlates with the onset of limb protraction (the movement of the limb rostral relative to the body), corresponding to the swing phase of walking. During protraction, both the knee and ankle flex, and then extend. With the onset of knee flexion, the limb is retracted (moved caudal relative to the body), corresponding to the stance phase of walking. During retraction, the knee is flexed while the ankle extends. Thus as in walking, a circular pattern is produced by the out-of-phase movements of knee and ankle (Fig. 4.8, A and B). This circular pattern is larger during swimming than the pattern for walking (Figure 4.3B) due to the larger range of motion at the ankle joint and to the greater degree of out-of-phase movement of the knee and ankle.

 Twenty-four hours after hemisection, the left leg moved very little during swimming trials and trailed passively behind the body, although the right leg continued to move normally. The left knee was held in a flexed position and the ankle moved cyclically through a limited range of motion. The range of motion of both the knee and ankle joints were greatly reduced, more so than for walking (Fig. 4.4; F=15.5, p<0.001 for knee, F=5.44, p<0.01 for ankle). This was the result of significant reduction in the amount of knee extension and ankle flexion compared to pre-operative values (Fig. 4.5; F=13.4, p<0.001 for knee, F=9.26, p<0.001 for ankle). Likewise, the normal pre-operative coordination between knee and ankle joints was absent one day post-operatively (Fig 4.8C). The left leg was first moved in an abbreviated cyclical manner at 5 days post-operative, although protraction was limited. At 14 days post-operative, the animals were able to move the left leg slightly more rostrally during protraction. Range of knee and ankle motion increased slightly over the 14 day recovery period, but did not reach pre-operative values (Fig. 4.4, p<0.05).
Figure 4.6
Limb positions of chick swimming with phasic cutaneous stimulation 1 day prior to left thoracic cord hemisection (A), 1 day after hemisection (B) and 14 days after hemisection (C). From left to right, each figure illustrates 1) end of retraction for left leg, 2) beginning of protraction, 3) end of protraction for left leg, and 4) mid-retraction for left leg. In (B), the lack of left knee extension results in insufficient protraction of the left limb. In (C), after 14 days of swim training with phasic cutaneous stimulation, the extension of the left knee recovers to normal pre-operative values.
Figure 4.7

Joint angles of left limb during swimming without cutaneous stimulation plotted against normalized cycle duration for hip, knee and ankle at one day pre-operative (A), 1 day after left thoracic cord hemisection (B) and 14 days post-operative (C). Solid lines represent 6th order regression line through data for all animals (n=8) (see methods). Thin dotted lines represent 99% confidence limits.
Figure 4.8

Knee angle vs ankle angle during swimming without phasic cutaneous stimulation (B,C,D) and with phasic cutaneous stimulation (E,F,G). The diagram in A was generated from the data in B indicating the retraction phase (small shallow crescent shaped segment of the loop) and protraction phase (large crescent shaped segment of the loop), as well as the approximate limb positions during the swim cycle. Data obtained one day prior to hemisection are illustrated in B and E; data obtained 1 day post-operative are illustrated in C and F; and 14 day post-operative data is shown in D and G. All data in B, C, and D were obtained from a single animal, as were the data in E, F and G. Each graph displays data from 5 to 8 swim cycles.
Figure 4.9

Joint angles of left leg during swimming with phasic cutaneous stimulation plotted against normalized cycle duration for hip, knee and ankle at one day pre-operative (A), 1 day after hemisection (B) and 14 days post-operative (C). Solid lines represent 6th order regression line through data for all animals (n=6) (see methods). Thin dotted lines represent 99% confidence limits.
Maximum knee extension and minimum ankle flexion remained significantly different from pre-operative values throughout the recovery period (Fig. 4.5, p<0.05). Coordination between left knee and ankle remained limited and the two joints essentially extended and flexed synchronously over a restricted range, similar to that observed during the immediate post-operative period for walking (compare Fig. 4.8D and 4.3C). The swimming abilities of hemisected chicks, at all stages of recovery, remained significantly worse than age-matched, sham-operated animals.

Recovery of swimming with phasic cutaneous input

When phasic cutaneous stimulation was provided for hemisected chicks during each swimming session, the chicks showed improvement in their swimming abilities as early as 2 - 3 days post-operative, and by 14 days post-operative, the leg movements of hemisected chicks could not be distinguished from those of sham-operated chicks of the same age (compare Figs. 4.9A and C, Fig. 4.6C). The improvement in limb motion initially occurred during the retraction phase of the swim cycle and took the form of enhanced extension of the left leg towards the bottom of the tank when the foot contacted the submersed tubes. This movement occurred occasionally on the first 2 - 3 days of recovery and became consistent for each hemisected chick by 5 days post-operative. At this time, the left leg generally moved in an increased arc of motion, comparable to the contralateral right leg. Range of knee and ankle motion after 5 days of recovery had returned to pre-operative levels (Fig. 4.4). Similarly, the amount of knee extension and ankle flexion returned to pre-operative values by 5 days post-operative (Fig. 4.5). If the tubes were then removed, the left leg immediately resumed the restricted movement of hemisected animals which were not provided with this form of phasic cutaneous feedback. Animals in which cutaneous stimulation was provided to only the ipsilateral leg (n=4, data not shown) showed improvement in left leg motion, comparable to that of animals provided with bilateral cutaneous stimulation. Animals provided with cutaneous stimulation to only the right leg (n=3, data not shown) did not show significant improvements in left leg motion. After
14 days with ipsilateral or bilateral cutaneous feedback, hemisected chicks maintained full limb motion whether they were swimming with or without the submersed tubes. Phasic cutaneous input also greatly improved the temporal coordination between the knee and ankle after 14 days of recovery (compare Fig. 4.8D with Fig. 4.8G).

Discussion

Ipsilateral joint motion immediately after hemisection

After hemisection, several acute changes occur in joint motion which are similar for both swimming and walking. The knee fails to extend normally during the limb cycle and the ankle joint remains hyperextended throughout the cycle (Figs. 4.2B, 4.7B). The consistency with which these patterns are repeated for both behaviours suggest that these joint angle changes are a direct effect of the hemisection, i.e. alteration of ipsilateral descending and ascending spinal projections, and not a secondary result derived by the animal to improve overground locomotion. The specific neuroanatomical or neurophysiological basis for these hemisection-induced joint angle changes is unknown, although some of the residual functioning of the left leg during walking could be attributed to undamaged pathways which cross at the lumbosacral level.

Recovery of walking and swimming behaviour after hemisection

After hemisection, limb motion during walking improves more rapidly than it does during swimming. Joint motion does improve for swimming, but has not returned to normal within 14 days. Several animals followed up to 3 weeks after hemisection showed some continued improvement in swimming abilities, but right and left limb movements still remained visibly asymmetric. It is possible that, if left for longer periods of time, limb motion during swimming in these chicks would eventually become indistinguishable from that of normal animals. The neural mechanisms responsible for the functional improvements seen in both walking and swimming could
include sprouting of undamaged inputs to the lumbosacral cord, eg. segmental afferent axons and brainstem-spinal axons which cross to the left side at the lumbosacral level, and/or gradual restoration of excitability levels on the left side of the lumbosacral cord.

It has been demonstrated that training has a positive effect on recovery of locomotor function after thoracic spinal transection (Barbeau and Rossignol, 1987; Edgerton, et. al., 1992). Adult cats which do not receive regular treadmill training throughout the recovery period do not recover full weightbearing locomotion as quickly as animals that have received training. However, the poor recovery of swimming does not appear to be due to insufficient training. I noted that hemisected chicks which did not receive swim training exhibited the same swimming abilities after 14 days as chicks which had been trained throughout recovery.

Recovery of swimming behaviour with added phasic cutaneous stimulation

In both humans and cats recovering from a spinal cord injury, increased weightbearing, which significantly activates proprioceptors, improves stepping ability on a moving treadmill (Barbeau and Rossignol, 1987; Barbeau, et. al., 1987; Edgerton, et al., 1992). However, both cutaneous and proprioceptive inputs are activated by foot contact and limb loading, respectively, during the stance phase. Thus it is difficult to determine the relative importance of these two classes of input to the recovery of locomotion after spinal injury. I attempted to separate these two forms of feedback by examining swimming, where there is reduced phasic cutaneous and proprioceptive feedback when compared to walking. By subsequently providing only phasic cutaneous stimulation to the plantar surface of the foot, I noted an improved recovery of joint motion compared to pre-operative values. Careful video analysis shows that the neutrally buoyant tubes moved away immediately upon contact. Thus the chick obtained negligible, if any, support and the limb remained relatively unloaded by the tubes.

Further confirmation that cutaneous feedback alone is responsible for this improvement in limb
action could be obtained by local anaesthesia of the skin of the foot for each training session. Local
anaesthesia of the dorsal surface of the foot reduces the contact placing response during walking in
cats (Prochazka, et al., 1978). Without input from cutaneous receptors, the limb pushes against an
obstacle and then is dragged over it, in contrast to an unanaesthetized limb which is flexed as soon
as foot contact occurs (Prochazka, et al., 1978). If cutaneous input is solely responsible for the
improved limb action seen in this study, I would expect to see no improvement in limb motion in
response to the presence of the submerged tubes after removal of skin sensation. I judged, however,
that it would be impractical to anaesthetize the entire plantar surface of the chicken's foot and toes
as would be required for this study, as this would necessitate multiple subcutaneous injections of
local anaesthetic repeated every 2 days for 2 weeks, likely producing significant tissue damage.

The neural mechanism by which the suspended tubes improve swimming recovery is unknown.
The fact that left leg motion improves when cutaneous stimulation is only provided to the left foot
indicates that the mechanism is largely mediated by ipsilateral afferent pathways. It has been shown
that weak stimulation of the pad and plantar surface of a cat's foot during the stance phase produces
an immediate increase in the amplitude and duration of ongoing ipsilateral extensor activity
(Duysens and Pearson, 1976). It is possible in hemisected chicks that, during the initial stages of
recovery, phasic stimulation of the plantar surface of the left foot during retraction causes a similar
reflexive activation of extensor muscles and results in further extension of the leg.

I have demonstrated that selective stimulation of cutaneous receptors, without the significant
increase in proprioceptive feedback associated with limb loading, is sufficient to increase limb
extension during swimming after spinal hemisection. With repeated training, this improvement in
limb extension translates into a permanent alteration in limb action. Behavioural plasticity is also
evident in the cat after manipulation of inputs to spinal locomotor circuits. When ipsilateral
hemisection is superimposed on chronic deafferentation in the cat, normal locomotor patterns
eventually return in those animals with partial deafferentation of the limb, but animals with complete peripheral deafferentation adopt novel locomotor patterns (Goldberger, 1988). Functional recovery after hemisection or incomplete deafferentation has been associated with anatomical sprouting of undamaged peripheral afferent projections (Goldberger and Murray, 1988; Helgren and Goldberger, 1993). Thus both anatomical and behavioural evidence suggests that alterations in the pattern of peripheral afferent feedback can be used to compensate for the loss of peripheral or central inputs to spinal locomotor circuits.
Chapter Five:
General Discussion

This thesis has demonstrated that posthatching chicks are capable of significant behavioural plasticity, both during normal development and after partial spinal injury. This chapter will discuss the neuroanatomical and neural circuitry changes which may be responsible for the observed behavioural plasticity. I will also outline methods which may manipulate CNS plasticity in order to enhance locomotor recovery after spinal cord injury in humans.

A. Anatomical basis for developmental plasticity - role of activity-dependent mechanisms

The normal ontogeny of neural circuitry underlying many behaviours involves the initial establishment of a basic pattern of synaptic connections which are then modified and refined later in development (for review, see Goodman and Shatz, 1993). The initial establishment of neuronal connections appears to occur primarily through molecular recognition processes, as growing neurites are guided along pathways to appropriate targets. Subsequent reorganization of these initial connections to produce stable functioning circuits occurs by distinct processes that depend largely upon experience and sensory input. These mechanisms are termed "activity-dependent", because the amount of neural activity within circuits determines the strength of circuit connections.

Developmental activity-dependent processes are of interest because they are thought to occur by mechanisms similar to those underlying learning and memory in the mature CNS (Constantine-Paton, et al., 1990). Thus, these same processes may be responsible for the locomotor plasticity demonstrated in young precocial animals such the chick, which possesses a relatively mature functional CNS. However, little is known regarding activity-dependent
plasticity during locomotor development. The developmental role of activity-dependent mechanisms have been extensively studied in the visual system. Activity-dependent processes are involved in the refinement of the initial coarse pattern of retinotectal projections in amphibians, to produce a fine-grained detailed topographic map of visual space in the tectum (Goodman and Shatz, 1993). When neural activity is blocked with tetrodotoxin (TTX), the formation of this detailed topographic map is altered. In mammals, neural activity is required for the establishment of ocular dominance columns in the visual cortex - closing the lids of one eye in a newborn cat causes the thalamic axons from the open eye to occupy proportionately more cortical area than axons from the closed eye (Hubel and Wiesel, 1970; Hubel, et al., 1977).

However, it is not neural activity per se which produces the detailed map of retinal projections on the tectum. For example, rearing animals in strobe lighting, which causes synchronous neural activity of all retinal cells, does not result in the production of a precise topographical map (Schmidt and Eisele, 1985). Under normal conditions, therefore, it is the local spatial and temporal pattern of neural activity that determines the final pattern of connections. At the synaptic level, this could occur if transmission at a particular synapse is strengthened when both the pre- and post-synaptic cells are active simultaneously (Hebb, 1949). Synapses with these properties have been demonstrated in the hippocampus by experimentally activating both pre- and post-synaptic cells and measuring the increased efficacy of synaptic transmission (Madison, et al., 1991). In a non-experimental situation, pre- and postsynaptic cells are active simultaneously when many inputs converging on the same cell are active together. For example, when an object in the visual field activates a local population of retinal ganglion cells, postsynaptic neurons in the tectum which receive converging input from this retinal population are also simultaneously active (Goodman and Shatz, 1993). Connections between these two groups of cells are selectively strengthened, so that, with sufficient visual experience, specific
positions in visual space are eventually mapped onto the tectum.

Similarities between developmental plasticity and mechanisms of learning are further supported by the fact that the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor has been shown to mediate both types of activity-dependent synaptic strengthening (Cline, et al., 1989; Madison, et al., 1991). NMDA receptors are capable of acting in this capacity by generating a calcium current when the postsynaptic cell is sufficiently depolarized, e.g. by simultaneous activation of converging inputs. Small changes in intracellular calcium can trigger second messenger pathways leading to strengthening of synaptic transmission by both pre- and post-synaptic mechanisms (Kullman and Siegelbaum, 1995). Blockade of NMDA receptors prevents long-term potentiation (an experimentally-induced correlate of learning) in the hippocampus (Madison, et al., 1991). NMDA blockade also disrupts the detailed patterning of retinotectal connections (Cline, et al., 1989).

In addition to changes in synaptic strength, neuroanatomical changes have been associated with activity-dependent plasticity in the CNS, most notably in the cerebral cortex (Kolb, 1995). There is clear evidence that cells in the cortex undergo experience-dependent morphologic changes. Laboratory rats raised in a stimulating environment, in contrast to those raised in standard laboratory cages, possessed larger cortical neurons with more highly branched dendrites, increased numbers and sizes of synaptic contacts, as well as increased number of glia and capillary vessels (for review, see Kolb, 1995). Alterations in behaviour accompanied neuroanatomical changes, in that animals raised in stimulating environments performed much better on learning tasks (Hebb, 1947). Importantly, these profound morphological changes are not due simply to a general increase in cortical activity, as it has been shown that specific learning experiences will influence the morphology of selective cortical regions (Kolb, 1995). For example, rats trained in a single limb reaching task showed increased dendritic branching in
the cortical motor area associated with the trained limb but not in the area associated with the untrained limb. Rats trained in a bimanual task show bilateral increases in the forelimb motor areas, comparable to the changes seen for a single trained limb (Kolb, Tomie and Ouellette, 1995, referenced in Kolb, 1995). Thus, similar to the production of detailed visual maps in the tectum, it is the specific local pattern of neuronal activity that produces neuroanatomical alterations in the cortex.

What relevance do the activity-dependent processes occurring in visual or cortical development have for the behavioural plasticity demonstrated in posthatching chicks? First, it is possible that the locomotor plasticity evident in the first weeks posthatching is mediated by similar processes. The development of basic locomotor circuitry, both spinal and supraspinal, is completed well before hatching in chicks (Okado and Oppenheim, 1985; Sholomenko and O'Donovan, 1995). Changes in behaviour after hatching therefore must be mediated by mechanisms other than development of new axonal projections. These mechanisms could include the local establishment of new synaptic connections through local axonal sprouting, with associated changes in dendritic morphology, number of glial supporting cells, etc., as described in the cerebral cortex (Kolb, 1995). In addition, modifications in the strength of existing connections could mediate locomotor plasticity. There is evidence that NMDA-mediated synaptic modifications are involved in refinement of spinal cord circuits in both chicks and mammals. For example, NMDA-mediated processes underlie the somatotopic organization of afferent projections in the chick spinal cord (Mendelsohn, et al., 1994). NMDA receptors may also be involved in the maturation of spinal networks in the postnatal rat (Maier, et al., 1995).

To determine whether similar activity-dependent processes are involved in gait maturation in the chick, both behavioural and anatomical methods can be employed. The role of experience in gait maturation can be determined by manipulating the amount and type of locomotor exercise
that young chicks receive. Outcomes can be investigated: 1) behaviourally, by monitoring the progress of gait development in groups of animals receiving different amounts of locomotor training, and 2) anatomically, through histological investigation of neuronal and glial morphology, including analysis of dendritic branching and synaptic numbers in the lumbosacral spinal cord of trained and untrained animals. In addition, experiments involving blockade of NMDA receptors at different times posthatching, with or without controlled amounts of gait training, could determine the role of NMDA-mediated synaptic processes during locomotor development in chicks.

Activity-dependent processes may also be involved in the recovery of walking behaviour which occurs after partial spinal injury in the chick. The repetition of leg action during daily walking trials may act to strengthen neural circuits involved in stepping and weight support. It is known that repetition of treadmill stepping after spinal cord injury in cats will improve the performance of stepping, such that the animals can step at faster treadmill speeds than untrained animals (Barbeau and Rossignol, 1987; Lovely, et. al., 1990). This improvement could not be attributed to changes in the skeletal muscular system (Edgerton, et al., 1991) and so must be related to activity-dependent alterations in neural circuitry.

The provision of cutaneous stimulation during swim training in hemisected chicks may also act through an activity-dependent mechanism. The presence of the tubes initially stimulated the affected leg to move through a greater range of motion. It is conceivable that the daily repetition of this motion strengthened the neural circuits involved in leg action during swimming. After 2 weeks of training, it is likely that the repetitive action of the limb, rather than the cutaneous stimulation per se, provided sufficient reinforcement of the neural pathways such that the motion was maintained even after the stimulus was removed. Similarly, spinal cats show improved stepping performance on a treadmill after appropriately timed afferent stimulation has
been enhanced during training sessions. This was accomplished by gently pulling down on the
tail to increase proprioceptive excitation during the stance phase (Lovely, et. al., 1990).
Experiments involving blockade of NMDA receptors during walking and swimming trials after
spinal cord injury in chicks could help determine the extent of NMDA-mediated processes in
recovery of these behaviours.

B. Sensorimotor stimulation to enhance locomotor recovery after spinal injury

The evidence that increased afferent stimulation leads to improved motor recovery in
experimental animals provides a compelling argument for use of similar techniques after spinal
cord injury in humans. The remainder of this chapter will examine the application of these
techniques toward the improvement of locomotor recovery in spinal cord injured patients.

The production of well coordinated stepping movements in spinal animals is generally
attributed to the existence of locomotor pattern generating circuits in the lumbosacral spinal
cord. Although there has been concern over the importance of these circuits in humans (Winter,
1989), recent reports have described involuntary coordinated limb alternation in spinal cord
injured patients (Calancie, et al., 1994, Wernig and Muller, 1992), suggesting the existence of
pattern generating circuits in the human spinal cord. One patient with partial spinal cord injury
experienced involuntary and forceful stepping movements upon hip extension. EMG recordings
showed that all muscles of the legs were rhythmically recruited in a pattern identical to that
recorded during voluntary stepping on a treadmill (Calancie, et al., 1994). Similarly, patients
with no voluntary activity in major leg flexor and extensor muscles show phasic EMG activity in
the same muscles during well coordinated stepping activity on a treadmill (Wernig and Muller,
1992; Dietz, et al., 1995). Of course, it is not possible in human patients to determine whether
the alternating output is generated completely from circuits in the spinal cord, because afferent
input remains intact after spinal cord injury. However, for the purposes of maximizing locomotor recovery, the fundamental source of the rhythmic output matters less than the fact that such output can perhaps be modified to improve functional recovery.

One well known method of improving locomotion after spinal cord injury is weight-supported treadmill training. It is now established that training with incremental increases in independent body weight support will improve locomotor performance (Barbeau and Wainberg, 1987; Dobkin, et al., 1992; Wernig and Muller, 1992). An increase in walking speed, stride length, single leg support time and EMG amplitudes are all seen when patients are trained to support ever-increasing proportions of their body weight during exercise on a moving treadmill (Visintin and Barbeau, 1989). Improved overground walking abilities have also been reported in patients receiving weight supported treadmill training (Wernig, et al., 1995). Improvements in locomotor abilities likely arise from two sources. First, gait training will increase the strength and endurance of leg muscles, especially extensor muscles, which undergo severe disuse atrophy after spinal injury (Gordon and Mao, 1994). Second, gradual increases in weight support throughout training may act to increasingly stimulate and strengthen the spinal neural circuitry involved in weight supported locomotor movements.

One of the reasons that weight-supported stepping is effective is that it allows stepping movements to be practiced without the need for control of posture and balance, which are often impaired after spinal cord injury. As described in Chapter 4 of this thesis, swimming is a behaviour which involves alternating stepping action of the limbs but which does not demand the degree of weight support or balance control required for overground locomotion. As such, swim training could be a useful alternative to weight supported locomotor training with a harness and treadmill. As demonstrated in Chapter 4, however, afferent inputs received during swimming may be insufficient to promote and maintain stepping action of the limbs after spinal
cord injury. Appropriate afferent stimulation could be provided underwater, either in the form of mild cutaneous stimulation described in Chapter 4, or simply as a solid platform upon which the patient could stand and partially support their bodyweight. This would provide appropriate cutaneous and proprioceptive stimulation to promote stepping action while the majority of bodyweight is supported by the water column.

Weight supported training is only one method of providing appropriately timed afferent input. Afferent excitation can also be enhanced by artificially stimulating peripheral nerves during locomotion. This form of functional electrical stimulation (FES) has been used to improve limb flexion in the swing phase and/or to restore reflex modulation during walking in spinal injured and spastic patients (Granat, et al., 1993a,b; Fung and Barbeau, 1994). For example, stimulation of the peroneal nerve elicits a flexion of the limb, in which the hip and knee are flexed and the ankle is dorsiflexed. When the peroneal nerve is stimulated at the end of the stance phase, the flexion response simulates the action of the leg during the swing phase and allows the foot to be lifted to prepare for the subsequent ground contact (Granat, et al., 1993b).

Afferent stimulation can also be used to restore reflex modulation in spinal cord injured patients (Fung and Barbeau, 1994). Many patients have hyperreactive reflexes and do not show the normal inhibition of soleus stretch reflexes which occurs during the early stance and swing phases (Capaday and Stein, 1986; Yang, et al., 1991). This leads to abnormal activation of the soleus during the step cycle, interfering with transitions from swing to stance. Cutaneomuscular stimulation to the medial plantar region of the foot in the early stance and swing phases of spinal cord-injured patients inhibited the soleus H-reflex at this time, leading to reflex modulation which simulated the normal pattern (Fung and Barbeau, 1994). This artificial reflex modulation was effective in reducing limb stiffness and toe drag during early stance and swing (Fung and Barbeau, 1994).
Another form of FES involves the direct stimulation of leg muscles, rather than the reflexive activation of muscle through afferent stimulation. Muscle groups such as the quadriceps, hip abductors, gluteals and/or hamstrings are activated using superficial electrodes. The particular group(s) of muscles activated, and the pattern of activation, are determined by the requirements of the individual patient (Granat, et al., 1993a).

The goal of FES is to restore locomotor function by artificially reproducing the leg action normally produced by spinal motor output. An FES stimulation sequence generally combines both direct and reflexive activation of muscles. For example, direct quadriceps stimulation permits weight support during the stance phase, while subsequent peroneal nerve stimulation produces flexion of the limb during the swing phase (Granat, et al., 1992, 1993a,b). However, the use of FES for the improvement of gait in SCI patients is still in the experimental stages, and has not been widely used clinically (Barbeau and Rossignol, 1994). This can be attributed in part to technical difficulties with the stimulation paradigm (Stein, et al., 1993) and the adverse responses of muscle and nerve to non-physiological stimulation. For example, direct muscle stimulation results in rapid muscle fatigue (Granat, et al., 1992), and the flexion withdrawal reflex shows a long latency and habituates rapidly (Granat, et al., 1992, 1993b). These difficulties, along with the large energetic cost of FES-assisted locomotion (Marsolais and Edwards, 1988), currently limit the duration of time that FES can be used safely and effectively.

In spite of difficulties, however, there have been reported improvements in locomotor abilities arising from FES training programs (Granat, et al., 1993a). After 5 months of FES training, patients with incomplete spinal cord injuries demonstrated an increase in voluntary muscle strength, reduced spasticity, reduced physiological cost of locomotion and an increased stride length during non-FES assisted gait. Interestingly, an increase in muscular strength was apparent both in directly activated muscles and in muscles which were activated only reflexively.
through flexion withdrawal responses. This increase in muscle strength was considered responsible for the increased stride length and reduced energy expenditure seen during non-FES assisted locomotion (Granat, et al., 1993a).

It appears then, that the long term benefits of FES-assisted training arise from effects on muscle physiology directly. However, it is possible that some benefits arise from repeated reinforcement of reflex pathways due to afferent stimulation. Repeated activation of afferent pathways may induce some reorganization of spinal neural circuitry, resulting in strengthening of the neural connections mediating the reflex in question. This strengthening could arise from local sprouting and increases in synaptic numbers, and/or activity-dependent modifications of existing synapses. It would be interesting to determine whether the reflex pathways involved can be modified sufficiently such that the effects of afferent stimulation, whether it be to improve limb flexion (Granat, et al., 1993b) or reflex modulation (Fung and Barbeau, 1994), are retained even after the artificial stimulation is removed.

Although it is beyond the scope of this thesis, pharmacological intervention for the enhancement of locomotion after spinal cord injury also plays an important role in rehabilitation of spinal cord injured patients. It is interesting that the most effective drugs appear to improve locomotor abilities largely by reducing spasticity rather than stimulating locomotor activity directly (Barbeau and Rossignol, 1994). Baclofen, a GABA$_B$ receptor agonist which reduces the stiffness of the stretch reflex (Capaday, 1995), has been used in the control of spasticity for over 20 years (Jones, et al., 1970). Drugs such as cyproheptadine (a serotonin antagonist) reduce the excitability of spinal reflexes and modulate the basic pattern of muscle activation during stepping (Barbeau and Rossignol, 1994). Thus, pharmacological therapy plays an important modulatory role, by reducing the adverse effects of spasticity on the generation of effective locomotion.
The previous discussion has been concerned with methods to enhance recovery of locomotor function after spinal injury in the absence of regeneration of damaged supraspinal projections. However, there has been some progress in experimental attempts to induce CNS axons to regenerate in the spinal cord. Most of these studies focus on manipulation of the adult CNS environment to make it more permissive for axonal outgrowth. Potential therapeutic interventions include application of growth promoting factors, such as neurotrophic factors (Schnell, et al., 1994) and transplants of fetal spinal tissue (Bregman, et al., 1993), or the neutralization of myelin-associated factors which inhibit axonal outgrowth (Schnell and Schwab, 1990; Keirstead, et al., 1995).

It is likely, however, that therapeutic interventions will not result in complete re-establishment of all descending projections to the spinal cord. It is therefore necessary to maximize the functional contributions of projections that do regenerate. As described in Chapter 1, regenerating supraspinal axons must establish stable synapses upon appropriate target neurons in the cord in order to contribute to functional recovery. This scenario can in some ways be considered a recapitulation of normal CNS development, when growing axons form initial patterns of connections which are subsequently remodelled to produce stable functioning circuits. As I have discussed previously in this Chapter, activity-dependent processes play a significant role in remodelling during normal development. After spinal cord injury, it is feasible that provision of appropriate patterns of activity within spinal locomotor circuits will assist in establishing functional connectivity between regenerating supraspinal axons and spinal neurons. Locomotor training, in combination with physical and electrical stimulation, can provide appropriate neural activity within spinal locomotor circuits and therefore has the potential to modify and refine the pattern of regenerating synaptic connections in an activity-dependent manner. Thus, methods previously described for enhancing locomotor recovery after spinal
injury in the absence of axonal regeneration can also play an important role in optimizing the functional contribution of regenerated supraspinal inputs.

A comprehensive rehabilitation program for the improvement of locomotion in spinal cord injured patients will undoubtedly involve a combination of the above approaches (Barbeau and Rossignol, 1994). In particular, repetitive training of weight-supported locomotor movements combined with optimization of sensory cues using physical and electrical stimulation show the most potential for exploiting spinal cord plasticity. I have demonstrated that these same approaches can improve motor recovery after spinal cord injury in chicks. I have also shown for the first time that locomotor plasticity is evident in young precocial animals during normal development. In both cases, the plasticity of locomotor behaviour is not due to outgrowth/regeneration of neuroanatomical projections but conceivably arises from functional reorganization of spinal circuitry, similar to the activity-dependent learning processes occurring during visual and cortical development. Further investigation into spinal mechanisms of activity-dependent plasticity is required so that we can promote and guide the learning capacity of the spinal cord, maximize the functional benefits of regenerating projections and facilitate long term improvements in locomotor abilities after spinal cord injury.
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