Innovative Methodology

QUANTIFICATION OF MOTOR CORTEX ACTIVITY AND FULL-BODY BIOMECHANICS DURING UNCONSTRUCTED LOCOMOTION

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cortex activity and full-body biomechanics during unconstrained locomotion. *J Neurophysiol* 00:
000-000, 2005. Recent progress in the understanding of motor cortex function has been achieved
primarily by simultaneously recording motor cortex neuron activity and the movement
kinematics of the corresponding limb. We have expanded this approach by combining high
quality cortical single-unit activity recordings with synchronized recordings of full-body
kinematics and kinetics in the freely behaving cat. The method is illustrated by selected results
obtained from two cats tested while walking on a flat surface. Using this method, the activity of
43 pyramidal tract neurons (PTNs) was recorded, averaged over 10 bins of a locomotion cycle,
and compared to full-body mechanics by means of principal component and multivariate linear
regression analyses. Patterns of 24 PTNs (56%) and 219 biomechanical variables (73%) were
classified into just four groups of inter-correlated variables that accounted for 91% of the total
variance, indicating that many of the recorded variables had similar patterns. The ensemble
activity of different groups of 2 to 8 PTNs accurately predicted the 10-bin patterns of all
biomechanical variables (neural decoding) and vice versa; different small groups of mechanical
variables accurately predicted the 10-bin pattern of each PTN (neural encoding). We conclude
that comparison of motor cortex activity with full-body biomechanics may be a useful tool in
further elucidating the function of the motor cortex.

**Keywords:** Pyramidal tract neurons, Kinematics, Kinetics, Neural encoding and decoding.
INTRODUCTION

Progress in understanding motor cortex function has been achieved largely by simultaneously recording the activity of motor cortex neurons and the mechanics of corresponding movements. Because of technical constraints, however, most studies have used motor tasks with one-, two- or three-degrees-of-freedom, such as manipulating objects with the hand (Evarts 1968), exertion of isometric forces (Ashe 1997), and reaching by the arm (Georgopoulos et al. 1982). These types of studies have revealed that the activity of the neuronal population of the motor cortex is related to different mechanical variables of motor tasks. For example, Evarts (1968) demonstrated in monkeys executing simple wrist movements that the activity of some neurons in the motor cortex was related to the activation of wrist flexor and extensor muscles. Motor cortex activity has also been found to be well correlated with other movement variables, e.g. the direction and velocity of movement (Georgopoulos et al. 1982 and Moran and Schwartz 1999, respectively), the direction and magnitude of the static force exerted by the arm (Ashe 1997), the hand location in space (Sergio and Kalaska 1997), the arm orientation (Scott and Kalaska 1997), and the joint torque and power (Scott et al., 2001).

Mechanically constrained paradigms employed in the abovementioned studies have allowed for the accurate and detailed determination of mechanical aspects of the task and their comparison with motor cortex activity. The motor cortex may also encode some parameters of multi-joint, full-body behavior (e.g. Kalaska and Drew 1993; Donchin et al. 2002). Simultaneous recording of motor cortex activity and body biomechanics in freely moving animals is required to test this suggestion, however.

Methods for recording the activity of the motor cortex in freely moving animals have been developed (Girman 1973; Armstrong and Drew 1984; Beloozerova and Sirota 1986;
Buzsaki et al. 1989). Using these methods in cats revealed that the activity of most pyramidal tract neurons (PTNs) during locomotion on a flat surface is rhythmically modulated with respect to phases of the step cycle. A detailed movement analysis is more challenging in freely moving animals than during tasks with a limited number of degrees of freedom. For this reason, motor cortex activity during unconstrained movements typically has been analyzed in conjunction with muscle activation and simple kinematics of the contralateral limb (e.g. Drew 1988).

We believe that simultaneous recordings and analyses of motor cortex activity and full-body biomechanics during unconstrained animal behavior will provide a better understanding of motor cortex functions. PTNs, which have widespread connections onto the interneurons and motoneurons of a number of muscles of the contralateral limb (Lawrence et al. 1985; Shinoda et al. 1986; Georgopoulos and Grillner 1989; Leblond et al. 2001), are likely to control different aspects of movement through complex spinal networks. In addition, extensive branching of corticospinal axons in the brainstem and spinal cord (Futami et al. 1979; Armand 1982; Armand et al. 1985; Canedo 1997) suggests that PTN activity may affect not only the motor aspects of the contralateral limb but also those of the other three limbs. Examples of such effects might include full-body postural responses to voluntary gait modifications made by one limb during obstacle overstepping (Lavoie et al. 1995) and posture correction responses on a tilting surface (Beloozerova et al. 2005). Recently, encoding and decoding of global movement variables have been reported for neurons of the dorsal spinocerebellar tract (Bosco and Poppele 1997) and sensory neurons in the L6 and L7 dorsal root ganglia (Stein et al. 2004) of the cat. Thus, it seems logical to examine the correlation between the activity of neurons of the major cortical descending tract, the PTNs, and global aspects of movement, including kinetic variables (i.e., joint moments and power) that appear to be important for interpreting motor cortical activity.
Experimental methods that can provide detailed full-body movement analyses in conjunction with recordings of motor cortex activity are necessary for this.

In this report we describe a new experimental paradigm that combines in vivo recordings of full-body kinematics and kinetics with high quality, single-unit extracellular recordings from the motor cortex in the cat. This paradigm allowed for direct comparisons between a variety of mechanical variables and cortical activity in unconstrained conditions. We hypothesized that such comparisons may reveal new relationships between the activity of the motor cortex and mechanical aspects of locomotor behaviors.

**MATERIALS AND METHODS**

Recordings of full-body movement mechanics and the activity of the motor cortex and selected muscles of the fore- and hindlimbs were obtained from two adult cats, a male and a female. All surgical and experimental procedures conformed to the principles of the care and use of animals adopted by the American Physiological Society and approved by the Animal Care and Use Committees of Barrow Neurological Institute and the Georgia Institute of Technology.

*Locomotion task*

Positive reinforcement (food) was used to habituate cats to the experimental situation and to engage them in locomotion behavior (Skinner 1938; Pryor, 1975). During the first phase of training, cats were fed their total daily ration in the experimental walkway (Fig. 1) and were allowed to explore the walkway at their own pace. The walkway was 3.0 m long and 0.4 m wide, and a black rubberized material (NovaMat) covered the floor. The walkway had clear Plexiglas walls (height 0.6 m, thickness 0.005 m) along both long sides and wooden walls on the short sides at each end. A clear Plexiglas sheet in the middle of the walkway separated it into two
corridors along its length (Fig. 1 A, B). When animals exhibited content and confident behavior in the walkway, the second phase of the training began. Food was associated with a brief audible signal (5 kHz) during this phase. When the cats clearly showed an orientation toward the sound, training proceeded to the third phase, in which walking from one end of the walkway to the other along one corridor and returning using the other corridor was associated with the audible signal and food reward.

Surgical procedures

After each cat was trained to walk in the walkway, surgery was performed under aseptic conditions using Isoflurane anesthesia. The methods for introduction of recording and stimulation electrodes into the brain have been, in part, reported earlier (Beloozerova and Sirota 1986, 1993; Sirota et al. 1988; Beloozerova et al. 2003). After immobilization of the cat’s head in a stereotaxic head holder, a longitudinal incision was made in the skin on the top of the head. The skin was then retracted to the sides of the head. The fascia was removed from much of the dorsal surface of the skull. At ten points around the circumference of the head, holes of 1.5 mm diameter were drilled approximately orthogonal to the surface of the skull, and stainless steel screws were inserted into these holes (Fig. 2 A, B). To protect the dura from penetration, the screws had a restraining shoulder 4 mm from the tip, and the tips of the screws were rounded (Fig. 2 C). The screws protruded below the inner surface of the skull by less than 1 mm. The heads of the screws extended 15 mm above the surface of the skull and were soldered to a copper wire; this apparatus was used as an electrical zero point while recording the activity of cortical neurons and limb muscles. The screw heads and the wire were inserted into a plastic cast to form a circular base (Fig. 2 A.1). This base later allowed the head of an awake animal to be fixed in a head-restraining device so that neurons could be isolated before recording their activity during
locomotion. Such fixation does not damage the skull or surrounding tissues. The firmness of the fixation of the base to the skull was mainly determined by the radial location of the screws around the skull, which minimize the stress applied along the axis of any one screw. The top portion of the base was also used to affix connectors, miniature micro-drives, preamplifiers, and a protective and electrically shielding cap (Fig. 2D).

To access the left motor cortex with recording electrodes, a portion of the os frontale, os ethmoidale, and dura above the left motor cortex over approximately 1.0 cm² were removed (Fig. 2 A.5). The motor cortex was visually identified by surface features and photographed. The aperture was then covered by an acrylic plate (Fig. 2 E.1) 1 mm thick in which approximately 200 holes, 0.3 mm in diameter, had been drilled and filled with a mixture of sterile bone wax and Vaseline. The plate was fixed to the surrounding bone with acrylic cement. Recording microelectrodes were later inserted into the motor cortex of the awake animal through the holes in the plate.

To access the pyramidal tract with stimulating electrodes, two 26 gauge hypodermic tubes were implanted above the medullary pyramids at the Horsley and Clarke coordinates (P 10; L 0.5) and (P 9.3; L 1.2) (Fig. 2 A.6) at a depth of H 0. Stimulating electrodes (200 µm OD), constructed of platinum-iridium wire insulated with Teflon to within 0.4 mm of the tip were introduced into the medullary pyramid through the guide tubes several days after the surgery in awake animals under physiological guidance (see below).

Electromyographic (EMG) electrodes were constructed from Teflon-insulated multi-strand stainless-steel wire (AS633, Cooner Wire). A pair of these wires was inserted into each of the following muscles of the right fore- and hindlimbs (contralateral to the motor cortex): m. brachialis (BR), the lateral head of the m. triceps brachii (TR), m. tibialis anterior (TA), and m. soleus (SO). Electrode placement was verified by stimulation through the implanted wires before
closure of the incision and also later during the terminal experiment. The electrode leads were led subcutaneously to the inside of the head base.

All exposed surfaces of the skull were covered with a thin layer of orthodontic resin (Densply Caulk). The space between the skull, the retracted skin, and the bottom of the base was filled with silicone elastomer (Factor II) to form a soft barrier between the inside and outside of the base. A protective and electrically shielding cap was fixed on the top portion of the base by bolts (Fig. 2 D). The cat was shaved. Permanent ink marks were made on the skin above all major limb joints. The animal was then allowed to recover for several days.

*Sampling of neuronal and muscle activity*

Several days after the surgery, a cat was placed on a table equipped with a comforting pad and encouraged to take a “sphinx” position. After the cat rested in this posture for several minutes, the base attached to the skull during surgery was fastened to an external frame so that the resting position of the head was approximated. This procedure minimized stress on the neck while the head was immobilized. After a few training sessions, all cats sat quietly with their head restrained. They did not seem to be disturbed, as they frequently fell asleep.

Before the first recording session, connectors for neuronal and EMG pre-amplifiers, sockets for stimulating and EMG electrodes, and the main output connector were positioned permanently on the caudal portion of the base (Fig. 2D). One or two manual microdrives (Fig. 2E) with dimensions 5x5x30 mm or 2x3x10 mm were also attached to the base. Each micromanipulator had an arm (Fig. 2 E.4), the position of which over the motor cortical plate (Fig. 2 E.1) could be adjusted by bending it or by changing its location on the moving nut (Fig.2 E.10) of the micromanipulator.
A conventional extracellular tungsten varnish insulated microelectrode (50-125 µm OD, FHC) or a quartz insulated microelectrode with platinum-tungsten core (40 µm OD) (Reitboeck 1983) connected to a micro-manipulator was inserted into the motor cortex through a hole in the plastic plate and advanced into the cortical tissue (Fig. 2 E). After the electrode reached the cortical depth where clear responses of many neurons to limb movements could be observed (presumably layer V), two platinum-iridium wires (200 µm outer diameter, insulated with Teflon to within 0.4 mm of the tip) were slowly lowered into the medullar pyramid through the guide tubes implanted during surgery. Pulses of graded intensity (in the range of 0.1-0.5 mA, 0.2 ms duration), were delivered through this bipolar electrode. The wires were fixed at the positions that most effectively elicited antidromic responses in motor cortex neurons, and they served as the pyramidal tract-stimulating electrodes for the remainder of the experiments with the subject. The criterion for identification of antidromic activation was the test for collision of spontaneous and evoked spikes (Bishop et al. 1962; Fuller and Schlag 1976).

Neuronal and muscle activity were pre-amplified using miniature preamplifiers positioned on the head base. The activity was additionally amplified and filtered (300 – 10000 Hz band pass for neurons and 30 – 1000 Hz band pass for EMG) using a CyberAmp 380 (Axon Instruments) amplifier. Neuronal activity was sampled at 30 kHz and muscle activity at 3 kHz. All signals were displayed on a computer monitor and archived on a computer hard drive in a single file. A waveform analysis was employed on- and off-line to discriminate between and identify the spikes of the neurons using the Power1401/Spike2 system (Cambridge Electronic Design, Cambridge, UK) waveform-matching algorithm. The identities of the neurons were periodically verified during locomotion using the collision test. A representative record of the
discharges of two neurons projecting to the pyramidal tract (pyramidal tract neurons, PTNs) along with the EMG activity of selected muscles during six walking steps is shown in Fig. 3A.

**Video recordings**

Light reflecting markers (diameter 6 to 10 mm) were placed on the following previously marked spots on the cat’s body using sticky double sided tape: ventral iliac spine, greater trochanter, approximate knee joint center, lateral malleolus, base of the fifth metatarsal, end of the distal phalanx of the two hindlimbs, vertebral border of the scapula, the greater tubercle, approximate elbow joint, lateral styloid process, base of the fifth metacarpal, and end of the distal phalanx of the two forelimbs (Fig. 4). Also, two markers were placed on each side of the head at approximate intersections of the long axis of the head and perpendiculars from the lateral corner of the eye and the anterior edge of the ear (Fig. 4). The midpoint between the two markers corresponds to the center of mass location of the head (Hoy and Zernicke 1985).

Locomotion was recorded using two electronically synchronized, high-speed video cameras (Peak Performance Technologies, Inc.) with a rate of 60 or 120 frames per second. A video camera was placed on each side of the walkway with the two cameras aligned perpendicular to the plane of progression (Fig. 1B.2). Sources of light (Fig. 1B.3) were positioned behind the two cameras to illuminate the reflective markers on the cat. Plywood screens were suspended from the ceiling to prevent light from the opposite side of the walkway from being seen in the camera’s field of view (Fig. 1B.4). The camera positions allowed for recordings of two to four complete step cycles depending on locomotion speed. Calibration scales (two markers with a 1 m distance between them) in both corridors of the walkway were subsequently used to calculate horizontal and vertical coordinates of the markers on the cat’s body with respect to the laboratory coordinate system.
Ground reaction force recording

Two miniature force platforms (Broker and Gregor 1990; Fowler et al. 1993; Gregor et al. 2001) were concealed in the center of the right side of the walkway (Fig. 1A). Mechanically separated from the surrounding walkway floor, the force platforms measured the vertical, anterior-posterior, and medial-lateral components of the ground reaction force vector and the coordinates of its point of application. This information was used as input to the inverse dynamics analysis of fore- and hindlimb movements. Prior to the measurements, the force platforms were calibrated using known weights. The sampling rate of the ground reaction forces was 1000 Hz.

Video recordings, ground reaction forces, and cortex and muscle activity recordings were synchronized by a common electronic impulse that appeared on both video and analog records.

Terminal experiment and histological procedures

At the termination of the experiments, the cats were deeply anesthetized with pentobarbital sodium, and several reference electrolytic lesions were made in the recording area in the left motor cortex. Positions of EMG electrodes in the muscles were verified. Segments’ lengths as defined in Hoy and Zernicke (1985) were measured using an anthropometer. The cats were perfused with isotonic saline followed by a 10% Formalin solution. Frozen sections of 50-µm thickness were cut in the regions of the motor cortex and medullary pyramid. The tissue was stained for Nissl substance with cresyl violet. Positions of tracks in the cortex were estimated in relation to the reference lesions. Stimulating electrode locations in the medullary pyramid were verified.
Inverse dynamics analysis

Fourteen markers on each side of the body were digitized using the Peak Performance Motion Analysis System and its software. We developed software for a full-body inverse dynamics analysis. The marker coordinates were filtered using a fourth order, zero phase lag, Butterworth low-pass filter. The cutoff frequency of the filter was determined separately for the horizontal and vertical coordinates of each marker using Fourier (harmonic) analysis and the method described by Lanczos (1956), which is based on the different properties of the signal (true coordinates of a marker) and noise. The cutoff frequencies found were between 4 and 10 Hz. After filtering marker coordinates, the coordinates of the knee and elbow joints were calculated using the coordinates of the ankle and hip, and the wrist and shoulder markers, respectively, and the lengths of the forming segments that were measured during the terminal experiment. This was done to correct the recorded knee and elbow marker coordinates, which are significantly influenced by skin movements (Goslow et al. 1973). Velocity, acceleration, and jerk of the markers were calculated using numerical differentiation (Lanczos 1956).

Other kinematic variables also calculated for each body segment included the coordinates of the segments' center of mass locations; their velocities and accelerations; segment angles with respect to the vertical axis and their velocities and accelerations; and joint angles and their velocities. To calculate the center of mass location of each segment, the relative location of the center of mass reported by Hoy and Zernicke (1985) for major segments of the cat fore- and hindlimbs were used. The position, velocity and acceleration of the general center of mass of the cat body were subsequently calculated.

Resultant joint forces, moments, and their power at four joints of each limb during motor behaviors were calculated using an inverse dynamics analysis typically employed for kinetic analyses of a single limb (Fowler et al. 1993; Prilutsky and Zatsiorsky 1994; McFadyen et al.
1999; Gregor et al. 2001; Scott et al. 2001). A two-dimensional model of the cat body consisting of 21 rigid-body segments (Fig. 4) was used for the calculations. The segments were assumed to have constant inertial properties and to be connected by frictionless hinge joints. The mass and moment of inertia of each segment were calculated from the total body mass and segments' length using the empirical regression equations of Hoy and Zernicke (1985). Knowing the position and acceleration of the limb at each time instant, the length, mass, moment of inertia, center of mass location of each segment, and the magnitude, direction, and point of application of the external force, the resultant joint forces and moments at the metatarsophalangeal/metacarpalphalangeal, ankle/wrist, knee/elbow, and hip/shoulder joints were calculated (Fowler et al. 1993; Prilutsky and Zatsiorsky 1994; McFadyen et al. 1999; Gregor et al. 2001). Joint power at each joint was calculated as the product of joint moment and the corresponding joint velocity. In total, mechanical variables were computed as a function of time.

Comparison between motor cortex activity and movement biomechanics

Many computational methods have been used to investigate whether and how cortex activity is related to movement characteristics (e.g. Evarts 1968; Georgopoulos et al. 1982; Drew and Doucet 1991; Kakei et al. 1999; Moran and Schwartz 1999 and others). One of these methods, the cross-correlation analysis (for a recent review of its neurophysiological applications, see Hamm et al. 2001), in particular allows for assessing the strength of relationships between time-dependent activity of the PTN and time-dependent mechanical variables, and a time delay between them (Miller and Houk 1995; Schwartz and Adams 1995). To demonstrate how motor cortex activity can be compared with full-body mechanics, we employed a related statistical analysis, a multivariate linear regression analysis, which has several advantages. First, it takes into account possible correlations among the large number of
mechanical variables obtained in this study. Second, it allows for potential predictions of PTN activity as a function of biomechanical variables (neural encoding) or for reconstructions of biomechanical variables from the PTN ensemble activity (neural decoding) (e.g. Stein et al. 2004).

To conduct the multivariate regression analysis on neural and biomechanical data, histograms of the activity of 43 single PTN neurons were calculated for each analyzed step cycle of locomotion using a 10%-step cycle bin width. The onset of the swing phase of the right forelimb determined from ground reaction force and/or video recordings was taken as the beginning of the step cycle. The duration of each step cycle was divided into 10 equal bins, and the number of spikes in each bin was counted. The discharge frequency in a bin was derived according to the method of Udo et al. (1982), which averages the instantaneous frequency of inter-spike intervals that fall within the bin and also accounts for those intervals that overlap with the bin’s beginning and end. Subsequently, the histograms were averaged over 20 to 40 step cycles (see, for example, Fig. 3B) to obtain a resultant histogram. All kinematic, kinetic, and EMG variables of cat locomotion (299 in total) were also averaged over the same 10 bins as PTN activity.

Given the number of the PTN histograms (43) and averaged biomechanical variables (299), it was expected that some variables might be inter-correlated, i.e., the data set could be redundant. The degree of redundancy was determined by a principal component analysis of the 342 10-bin patterns with a variance maximizing rotation, which also classified the variables according to their patterns. The eigenvectors (principal components) were extracted according to Cattell’s criterion using STATISTICA (StatSoft, Inc.) software. The correlations calculated between each variable and the extracted factors (factor loadings) were considered significant when they exceeded a value of 0.7.
PTN activity encoding and decoding during walking was analyzed using multivariate linear regression models similar to those of Stein et al. 2004. The average firing rate \( f_i \) during a step cycle of the \( i \)-th PTN was calculated as a linear function of averaged mechanical variables \( g_j, j = 1,2,\ldots,n \) (PTN activity encoding): \[ f_i = a_{0i} + a_{1i} g_1 + a_{2i} g_2 + \ldots + a_{ni} g_n. \] The averaged magnitude of selected biomechanical variables \( g_j \) in a step cycle was calculated as a linear function of ensembles of PTN firing rates (PTN activity decoding): \[ g_j = b_{0j} + b_{1j} f_1 + b_{2j} f_2 + \ldots + b_{mj} f_m. \] The regression coefficients \( a \) and \( b \) were calculated using the forward entry method of stepwise linear regression, which finds the best subsets of independent variables (out of 299 for encoding and 43 for decoding) based on a correlation coefficient value. No time lag between PTN activity and mechanical variables was incorporated in these regression models because the bin duration (60 – 75 ms) exceeded the minimum time required for microstimulation of the motor cortex to modify EMG of the forelimb muscles in the walking cat (11 – 14 ms; Armstrong and Drew 1985) or the time required to initiate the early PTN responses to unexpected perturbation of locomotion (20 – 100 ms; Marple-Horvat et al. 1993).

RESULTS

Potential applications of the developed methodology will be illustrated below. First, we present and discuss the complete 2D full-body mechanics of cat locomotion, which was obtained for the first time in this study. We will consider only selected biomechanical variables in detail and will concentrate mainly on their patterns. Second, we will characterize patterns of PTN activity and biomechanical variables during walking as classified by the principal component analysis. Finally, the results of encoding and decoding mechanical variables by PTN activity will be presented.
Full-body biomechanics of walking

SELECTED KINEMATICS. During walking, all cats demonstrated typical patterns of stance-swing phase sequences (2 phases for each leg in a cycle, 8 phases in total; Figs. 5 – 7, panels 1 and 8). Depending on walking speed, the number of feet on the ground at the same time during the 8 phases of a cycle was 2-3-4-3-2-3-4-3 at speeds approximately 0.5 m/s and below (Figs. 5 – 7) and was 3-2-3-2-3-2-3-2 at faster walking speeds (not shown; see Prilutsky et al. 2001).

Joint angles of the hind- and forelimbs recorded in this study underwent phases of flexion and extension during walking step cycles similar to those reported by other authors (Goslow et al. 1973; Kuhtz-Buschbeck et al. 1994; Shen and Popple 1995; McFadyen et al. 1999; Smith et al. 1998). For example, the ankle, knee, wrist and shoulder joints yielded in the first half of stance and extended in the second half of stance. In swing, these joints flexed first and extended second (Figs. 5 and 6, panel 2). Thus, joint angle patterns at the ankle, knee, wrist and shoulder joints had two flexion and extension peaks in the step cycle. Metatarsophalangeal (MTP), metacarpophalangeal (MCP), and wrist joint angles had one flexion peak at the end of stance and one extension peak in the middle of swing. Hip joint angles reached their maximum flexion in the middle of swing and maximum extension at the end of stance (Figs. 5 and 6, panel 2).

Horizontal velocities of all hind- and forelimb segments had a bell-like shape with the velocity peaks occurring at mid-swing (see, for example, toe horizontal velocity in Figs. 5 and 6, panel 3). Horizontal velocity peaks ranged between 0.5 – 1.0 m/s for proximal segments (scapula, thigh, upper arm, shank) and 1.2 – 2.5 m/s for distal segments (forearms, tarsals, carpals, and digits). The vertical velocity of limb segments ranged between ± 2.5 m/s and had more complex patterns with one negative and two positive peaks.
The jerk (the 3rd time derivative of displacement) was also calculated. For example, the horizontal jerk of the forelimb paw had two positive and negative peaks in the step cycle. The largest, negative peak approaching -300 – -400 m/s^3 occurred approximately in the middle of the swing phase and coincided with joint angle peaks (Figs. 5 and 6, panel 3).

Positive peaks of vertical velocity of the general center of mass appeared to coincide with mid-stance of hindlimbs, whereas negative peaks occurred in late stance (Fig. 7, panels 4 and 8). Patterns of horizontal velocity of the general center of mass were less stereotyped and dependent on whether the cat maintained a constant average speed of progression.

SELECTED KINETICS. The horizontal and vertical components of the ground reaction forces applied to the hind and fore digits had patterns typical for mammalian locomotion (Fig. 7, panel 5). During the entire stance phase the vertical force was positive and reached values of 13–16 N (for hindlimbs) and 18–22 N (for forelimbs). The horizontal force component was negative during the first half of stance (i.e., contributed to deceleration of the body) and positive in the second half. Horizontal force peaks were between ±2 and ±6 N depending on locomotion speed. The higher forelimb vertical forces compared to the hindlimb forces (Fig. 7, panel 5) are consistent with literature reports and result from the general center of mass being located closer to the forelimbs (see also Manter 1938; Alexander 1980; Pandy et al. 1988).

Because of higher forelimb forces, the absolute values of joint moment peaks were generally larger for forelimbs than hindlimbs (Figs. 5 and 6; see also Manter 1938; Pandy et al. 1988). Patterns of moments and powers at hindlimb joints (Fig. 6) are also consistent with those reported for locomotion in cats by Fowler et al. (1993), McFadyen et al. (1999), and Gregor et al. (2001). In particular, values of moments and power at the hindlimb joints (Fig. 6, panels 4, 5) were relatively small during the swing phase of locomotion. During initial stance, all joints
exerted extensor moments, and the MTP, ankle and knee joints absorbed mechanical energy (joint power was negative). In the second part of stance, all joints except the hip generated positive power contributing to cat propulsion.

Similar patterns of joint moments and power were observed at the forelimb joints (Fig. 5, panels 4 and 5). The MCP, wrist, and elbow exerted mostly extensor moments with their peaks around mid-stance. The shoulder moment changed direction from extensor to flexor in the middle of stance, whereas the elbow moment switched from extensor to flexor at the end of stance. Among the forelimb joints, the elbow contributed the most to positive power generation and thus to forward propulsion (Fig. 5, panel 5).

EMG ACTIVITY. Increased activity of an elbow extensor, the lateral head of the *m. triceps brachii* (TR), coincided with an extensor elbow moment during stance, suggesting its agonist role at this joint in stance. An elbow flexor, *m. brachialis* (BR), was active at the end of stance and early swing, which also coincided with a flexor elbow moment (Fig. 5, panel 6). The ankle extensor *m. soleus* (SO) was primarily active during the generation of the extensor (plantar flexor) moment at the ankle in stance (Fig. 6, panel 6).

*Patterns of neural and biomechanical variables during walking*

The principal component analysis conducted on PTN activity histograms and on biomechanical variables averaged over 10 bins of a step cycle revealed four factors that accounted for 91% of the variance (Table 1). As a result, many neural and biomechanical variables were classified by the analysis into one of the four groups. Variables within each group had high factor loadings (> 0.7), i.e. high negative or positive correlations with the corresponding eigenvectors. The first group accounted for 34% of the variance (Table 1) and included
histograms of 13 PTNs and 106 biomechanical variables (e.g., segments’ angular orientations, BR EMG, forelimb joint angles and moments, etc.) whose patterns had one broad maximum/minimum in swing/stance (Fig. 8A). The second factor accounted for 27% of the variance (Table 1) and consisted of histograms of 8 PTNs and 69 biomechanical variables (e.g., ground reaction force, joint velocities, etc.) with a maximum/minimum at the stance-swing transition (Fig. 8B). Variables corresponding to the third factor (2 PTNs and 16 biomechanical variables: e.g., toe jerks, displacement of the general center of mass (GCM), etc.) accounted for 16% of the variance and had a more complex pattern: two maximums and two minimums in early/late swing and stance (Fig. 8C). The fourth factor included 1 PTN and 28 mechanical variables (e.g. joint velocity, linear velocity of the GCM) and also had two minimums and maximums in the step cycle (Fig. 8D). This factor accounted for 14% of the variance (Table 1).

The patterns of 19 PTNs (out of 43, or 44%) and 80 biomechanical variables (out of 299, or 27%) accounted for less than 10% of the variance and were not classified into the four groups.

*Encoding and decoding mechanical variables in/by PTN activity*

The ensemble activity of different groups of a relatively small number of PTNs (typically between 2 and 8) could predict patterns of any biomechanical variable with very high accuracy ($R^2=0.99$). Selected examples of predicted variables (toe jerk of the right forelimb, left shoulder moment, triceps EMG) are shown in Fig. 9 A,B,C. Neuron ensembles decoding biomechanical variables typically included at least one neuron from the group to which the biomechanical variable belonged (see Fig. 8). Each PTN contributed to decoding of at least one biomechanical variable. No other trends in neural decoding were apparent.

Similarly, the activity of each PTN averaged over 10 bins of a step cycle was accurately predicted by a relatively small number of biomechanical variables (typically between 2 and 7).
For example, the vertical ground reaction force under the right hindlimb, MCP power at the left forelimb, and horizontal acceleration of the trunk predicted the firing rate of PTN 1 (Fig. 9D); angular orientation of the head/neck segment and power at the left hip joint predicted the firing rate of PTN 9 (Fig. 9E); and a combination of trunk orientation, MCP joint power of the right forelimb, horizontal velocity of the right scapular, and angular acceleration of the left shank predicted the activation of PTN 29 (Fig. 9F).

Thus, the 10-bin pattern of any biomechanical variable of walking obtained in this study could be predicted by ensemble activity of a relatively small number of PTNs. Likewise, the 10-bin firing rate pattern of any of the 43 PTNs recorded here could be predicted by a relatively small number of biomechanical variables.

**DISCUSSION**

This manuscript describes a new experimental paradigm that combines the synchronized recordings of full-body mechanics and motor cortex activity during unrestrained locomotion in cats. This is the first report on a method of such simultaneous recordings.

*Biomechanics of full-body locomotion*

Although the inverse dynamics analysis employed here is well established, in studies of quadrupedal locomotion it has been primarily performed either on one hindlimb (Fowler et al. 1993; McFadyen et al. 1999; Gregor et al. 2001) or on the hind- and forelimb of one side of the body (Manter 1938; Pandy et al. 1988). Only a limited number of biomechanical variables from the 299 obtained in this study can be found in and compared with the published data. This comparison demonstrated a general agreement with the published data (see the Results section), but some differences exist.
In accordance with the results of Manter (1938), our positive and negative peaks of horizontal accelerations of hindlimb segments occurred in late and early stance, respectively. However, contrary to Manter’s data, our results suggest a clear dependence of acceleration peaks on segment proximity (the more distal the segment, the higher the acceleration peak; Fig. 7, panel 3 and Manter’s Fig. 5d). Also, Manter’s horizontal acceleration peaks ($\pm 10 \text{ m/s}^2$ for the shank and thigh and $\pm 20 \text{ m/s}^2$ for the hind foot) are substantially higher than ours ($\pm 3$ to $\pm 12 \text{ m/s}^2$, Fig. 7, panel 3) for about the same walking speeds. Different methods of estimating the time derivatives probably cause these differences. Manter used graphic differentiation, which is not an accurate method for obtaining velocities and accelerations (Lanczos 1956). This also can explain differences in peak values of segments’ angular velocities (see our data in Fig. 7, panel 2; Manter’s are in Fig. 6a of his paper). The patterns of angular segment velocities were similar, however; peak positive values occurred in the middle of swing.

The pattern of vertical velocity of the center of mass presented in Fig. 7, panel 4, is similar to that reported by Manter, but he did not determine the coordinates of the center of mass directly. He used a marker on the trunk near the assumed center of mass instead.

Patterns of neuronal activity and biomechanical variables, and their correlations

Which mechanical or physiological variables are encoded by motor cortex activity has been a central question in motor cortex physiology for decades. Many researchers have correlated cortex activity with different mechanical parameters and muscle activity (Evarts 1968; Georgopoulos et al. 1982; Houk et al. 1987; Scott and Kalaska 1997; Sergio and Kalaska 1997; Moran and Schwartz 1999; Reina et al. 2001; Holdefer and Miller 2002; Scott et al. 2001). Since many physiological (e.g. EMG) and mechanical variables (e.g. joint moments, limb velocity and acceleration) are correlated with each other (as also demonstrated in this study; Fig. 8), it is not
easy to elucidate the primary variables encoded in motor cortex activity. This might partly explain why this problem remains unresolved despite significant effort. In addition, the motor cortex contains groups of cells that appear to encode different aspects of motor behavior, including non-motor aspects, in both extrinsic and different intrinsic (joint angle, muscle) coordinates (Alexander and Crutcher 1990; Carpenter et al. 1999; Kakei et al. 1999). Some of these cells might be among the 19 PTNs not classified into the four groups by the principal component analysis in this study. Still another problem is that the discharge of some PTNs appears to encode different parameters under different conditions (e.g. Thach 1978; Sergio and Kalaska 2003; Kakei et al. 2003; Beloozerova et al. 2005).

Despite their limitations, correlation and in particular multivariate regression analyses (Houk et al. 1987; Holdefer and Miller 2002, Schwartz and Adams 1995; Hamm et al. 2001; Stein et al. 2004) have been used extensively and have provided valuable information regarding representations of movement variables in motor cortex activity.

The developed method of simultaneously recording PTN activity and full-body mechanics provided new information on possible relationships between motor cortex activity and movement biomechanics. The fact that 73% of the full-body mechanical variables were highly correlated with one of the four eigenvectors, as revealed by the principal component analysis (Fig. 8), indicates that these patterns are redundant, i.e., many of the variables are closely correlated. As a consequence, a comparison of a limited number of mechanical variables with neural activity might yield misleading results. On the other hand, activation patterns of only 56% of sampled PTNs could be related to the four eigenvectors. The remaining 44% of neurons have patterns that differ from the majority of full-body mechanical variables and might encode information that is not directly related to motor patterns. These PTN activation patterns could also be related to muscles not sampled in this study.
Given the great number of mechanical variables obtained in this study, perhaps it is not surprising that it was possible to find subsets of variables that could accurately predict the firing rate of each sampled PTN. Accurate predictions of mechanical variables by the ensemble activity of a small number of PTNs could also be explained by the majority of these variables having relatively simple patterns (with one to three peaks per cycle). However, whether or not these predictions are real and not caused by chance will require further studies.

**Limitations and advantages of the method**

The main limitations of the described experimental approach are the high complexity of the employed experimental procedures and the huge volume of information to be analyzed. The experimental procedures involve a combination of two innovative methods, analysis of full-body mechanics and recording of PTN activity in unrestrained animals, and thus require expertise in both areas of research. The motion analysis requires digitizing the coordinates of 28 markers on the animal body and is time consuming, but it can be substantially simplified by using motion analysis systems with automatic digitizing capabilities, which are commercially available. The results presented in this report show that despite the limitations, the new experimental approach is manageable and can yield unique and useful data on the functions of PTNs.

In particular, the developed method allowed us for the first time (i) to compare the kinetics (ground reaction forces, joint forces, moments, power) of the contralateral limb with PTN activity during unconstrained cat locomotion, (ii) to obtain and compare PTN activity with the kinematics and kinetics of the other three limbs, and (iii) to obtain and compare PTN activity with movement characteristics of the general center of mass in the cat. These new developments have the potential to reveal new information about the functions of the motor cortex. We wish to
stress, however, that any single method must be complemented with other techniques to obtain any definite information regarding physiological functions.

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REFERENCES


Holdefer RN and Miller LE. Primary motor cortical neurons encode functional muscle

Houk JC, Dessem DA, Miller LE, and Sybirskia EH. Correlation and spectral analysis of
relations between single unit discharge and muscle activity. J Neurosci Methods 21: 201-224,
1987.

Hoy MG and Zernicke RF. Modulation of limb dynamics in the swing phase of locomotion. J

Kakei S, Hoffman DS, and Strick PL. Muscle and movement representations in the primary

Kakei S, Hoffman DS, and Strick PL. Sensorimotor transformations in cortical motor areas.

Kalaska JF and Drew T. Motor cortex and visuomotor behavior. Exercise Sport Sci Rev 21:

Kuhtz-Buschbeck JP, Boczek-Funcke A, Illert M, and Weinhardt C. X-ray study of the cat


Lavoie S, McFadyen B, and Drew T. A kinematic and kinetic analysis of locomotion during

Lawrence DG, Porter R, and Redman SJ. Corticomotoneuronal synapses in the monkey: light
microscopic localization upon motoneurons of intrinsic muscles of the hand. J Comp Neurol

Leblond H, Menard A, and Gossard JP. Corticospinal control of locomotor pathways


FIGURE LEGENDS

Figure 1. Experimental setup. A: The walkway with two force platforms (view from above). A black rubberized material (NovaMat) covered the floor, and three clear Plexiglas sheets formed longitudinal walls and a middle divider of the walkway. Cats walked counterclockwise along the two corridors in the direction indicated by arrows. After completing each round, the cats received a few pellets of dry cat food in the corner of the walkway and then proceeded to the next round. B: View perpendicular to the direction of cat progression. (1) walkway with two transparent Plexiglas walls and a transparent divider in the middle; (2) two high-speed, synchronized video cameras; (3) sources of light for illuminating reflective markers on the cat; (4) plywood screens that block light from the camera on the opposite side; (5) flexible cable.

Figure 2. Assembly for recording cortical activity in freely walking cat. A: The skull with 10 screws implanted around its circumference approximately orthogonal to the skull surface (top view). The heads of the screws were inserted into a circular plastic cast that formed a base (1). The base had nut holes (2, 3) for fixation to the head restraining device and for fixation of the protective and shielding cap. The surface of the skull inside the base (4) was covered with a thin layer of orthodontic resin. An approximately 1.0 cm² opening (5) was made in the skull above the left motor cortex, and the removed bone was replaced with a plastic plate that allowed insertion of recording electrodes into the motor cortex (1 in E, also see the text). An approximately 1.5 mm diameter opening (6) was made in the skull above the left medullary pyramid to provide access for stimulating electrodes (see the text). B: The skull with 10 screws implanted around its circumference (view from the back). C: Drawing of the screw with the dimensions indicated. D: The base with a variety of connectors and the shielding cap fixed to it (view from the back). E: The method of insertion and advancement of electrodes into the motor
cortex. (1) A plastic plate that replaces the bone over the motor cortex has ~200 predrilled openings of 0.3 mm O.D.; openings are filled with wax. (2) An electrode is manually inserted into one of the openings and soldered (3) to an arm (4) of a micromanipulator. A thin multistrained wire (5) that leads to a connector for a preamplifier in the back of the base (see D) is also soldered to the arm (6). In the micro-manipulator, the screw (8) is held by a frame (9) that is fixed to the plastic cover of the skull (4 in A) or to the base (1 in A). As the screw turns, a nut (10) with the arm (4) soldered to it moves up or down, gliding along the frame (9) and moving the electrode (2) up or down in the brain. One revolution of the screw results in a 150-200 μm advancement of the electrode depending on the screw thread.

**Figure 3.** Examples of pyramidal tract neuron (PTN) activity during walking. **A:** A raw recording of activity of PTN 42 (from the forelimb area of the motor cortex) and 43 (from the hindlimb area of the motor cortex) along with EMGs of the right (contralateral) *m. brachialis* (BR), the lateral head of right *m. triceps brachii* (TR), and the right *m. soleus* (SO) during six steps. **B:** Activity of the same PTNs recorded during 20 steps, presented in raster and histogram formats. In the rasters, the step cycle duration is normalized to 100%, and the raster is rank-ordered according to the duration of the swing phase. Swing (Sw) is separated from stance (St) by an open circle. The dashed line indicates the mean beginning of the stance phase. The vertical scale bar equals 30 imp/s.

**Figure 4.** Definitions of body segments, joint angles and segments’ orientation. Two-dimensional model of the full body of the cat. It consists of two hindlimbs (comprised of four segments each: digits, tarsals, shank, thigh), two forelimbs (comprised of five segments each: digits, carpals, forearm, upper arm, scapular), pelvis, trunk with neck, and head. Each segment is
assumed to be a rigid body with known mass, center of mass location, and moment of inertia at the center of mass. Segments are linked by frictionless hinge joints. Black dots indicate positions of reflective markers on each side of the body. Arcs connecting adjacent segments define joint angles.

**Figure 5.** Selected kinematic and kinetic variables of the right forelimb (RF) and corresponding EMG and PTN activity during two consecutive cycles of walking. From top to bottom: (1) Cat stick figures (thicker solid lines show the right-side limbs, trunk and head; thinner solid lines show the left-side limbs; dashed thicker and thinner lines denote the ground reaction force vector applied to the right or left limb, respectively). (2) Angles at the metacarpophalangeal (dashed line), wrist (thin line), elbow (dot-dashed line), and shoulder (thick line) joints; see Fig. 4A for definition of angles. (3) Horizontal velocity and jerk of the toe of the RF. (4) Moments at the metacarpophalangeal, wrist, elbow, and shoulder joints (negative/positive values correspond to extension/flexion at the joints (plantar/dorsi flexion for the MCP and wrist)). (5) Power at the metacarpophalangeal, wrist, elbow, and shoulder joints (negative/positive power corresponds to absorption/generation of power). No joint moments and powers were calculated for the second step cycle because in this cycle the cat RF did not touch the force plate. (6) EMGs of right *m. brachialis* (BR) and the lateral head of the right *m. triceps brachii* (TR). (7) Activity of PTN 42 shown in the form of standard pulses. (8) Step diagrams, where stance is represented by black line and swing by no line; LF -left forelimb, LH - left hindlimb, RF - right forelimb, and RH - right hindlimb. Vertical solid lines indicate RF toe take-off; interrupted lines indicate RF touch down.
**Figure 6.** Selected kinematic and kinetic variables of the right hindlimb (RH) and corresponding EMG and PTN activities during two consecutive cycles of walking. In all the panels, except where indicated, dashed lines represent MTP joints, thin lines represent ankle joints, dot-dashed lines represent knee joints, and thick lines represent hip joints. SO indicates EMG of the right *m. soleus*. Other designations are as in Figure 5.

**Figure 7.** Selected kinematic and kinetic variables of the left forelimb (LF), left hindlimb (LH) and the general center of mass presented along with integrated EMGs of the muscles and histograms of PTN activity during two consecutive cycles of walking. From top to bottom: (1) Cat stick figures. (2) Angular velocity of metacarpals, forearm, upper arm and scapular of the LF (positive values correspond to counterclockwise segment rotation with respect to the proximal joint). (3) Horizontal acceleration of hind digits, metatarsals, shank and thigh of the LH. (4) Horizontal (*V_x*) and vertical (*V_y*) velocity of the general center of mass. (5) Horizontal (subscript x) and vertical (subscript y) components of the ground reaction force vector applied to the right forelimb (RF), left forelimb (LF), right hindlimb (RH) and left hindlimb (LH). (6) Integrated EMG of the right *m. brachialis* (BR) and the lateral head of the right *m. triceps brachii* (TR). (7) 10-bin/cycle-histograms of activity of two PTNs that are depicted in Fig. 3. Vertical bars correspond to 100 imp/s. (8) Step diagrams for the LF, LH, RF, and RH limbs in which stance is represented by black line and swing by no line.

**Figure 8.** Four groups of patterns of PTN activity and full-body biomechanical variables in walking revealed by principal component analysis. A: Patterns of neural activation (left panel) and selected mechanical variables (right panel) that are highly correlated with the first eigenvector. BrR, EMG of the right brachioradialis; DiR, angular orientation of the right
hindlimb digits; ThR, angular orientation of the right thigh; WrL and McL (open symbols), left wrist and left MCP angles; WrL and ElL (closed symbols), left wrist and elbow moments. The variables within this group account for 34% of the variance and include 13 PTNs and 106 biomechanical variables. B: Patterns of neural activation (left panel) and selected mechanical variables (right panel) that are highly correlated with the second eigenvector. WrR and HiL, joint velocities of the right wrist and the left hip; FyRH, the vertical component of the ground reaction force under the right hindlimb; ElR, power at the right elbow; DyTrRH and DyDiRH, vertical displacements of the right hindlimb digits and tarsals. The variables within this group account for 27% of the variance and include 8 PTNs and 69 biomechanical variables. C: Patterns of neural activation (left panel) and selected mechanical variables (right panel) that are highly correlated with the third eigenvector. DyGCM, vertical displacement of the general center of mass; JxRH and JxLH, horizontal jerk of the toes of the left and right hindlimbs. The variables within this group account for 16% of the variance and include 2 PTNs and 16 biomechanical variables. D: Patterns of neural activation (left panel) and selected mechanical variables (right panel) that are highly correlated with the fourth eigenvector. VyTiLH, VyTiRH, vertical velocities of the left and right thighs; VyGCM, vertical velocity of the general center of mass; ShoL, joint velocity of the left shoulder. The variables within this group account for 14% of the variance and include 1 PTN and 28 biomechanical variables. The vertical dashed lines indicate the end of swing and beginning of stance phase. The standard deviations are not shown because their values in most cases correspond to the symbol size.

**Figure 9.** Examples of PTN activity decoding (A,B,C) and encoding (D,E,F) mechanical variables averaged over 10 bins of a simple locomotion cycle. The solid and dashed lines are recorded and predicted patterns, respectively. A: Horizontal jerk of the right hindlimb toes
predicted from the ensemble activity of 5 PTNs using a multivariate linear regression model ($R^2=0.99$). **B**: Moment at the left shoulder predicted from the ensemble activity of 4 PTNs ($R^2=0.99$). **C**: EMG of the right triceps predicted from the ensemble activity of 5 PTNs ($R^2=0.99$). **D**: The firing rate of PTN 1 predicted from 3 biomechanical variables ($R^2=0.99$): the vertical ground reaction force under the right hindlimb, power at the left MCP, and horizontal trunk acceleration. **E**: The firing rate of PTN 9 predicted from the angular orientation of the neck/head segment and power at the left hip joint ($R^2=0.99$). **F**: The firing rate of PTN 29 predicted from the angular orientation of the trunk, power at the right MCP joint, horizontal velocity of the right scapular, and angular acceleration of the left shank. The vertical dashed lines indicate the end of swing and beginning of stance.
**Table 1.** Results of the principal component analysis of the neural and motor patterns calculated for 10 bins of a walking cycle. Eigenvalues and the accounted total and cumulative variance.

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Fig. 5

- (1) Velocity
- (2) Joint angle, deg
- (3) Velocity
- (4) Jerk
- (5) Joint moment, Nm
- (6) Joint power, W
- (7) BR, TR, PTN
- (8) LF, LH, RF, RH

Time: 0.0 - 1.5 s
Fig. 6
Fig. 8 A, B
Fig. 8 C, D
Fig. 9

A

B

C

D

E

F

Cycle time, bin

Cycle time, bin