The Control of Interlimb Coordination during Left-Right and Transverse Split-Belt Locomotion in Intact and Spinal Cord-Injured Cats

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Abstract. Proper coordination of the four limbs, or interlimb coordination, is a fundamental requirement for locomotion in terrestrial mammals. The control of interlimb coordination during quadrupedal locomotion was studied in adult cats by independently controlling the speed of the left and right sides, or of the fore- and hindlimbs, using a treadmill with four independent running surfaces. Here, we briefly present some of our recent findings pertaining to the control of interlimb coordination during quadrupedal locomotion in intact and spinal cord-injured adult cats.

1 Introduction

Precise and flexible coordination of the four limbs (i.e. interlimb coordination) is a fundamental requirement for quadrupedal or bipedal locomotion. Maintaining dynamic stability in a changing environment requires constant adjustments in interlimb coordination. Despite its importance, the neural control of interlimb coordination in mammalian systems is largely unknown, although genetic manipulations in mice have begun to identify some of the neuronal populations involved [1,2]. It has been well documented that humans have conserved a quadrupedal-like control of interlimb coordination during bipedal walking [3,4]. As such, studies in quadrupedal terrestrial mammals that walk on all four limbs, such as the cat, are appropriate and useful translational models to uncover neurophysiological and biomechanical mechanisms involved in the control of interlimb coordination during human locomotion. To study interlimb coordination during locomotion, we recently devised a series of experimental paradigms whereby the speeds of the left and right sides [5] or of the fore- and hindlimbs [6] were independently controlled, respectively. Here, we briefly review recent findings during left-right (i.e. unequal speeds for the left and right sides) and transverse (i.e. unequal speeds for the fore- and hindlimbs) split-belt locomotion paradigms in intact and spinal cord-injured cats.

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2 Methods

2.1 *Animals and Surgical Procedures*

All procedures were approved by the Animal Care Committee of the Université de Sherbrooke and were in accordance with policies and directives of the Canadian Council on Animal Care. Before and after experiments, animals were housed and fed within designated areas. Five adult cats (1 male, 4 females) weighing between 3.5 and 7.7 kg were used. Cats were trained to walk on an animal treadmill with four independently controlled running surfaces 120 cm long and 30 cm wide (Bertec Corporation, Columbus, Ohio). A Plexiglas separator (120 cm long, 3 cm high, 0.5 cm wide) was placed between the left and right belts for left-right split-belt locomotion. Two Plexiglas separators (120 cm long, 50 cm high) were also placed 30 cm apart to constrain the animal to walk with the left and right sides separately on the two belts during left-right split-belt locomotion or at the midline between the front and rear belts on the left side of the treadmill for transverse split-belt locomotion. Cats were given food and affection as reward.

Implantation and spinal transection surgeries were performed under aseptic conditions in an operating room with sterilized equipment. Prior to surgery, the cat was sedated with an intramuscular (i.m) injection of Butorphanol (0.4 mg/kg), Acepromazine (0.1 mg/kg), and Glycopyrrolate (0.01 mg/kg). Induction was done with Ketamine/Diazepam (0.11 ml/kg in a 1:1 ratio, i.m.). Once anesthetized, the cat was quickly intubated with a flexible endotracheal tube and anesthesia was maintained by adjusting isoflurane concentration as needed (1.5 - 3%). The fur overlying the back, stomach, and hindlimbs was shaved with electric clippers and loose hair was vacuumed. The level of anesthesia was confirmed and adjusted throughout the surgery by monitoring cardiac and respiratory rates, by applying pressure to the paw to detect limb withdrawal, and by assessing jaw tone. Body temperature was monitored using a rectal thermometer. In two cats (2 females), the spinal cord was completely transected (i.e. spinalized) at low thoracic levels. A small laminectomy was performed between the junction of the 12th and 13th vertebrae, the dura was removed, and after local lidocaine application (Xylocaine, 2%), the spinal cord was transected with surgical scissors. Hemostatic material (Surgicel) was inserted within the gap, and muscles and skin were sewn back to close the opening in anatomic layers.

For electromyography (EMG), pairs of Teflon insulated multistrain fine wires (AS633; Cooner wire, Chatsworth, CA, USA) were directed subcutaneously from a head-mounted 24 pin connector (Hirose Electric Co Ltd) and sewn into the belly of selected hindlimb and forelimb muscles for bipolar recordings. During surgery an antibiotic (Convenia, 0.1 ml/kg) was injected subcutaneously and a transdermal fentanyl patch (25 mcg/hr) was taped to the back of the animal 2-3 cm from the base of the tail. During surgery and approximately seven hours later, another analgesic (Buprenorphine 0.01 mg/kg) was administered subcutaneously. After surgery, cats were placed in an incubator and closely monitored until they regained consciousness.

For the spinalized cats, the bladder was manually emptied 1–2 times each day. The hindlimbs were frequently cleaned by placing the lower half of the body in a warm soapy bath. After a few days, cats were trained 5 times a week to walk on the treadmill. Early after spinalization, training consisted of two experimenters moving the hindlimbs over the moving treadmill belt to simulate locomotion with similar joint kinematics and paw contacts while the forelimbs were positioned on a fixed platform located ~1 cm above the belt. After a few days, the skin of the perineal region was stimulated to evoke stepping movements. A Plexiglas separator was placed between the hindlimbs to prevent them from impeding each other because of increased adductor activity. Initially, the experimenter supported the hindquarters by lifting the tail. Recording sessions started once the animals attained a stable locomotor pattern with full weight bearing and consistent plantar foot placement. The experimenter provided equilibrium by holding the tail.

2.2 Experimental Paradigms

Left-right split-belt locomotion: Each cat performed two sessions that consisted of several episodes of tied-belt (i.e. equal speeds for the left and right sides) and left-right split-belt locomotion. In spinalized cats only the hindlimbs moved, with the forelimbs on a fixed platform. During tied-belt locomotion, speeds ranged from 0.3 to 1.0 m/s in 0.1-m/s increments. During left-right split-belt locomotion, one side walked at a constant speed of 0.4 m/s, 0.5 m/s or 0.6 m/s while the other side varied its speed from 0.3 to 1.0 m/s in 0.1-m/s increments. Episodes of tied-belt or left-right split-belt locomotion at the different speeds were presented randomly from one session to another and approximately 30 seconds of rest were given between episodes. Each episode lasted approximately 20 s to obtain 10-15 cycles. Left and right treadmill speeds were increased with an acceleration of 0.1 m/s and data collection started when the desired speeds were attained.

Transverse split-belt locomotion: Each cat performed five sessions of several locomotor episodes in five conditions: (1) fore- and hindlimbs walking at equal speeds (i.e. tied-belt locomotion) from 0.4 m/s to 0.8 m/s in 0.1-m/s increments; (2, 3) forelimbs walking at a constant speed of 0.4 m/s or 0.8 m/s with the speed of the hindlimbs increasing from 0.4 to 0.8 m/s in 0.1-m/s increments; (4, 5) hindlimbs walking at a constant speed of 0.4 m/s or 0.8 m/s with the speed of the forelimbs increasing from 0.4 to 0.8 m/s in 0.1-m/s increments. In a few sessions, faster speeds (up to 1.4 m/s) were used to evaluate the effects of increasing the speed difference between the front and rear belts. Only episodes where the animal had its forelimbs and hindlimbs on their respective belts were retained for analysis. Each episode lasted approximately 20 s to obtain 10-15 cycles. Front and rear treadmill speeds were increased with an acceleration of 0.1 m/s and data collection started when the desired speeds were attained.

2.3 *Data Acquisition and Analysis*

Videos of the left and right sides were captured with two cameras (Basler AG) at 60 frames per second. A custom-made Labview program acquired the images and synchronized the cameras. Videos were analyzed off-line. Cycle duration was measured from successive paw contacts. Paw contact was defined as the first frame where the paw made visible contact with the treadmill surface. Stance duration corresponded to the interval of time from paw contact to the most caudal displacement of the paw relative to the hip or the shoulder, while swing duration was measured as cycle duration minus stance duration.

3 Results

3.1 *Left-Right Split-Belt Locomotion*

One form of adjustment in interlimb coordination occurs when walking along a circular path, where the outer leg must walk faster than the inner leg [7]. One way to simulate some features of circular path walking is by independently controlling the speed of the left and right sides (i.e. left-right split-belt locomotion). During left-right split-belt locomotion, the stance and swing phases on the slow side are increased and decreased, respectively, while the stance and swing phases on the fast side are decreased and increased, respectively [5,8,9]. We recently studied bilateral changes in hindlimb stance and swing phases by measuring the slopes of the linear regressions between stance duration and cycle duration (r_{STA}) and between swing duration and cycle duration (r_{SW}) during left-right split-belt and tied-belt (i.e. equal speeds on left and right sides) locomotion in intact and chronic spinalized adult cats [5]. The slopes of the regressions between the phases and cycle duration quantify how the stance and swing phases vary as a function of cycle duration. During tied-belt locomotion, r_{STA} was significantly greater than r_{SW} bilaterally, as shown previously [10,11]. During left-right split-belt locomotion, r_{STA} and r_{SW} were respectively decreased and increased on the constant side, compared to values obtained during tied-belt locomotion, whereas on the varying side, r_{STA} and r_{SW} were respectively increased and decreased. Thus, phase variations were differentially modulated in both hindlimbs concurrently. Results were similar in intact and chronic spinalized cats, indicating that the bilateral control of phase variations is mediated at the level of the spinal cord, most likely by a sensory mechanism.

3.2 *Transverse Split-Belt Locomotion*

While left-right split-belt locomotion alters some features of interlimb coordination, independently controlling the speed of the fore- and hindlimbs primarily alters inter-girdle coordination. The adaptation to transverse split-belt locomotion is strikingly different if it is the forelimbs or the hindlimbs that are walking faster [6]. If the forelimbs walk faster than the hindlimbs, there is often an uncoupling of the forelimb and hindlimb rhythms (i.e. unequal cycle durations) with the forelimbs taking two or more steps for every hindlimb cycle. As a result, the sequence of limb contacts (i.e. footfall patterns) that normally proceeds with a hindlimb contact followed by contact of the homolateral forelimb, termed a lateral sequence [12], is altered. When the forelimbs walk faster than the hindlimbs, a diagonal sequence can emerge whereby contact of the hindlimb is followed by contact of the contralateral forelimb. In contrast, if the hindlimbs walk faster than the forelimbs, cycle duration is equal at both girdles, even with hindlimb speeds up to 1.4 m/s. The footfall pattern also maintains a normal lateral sequence. From these results, it is clear that inter-girdle coordination is organized asymmetrically in quadrupeds.

4 Conclusion

Left-right and transverse split-belt locomotion are useful tools to study interlimb coordination during quadrupedal locomotion. We recently showed that phase variations are bilaterally altered during left-right split-belt locomotion and that a spinal mechanism is involved [5]. We have also shown that inter-girdle coordination is organized asymmetrically and that an uncoupling of the forelimb and hindlimb rhythms occurs when the forelimbs are made to walk at a faster speed than the hindlimbs [6]. An uncoupling of the forelimb and hindlimb rhythms also occurs following incomplete thoracic [13] or cervical [14] spinal cord injuries and it is always the forelimbs that take extra steps, similar to what occurs with transverse split-belt locomotion with the forelimbs walking faster. Transverse split-belt locomotion could help elucidate the mechanisms involved in this uncoupling and its potential role in adapting interlimb coordination to certain environmental demands. In coming years, we will determine how the control systems regulating interlimb coordination are modified and adapt following incomplete spinal cord injuries and other types of injuries or diseases that disrupt the control of locomotion.

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