Body stability and muscle and motor cortex activity during walking with wide stance

Brad J. Farrell, Margarita A. Bulgakova, Irina N. Beloozerova, Mikhail G. Sirota, and Boris I. Prilutsky

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Farrell BJ, Bulgakova MA, Beloozerova IN, Sirota MG, Prilutsky BI. Body stability and muscle and motor cortex activity during walking with wide stance. J Neurophysiol 112: 504–524, 2014. First published April 30, 2014; doi:10.1152/jn.00064.2014.—Biomechanical and neural mechanisms of balance control during walking are still poorly understood. In this study, we examined the body dynamic stability, activity of limb muscles, and activity of motor cortex neurons [primarily pyramidal tract neurons (PTNs)] in the cat during unconstrained walking and walking with a wide base of support (wide-stance walking). By recording three-dimensional full-body kinematics we found for the first time that during unconstrained walking the cat is dynamically unstable in the forward direction during stride phases when only two diagonal limbs support the body. In contrast to standing, an increased lateral between-paw distance during walking dramatically decreased the cat’s body dynamic stability in double-support phases and prompted the cat to spend more time in three-legged support phases. Muscles contributing to abduction-adduction actions had higher activity during stance, while flexors muscles had higher activity during swing of wide-stance walking. The overwhelming majority of neurons in layer V of the motor cortex, 82% and 83% in the forelimb and hindlimb representation areas, respectively, were active differently during wide-stance walking compared with unconstrained condition, most often by having a different depth of stride-related frequency modulation along with a different mean discharge rate and/or preferred activity phase. Upon transition from unconstrained to wide-stance walking, proximal limb-related neuronal groups subtly but statistically significantly shifted their activity toward the swing phase, the stride phase where most of body instability occurs during this task. The data suggest that the motor cortex participates in maintenance of body dynamic stability during locomotion.

Body stability; muscle and motor cortex activities; PTNs

The ability to control body balance and stability during locomotion is essential to prevent falls and recover from perturbations. Maintenance of stable standing and locomotion is complicated by injury (Day et al. 2012; Duong et al. 2004; Holder-Powell and Rutherford 2000), aging (Schrager et al. 2008), fear of falling (Chamberlin et al. 2005; Dunlap et al. 2012), and other factors. Several motor strategies have been found to help maintain stability while walking on complex terrain. They include 1) reducing walking velocity (Chamberlin et al. 2005; Dingwell et al. 2000; Gálvez-López et al. 2011; Maki 1997), 2) reducing stride length and increasing stance width (Dunlap et al. 2012; Maki 1997; Misiaszek 2006), and 3) lowering the center of mass (Gálvez-López et al. 2011; McAndrew Young et al. 2012; Schmidt and Fischer 2010) and prolonging the double-support phase (Chamberlin et al. 2005; Maki 1997). While stability of the human body during locomotion has been analyzed in detail, little is known about static and dynamic stability of quadrupedal animals, particularly the cat, an animal model widely used to study neural control of locomotion. Therefore, the first goal of this study was to characterize body stability of the cat during unconstrained walking on a flat surface. We hypothesized that while the cat body was stable in the sagittal plane at all times during normal walking, it would experience transient periods of lateral (frontal plane) instability during ipsilateral double-support phases.

Complex natural environments present significant challenges to the postural control system of walking animals, including humans. Of all the strategies used to maintain stable locomotion in such environments noted above, increasing stance width is the one that can be easily employed to improve stability in the frontal plane. Indeed, a larger stance width increases support area when more than one foot is in contact with the ground and may improve gait stability (see, e.g., Hof et al. 2005; McAndrew Young and Dingwell 2012), whereas decreasing the stance width when walking on a narrow path destabilizes gait in humans (McAndrew Young and Dingwell 2012), rats (Schmidt and Fischer 2010), and dogs (Gálvez-López et al. 2011) but not in cats, as shown in a recent study by Gálvez-López and colleagues (2011) and in our preliminary report (Farrell et al. 2011). The latter results suggest that in cats maintenance of body balance in the frontal plane may be organized differently. Thus the second goal of this study was to characterize in detail the body stability of the cat and contributing activities of limb muscles during walking with a wide lateral distance between paws (wide-stance walking). We hypothesized that during wide-stance walking the cat’s body stability would be higher than during walking with a self-selected (unconstrained) between-paw distance. We further hypothesized that muscles contributing to frontal-plane joint moments would show altered activity patterns during wide-stance walking.

While the contribution of passive body mechanics to regulation of balance during standing and locomotion may be substantial (Bunderson et al. 2010; Scrivens et al. 2006; Ting et al. 2009), active mechanisms of the spinal cord, brain stem, and motor cortex are critically important for maintaining stable posture and locomotion (Beloozerova et al. 2005; Honeycutt and Nichols 2010; Karayannidou et al. 2009; Macpherson and...
Fung 1999; Musienko et al. 2012; Stapley and Drew 2009; Zelenin et al. 2010). In our previous studies we found that in standing quadruped animals, the rabbit and the cat, neurons from both fore- and hindlimb representations in the motor cortex modulate their activity in response to lateral tilts of the supporting surface (Beloozerova et al. 2003b, 2005). It is also known that during walking neurons in the motor cortex modulate their discharges in respect to the stride cycle (e.g., Armer et al. 2013; Beloozerova et al. 2003a; Beloozerova and Sirota 1993a, 1993b; Drew 1993; Fitzsimmons et al. 2009; Karayannidou et al. 2009; Prilutsky et al. 2005; Stout and Beloozerova 2012, 2013; Widajewicz et al. 1994). Recently we have found that when the surface on which the cats walked was tilted laterally more than half of pyramidal tract projecting neurons (PTNs) changed the magnitude of their locomotion-related activity modulation compared with walking on a level surface, thereby responding to this perturbation of posture during walking (Beloozerova et al. 2005; Karayannidou et al. 2009). In that study, however, the base of support during locomotion in different conditions was not analyzed. Thus it remains unclear whether the cat’s body stability was actually diminished in the task of walking on the inclined surface, and whether cortical neurons responded to a change in demand for the body balance in the frontal plane. Therefore, the third goal of this study was to examine the activity of neurons in the motor cortex, an area known to contribute to control of both locomotion and posture, during walking with both unconstrained and wide-between-paw distance. We hypothesized that walking with a wide between-paw distance would be associated with lower demands for active cortical contribution to balance control in the frontal plane.

We found, quite unexpectedly, that during normal unrestrained walking on a level surface the cat’s body was dynamically unstable in the sagittal plane twice per gait cycle: immediately before ground contact by each forepaw (phases of single support) laterally more than half of pyramidal tract projecting neurons responded to the demand to maintain compromised body stability of locomotion-related frequency modulation, apparently in the motor cortex changed their activity, most often the depth of locomotion-related frequency modulation, and wide separation of limbs (paw separation > 9 cm, wide-stance walking). One cat (cat Zv, Table 1) was trained to perform the same tasks on a treadmill. The walking path for wide-stance walking was created by placing a 9 cm wide × 4 cm tall triangular prism in the center of the walkway or treadmill, thus leaving areas for paw placement on either side of the prism separated by at least 9 cm in the frontal plane (Fig. 1A). Cats that participated in cortical recording experiments were trained to wear a cotton jacket, a light backpack with connectors, and an electromechanical sensor on the right forepaw for recording swing and stance phases of the stride cycle (Beloozerova and Sirota 1993a).

During each experimental session, both the unconstrained and wide-stance walking tasks were recorded without changing markers or the EMG cable. The order of tasks was randomized between sessions and animals. Recordings lasted for several weeks 5 days a week until a sufficient number of trials was collected.

**Surgical Procedures**

After animals were trained, they were all implanted with EMG electrodes in selected limb muscles and two cats additionally were implanted with guide tubes for access to the motor cortex and pyramidal tract (for details see Beloozerova et al. 2010; Prilutsky et al. 2005, 2011). In summary, the surgery was performed in aseptic conditions and under general anesthesia (ketamine 10 mg/kg sc, atropine 0.05 mg/kg sc, and isoflurane inhalation, induction at 5%, maintained at 1–3%). The animal was continuously monitored for temperature, respiration, heart rate, and blood pressure throughout the surgery. All limbs, back, and skull were shaved and cleaned with a surgical disinfectant. Skin incisions were made in the skull, lower back, thigh and shank, and forearm and upper arm. Teflon-insulated multistranded stainless steel wires (100-μm diameter; Cooner Wire) were passed subcutaneously along the back from a connector mounted on the skull to the muscles of interest. Pairs of wires with a small strip of insulation removed were secured in the midbelly of each muscle 5–10 mm apart. The implanted muscles included soleus (SO), ankle extensor; medial (MG) and lateral (LG) gastrocnemii, ankle extensors and knee flexors; tibialis anterior (TA), ankle flexor; vastus medialis (VM) and lateralis (VL), knee extensors; rectus femoris (RF), knee

### Table 1. Animal characteristics and analyses conducted

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Hindlimb length is defined as the sum of tarsal, shank, and thigh lengths; forelimb length is defined as the sum of carpal, forearm, and upper arm lengths.

**METHODS**

**Ethical Approval**

All experimental and surgical procedures were consistent with US Public Health Service Policy on Humane Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committees of Georgia Institute of Technology and Barrow Neurological Institute.

**Locomotion Tasks**

With positive reinforcement by food, seven adult female cats (Table 1) were trained to walk on a Plexiglas-enclosed walkway (3.0 × 0.25 m²) with self-selected stance width (unconstrained walking) and wide separation of limbs (paw separation > 9 cm, wide-stance walking). One cat (cat Zv, Table 1) was trained to perform the same tasks on a treadmill. The walking path for wide-stance walking was created by placing a 9 cm wide × 4 cm tall triangular prism in the center of the walkway or treadmill, thus leaving areas for paw placement on either side of the prism separated by at least 9 cm in the frontal plane (Fig. 1A). Cats that participated in cortical recording experiments were trained to wear a cotton jacket, a light backpack with connectors, and an electromechanical sensor on the right forepaw for recording swing and stance phases of the stride cycle (Beloozerova and Sirota 1993a).

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Extensor and hip flexor; sartorius medial (SAM), knee and hip flexor; iliopsoas (IP), hip flexor; biceps femoris anterior (BFA), hip extensor; biceps femoris posterior (BFP), hip extensor and knee flexor; adductor femoris (magnus) (AF), hip adductor and extensor; gluteus medius (GLM), hip abductor and extensor; triceps brachii lateral head (TRL), elbow extensor; extensor digitorum communis (EDC), wrist and digits dorsiflexor; and flexor carpi ulnaris (FCU), wrist plantar flexor. EMG electrode placements were verified by electrical stimulation through their leads. Length of each limb segment and width of limb joints were measured with a caliper.

In two cats that were prepared for recording of the activity of the motor cortex, a portion of the skull and dura above the left motor cortex was removed and the motor cortex was identified by the surface features and photographed (Fig. 2A). The exposed motor cortex was covered by a 1-mm-thick acrylic plate with predrilled holes of 0.36-mm diameter spaced 0.5 mm apart. Holes in the plate were prefilled with bone wax. For the purpose of physiological identification of PTNs, two 26-gauge hypodermic guide tubes for a later insertion of stimulating electrodes under physiological guidance in the motor cortex, a portion of the skull and dura above the left motor cortex, a portion of the skull and dura above the left motor cortex and covered with a plastic cap electrically shielded and mechanically protected head implants. Three white circle markers on each limb were used to define unit vectors v, u, and w and the limb plane (see text for further explanations). Large black and white circles indicate position of cat center of mass (CoM) and its projection on the ground. C: dorsal view of the cat during walking: large black and white circle, CoM; black and white square, extrapolated center of mass (XCoM). Paw prints indicate paws on the ground that form the base of support, a lighter gray area. Filled circles are markers on iliac crests, scapulars, the corner of each eye and ear. D: definitions of the base of support, index of static stability (ISS), and index of dynamic stability (IDS) and its components, i.e., index of dynamic stability in the frontal plane (IDSf) and index of dynamic stability in the sagittal plane (IDSs) (see text for further explanations).

Biomechanical and EMG Recordings

Full-body kinematics and ground reaction forces were collected during unconstrained overground locomotion as described previously (Beloozerova et al. 2010; Prilutsky et al. 2005). Briefly, three-dimensional (3D) positions of 28 light-reflective markers on the cat’s body were recorded at 120 frames/s with a six-camera motion capture system (Vicon Motion Systems). The markers were attached by double-sided adhesive tape to the shaved lateral aspects of the limb joints [the greater trochanters (hip joints), approximate knee joint centers, lateral malleoli (ankle joints), base of the fifth metatarsals (metatarsophalangeal joints, MTP), the greater tubercle of the humerus (shoulder joints), approximate elbow joint centers, ulna styloid process (wrist joints), base of the fifth metacarpals (metacarpophalangeal joints, MCP), trunk anatomical landmarks (left and right iliac crest and top aspect of left and right scapula), and lateral aspects of the head] (Fig. 1, A–C). The three components of the ground reaction force vector and point of force application [center of pressure (CoP)] to the paw were recorded by three small force platforms (0.16 × 0.11 m² and 0.11 × 0.07 m²; Bertec) at a sampling rate of 360 Hz. During cortical activity recordings, cats walked on an electroconductive rubberized material, which allowed for measurements of the electrical resistance between the electromechanical sensor on the right forepaw and the floor in order to detect the stance and swing phases of the stride (Fig. 2B).

The EMG signals were sampled at 3,000 Hz, amplified, and saved on a hard drive of a PC computer for subsequent analysis. Examples of raw myoelectric signals recorded during unconstrained and wide-stance walking are shown in Fig. 2B.
Fig. 2. The area of recording in the motor cortex and an example of the activity of a neuron along with selected muscles during unconstrained and wide-stance walking. 

A: areas of recording in the left motor cortex. Microelectrode entry points into the cortex were combined from both cats and are shown as circles on the photograph of cat Mc’s cortex: cat Mc entry points are depicted by white symbols, whereas those of cat Zv are shown by black symbols. Squares designate tracts where neurons with receptive fields spanning both the fore- and hindlimb were recorded. The star marks the track from which the neuron whose activity during walking is shown in fragments B–F was recorded. B–F: a typical example of the activity of a neuron (PTN 5126) and selected right fore- and hindlimb muscles during unrestrained and wide-stance walking. B: activity of the neuron and muscles during walking first unconstrained and then, after a turn, with wide between-paw distance. Bottom trace shows the stance (St) and swing (Sw) phases of the stride of the right forelimb that is contralateral to the recording site in the cortex. TRL, triceps brachii lateral head; EDC, extensor digitorum communis; FCU, flexor carpi ulnaris; GLM, gluteus medius; VL, vastus lateralis. C and D: the activity of the same neuron during unconstrained walking is presented as a raster of 50 strides (C) and as activity distribution (D). The duration of each stride is normalized to 100%. In the raster, the end of swing and the beginning of stance in each cycle is indicated by an open triangle. In the activity distribution, the horizontal black bar shows the period of elevated firing (PEF) as defined in METHODS. Horizontal dashed line indicates the level of activity during standing. Vertical dashed line indicates the end of the swing and beginning of the stance phase. E and F: raster and distribution of the activity of the same neuron during wide-stance walking.

**Neuronal Recording and Identification**

Extracellular single-neuron activity recordings were obtained from the rostral and lateral sigmoid gyrus (forelimb representation area) as well as from the postcruciate cortex within the fold of the cruciate sulcus (hindlimb representation) (Fig. 2A). These areas are considered to be the motor cortex based on a considerable body of data obtained by means of inactivation, stimulation, and recording techniques (Armstrong and Drew 1984, 1985a, 1985b; Beloozerova and Sirota 1993a; Drew 1993; Martin and Ghez 1985, 1993; Nieoullon and Rispal-Padel 1976; Phillips and Porter 1977; Vicario et al. 1983; Widajewicz et al. 1994). Recordings were obtained with tungsten varnish-insulated microelectrodes with outer diameter of 120 μm and impedance of 1–3 MΩ at 1,000 Hz (Frederick Haer). Electrodes were inserted into cortex through 0.36-mm holes in the plastic plate implanted above the cortex as explained above. The electrode was advanced into the tissue by means of a custom-made miniature manual microdrive (2.5 g) firmly attached to the head base. The signals recorded at 30 kHz were preamplified by a miniature custom-made preamplifier on the cat’s head and subsequently further amplified with a CyberAmp 380 stationary amplifier (Axon Instruments). The amplified signals were filtered (0.3- to 10-kHz band pass), fed to a screen and audio monitor, and saved on the hard drive of a PC computer with data acquisition system Power 1401/Spike 2 System (Cambridge Electronic Design). Examples of recordings from a PTN during unconstrained and wide-stance walking are shown in Fig. 2, B–F.

Each encountered neuron was tested for antidromic activation with pulses of graded intensity (0.2-ms duration, up to 0.5 mA) delivered through the bipolar stimulating electrode in the medullary pyramidal tract. Construction and insertion of this electrode have been detailed previously (Prilutsky et al. 2005). The cells that passed the test for collision of spikes (Bishop et al. 1962; Fuller and Schlag 1976) were identified as PTNs. Each recorded neuron was tested for antidromic activation before, during, and after each locomotion test. In addition, waveform analysis was employed to identify and isolate the spikes of a single neuron with the Power 1401/Spike 2 System waveform-matching algorithm.

The distance between electrodes in the medullary pyramidal tract and at recording sites in the pericruciate cortex was estimated at 51.5 mm, which includes the curvature of the pathway, as well as the spread of current and the refractory period at the site of stimulation. Neurons were classified as fast or slow conducting with the criteria of Takahashi (1965): neurons with axonal conduction velocity of 21 m/s or higher were considered to be fast conducting, while those with lower conduction velocities were considered to be slow conducting.

Somatic receptive fields of the neurons were mapped while the animals were resting on a pad with their head restrained. Somatosensory stimulation was produced by palpation of muscles and tendons and by passive movements of joints. Responses of individual cells and populations of neurons were detected by listening to an output of the audio monitor. Neurons responsive to passive movements of joints were assessed for directional preference.
Terminal Experiments

After completion of the experiments the animals were deeply anesthetized, and in cats used for cortical activity recordings several reference lesions were made in the motor cortex in the vicinity of areas from which neurons were recorded and in the pyramidal tract where stimulation electrodes were located. Cats were then euthanized with an overdose of pentobarbital sodium (120–180 mg/kg iv) and immediately perfused with isotonic saline followed by a 3% formalin solution. Frozen brain sections of 50–µm thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet, and the positions of recording and stimulation electrodes were verified. The cadavers of cats were used to verify segment lengths and joint widths as well as locations of implanted EMG wires.

Kinematic and Kinetic Analysis

Recorded strides along the walkway were examined for steadiness of locomotion, and trials in which cats significantly slowed down or accelerated while walking were discarded. Recorded 3D positions of body markers were low-pass filtered (4th-order, zero-lag Butterworth filter, cutoff frequency 6 Hz). To estimate locations of joint centers from skin markers on the fore- and hindlimbs, limb planes were defined for each video frame based on the hip, knee, and ankle markers for the hindlimbs and on the shoulder, elbow, and wrist markers for the forelimbs (Fig. 1B; white circles indicate markers used to define limb planes). Each limb plane was associated with the unit vector triad \( \mathbf{u}_i, \mathbf{v}_j, \mathbf{w}_k \):

\[
\mathbf{u}_i = \left( \mathbf{p}_{2i} - \mathbf{p}_{1i} \right) / \left( \left| \left( \mathbf{p}_{2i} - \mathbf{p}_{1i} \right) \right| \right), \quad \mathbf{v}_j = \left( \mathbf{p}_{3j} - \mathbf{p}_{1j} \right) \times \left( \mathbf{p}_{2j} - \mathbf{p}_{1j} \right) / \left( \left| \left( \mathbf{p}_{3j} - \mathbf{p}_{1j} \right) \times \left( \mathbf{p}_{2j} - \mathbf{p}_{1j} \right) \right| \right), \quad \mathbf{w}_k = \mathbf{v}_j \times \mathbf{u}_i \tag{1}
\]

where \( \mathbf{u}_i \) and \( \mathbf{v}_j \) are orthogonal unit vectors in the limb plane and \( \mathbf{w}_k \) is a unit vector orthogonal to limb plane \( i \) (Fig. 1B); \( \mathbf{p}_{1i} \), \( \mathbf{p}_{2i} \), and \( \mathbf{p}_{3i} \) are position vectors defining locations of three limb markers in the global coordinate frame \( OXYZ \). To minimize errors caused by skin movement around knee and elbow joints (Miller et al. 1975), positions of knee and elbow markers within the limb planes were recalculated with measured positions of hip and ankle and shoulder and wrist markers, respectively, and the corresponding segment lengths (thigh, shank and upper arm, forearm). Location of centers at distal limb joints (MTP, ankle, knee, MCP, wrist, elbow) and paw center of mass location were calculated by shifting the joint marker position along the line passing through the marker and parallel to unit vector \( \mathbf{w}_p \), which is perpendicular to the limb plane (Fig. 1, A and B). The shift distance was equal to the radius of the marker plus half the measured width of the joint. Hip and shoulder joint center locations were determined by shifting the marker position medially along the line connecting the two hip and two shoulder markers, respectively. For the hip joints the shift length was equal to the radius of the marker plus the distance from the greater trochanter to the femoral head center and for the shoulder the radius of the marker plus the distance from the greater tubercle of the humerus to the humeral head center (Fig. 1A). The joint and bone dimensions were measured while the animal was sedated during surgery and again postmortem.

To determine displacement of the center of mass (CoM) of an 18-segment model of the cat in three dimensions (Fig. 1, B and C), segment mass \( m_i \) and relative location of segment center of mass on the long segment axis \( \lambda_i \) were calculated for each segment based on measured cat mass \( m \) and segment length (Table 1) with the regression equations developed in Hoy and Zernicke (1985). Position of CoM location for each segment in the global coordinate system was computed as

\[
p_{\text{CoM}i} = p_{2i} + \lambda_i (p_{3i} - p_{1i}) \tag{2}
\]

where \( p_{\text{CoM}i}, p_{1i}, \) and \( p_{3i} \) are vectors describing positions of the CoM, proximal and distal joint centers of segment \( i \), respectively. The 3D position of the general CoM of the full-body model \( p_{\text{CoM}} \) for each video frame was then computed as

\[
p_{\text{CoM}} = \sum_{i=1}^{18} m_i p_{\text{CoM}i} \tag{3}
\]

or in scalar form

\[
X_{\text{CoM}} = \sum_{i=1}^{18} m_i x_i, \quad Y_{\text{CoM}} = \sum_{i=1}^{18} m_i y_i, \quad Z_{\text{CoM}} = \sum_{i=1}^{18} m_i z_i \tag{3a}
\]

where \( x_i, y_i, \) and \( z_i \) are the coordinates of the \( i \)th segment CoM, \( m_i \) is the mass of the \( i \)th segment, \( m \) is the total mass of the cat, and \( X_{\text{CoM}}, Y_{\text{CoM}}, \) and \( Z_{\text{CoM}} \) are the coordinates of the general CoM. The 3D CoM velocity and acceleration were calculated as the first and second derivative of CoM position, respectively.

The frontal-plane shoulder angle was defined as the angle between the line connecting the two shoulder joint centers and the line from the shoulder center to the MCP joint center (Fig. 1A; see also Misiaszek 2006). The hip frontal-plane angles were determined similarly. Stride parameters such as stride length, stance width, mean forward velocity of the general CoM, stance and swing duration, and duty factor (the ratio of stance duration and stride duration) were calculated based on recorded waking kinematics. Stance width for fore- and hindlimbs was defined as the distance between the left and right paw centers during stance. The timing of stance onset (paw contact with the ground) and offset (paw lift-off) was determined based on ground reaction force (when the paw contacted a force platform), using the paw forward velocity (for details see Pantall et al. 2012), or, in experiments with cortical activity recordings, based on readings from the electromechanical sensor on the right forepaw.

The frontal-plane resultant muscle moments at the hip and shoulder joints (\( M_j \)) were computed as the cross product of the position vector from the joint center to the point of ground reaction force application (\( p \)) and the ground reaction force vector applied to the paw (\( F \)):

\[
M_j = - (p \times F) \tag{4}
\]

where subscript \( j \) designates the joint number. We used this simplified equation that does not include inertial terms \( ma \) and \( J_\alpha \) (\( m \), \( I \), segment mass and moment of inertia; \( a \), and \( \alpha \), \( i \)th segment linear and angular acceleration; for details see, e.g., Zatsiorsky 2002) because moments of inertia with respect to the anterior-posterior and longitudinal axes for cat segments are not known and because segment accelerations during the stance phase of walking are relatively small (see DISCUSSION and Fig. 6B). The frontal joint moments were normalized to the product of the body weight (in N) and the corresponding leg length (in m).

Analysis of Body Stability

The animal is considered statically stable if the vertical projection of its general CoM is within the support area. During locomotion this condition is always satisfied in hexapedal terrestrial insects (Ting et al. 1994), whereas in humans the CoM projection is outside the support area for a portion of the cycle, making human locomotion statically unstable (Hof et al. 2005; Winter 1995). To extend the notion of static stability to dynamic situations including locomotion, Hof introduced the notion of the “extrapolated center of mass position” (\( X_{\text{CoM}} \)) and a measure of dynamic stability, i.e., the distance between \( X_{\text{CoM}} \) and CoP within the boundaries of the base of support (BoS) (Hof et al. 2005, 2007). The \( X_{\text{CoM}} \) vector in the horizontal plane is calculated as

\[
X_{\text{CoM}} = \mathbf{p}_{\text{CoM}} + \frac{\mathbf{v}_{\text{CoM}}}{\omega_0} \tag{5}
\]

where \( \mathbf{p}_{\text{CoM}} \) and \( \mathbf{v}_{\text{CoM}} \) are the general CoM position and velocity vectors in the horizontal plane, \( \omega = \sqrt{g/I} \) is the eigen (natural)
frequency of the pendulum with mass equaled body mass \( m \) suspended on a massless stick of length \( l \), equaled leg length, and \( g \) is acceleration of gravity. Because it was not possible to record CoP for the entire walking cycle with three force plates available, we evaluated body dynamic stability as the shortest distance between \( X_{CoM} \) and the edge of BoS [index of dynamic stability (IDS); Fig. 1, C and D]. The animal was considered dynamically unstable if \( X_{CoM} \) was outside BoS (IDS < 0). This instability condition corresponds to a situation when CoP cannot be moved in front of \( X_{CoM} \) without taking a step to reverse \( X_{CoM} \) movement away from an instability region. In this situation the animal must take a step and place the paw in front of \( X_{CoM} \) to prevent falling (see condition C, p. 3 in Hof et al. 2005). When IDS is inside BoS (IDS > 0), the body may still be dynamically unstable, especially if \( X_{CoM} \) is close to a BoS border; this follows from the analysis of the inverted pendulum model provided by Hof et al. (2005). The IDS was partitioned into two perpendicular line segments representing indexes of dynamic stability in the frontal and sagittal planes, respectively (Fig. 1D). In addition, the index of static stability (ISS) was calculated as the shortest distance between \( X_{CoM} \) and the edge of BoS (Fig. 1D).

Time histories of mechanical variables of individual limbs were time normalized to 100% of the limb stride duration; mechanical variables describing motion of the general center of mass and stability measures were time normalized to stride duration of the right forelimb. All time-normalized mechanical variables were averaged across walking cycles of individual cats and then across cats for unconstrained and wide-stance walking conditions.

**Analysis of EMG**

EMG signals were band-pass filtered (30–1,000 Hz, 3 dB) and full-wave rectified. EMG burst onset and offset times were determined using a threshold EMG value defined as the mean EMG in between burst periods plus 2 SD (Gregor et al. 2006; Prilutsky et al. 2011). The mean magnitude of EMG bursts was calculated for all analyzed strides and normalized to the maximum mean EMG value found across all strides of unconstrained walking within each cat. The linear EMG envelopes were generated for each cycle with a 4th-order low-pass, zero-lag Butterworth filter with 20-Hz cutoff frequency, time normalized to stride duration, and averaged across all selected cycles.

**Analysis of Cortical Activity**

Instances of paw-off and ground contact of the right forelimb were detected with an electromechanical sensor on the paw (Fig. 2B), and the stride duration was divided into 20 equal bins. For forelimb-related neurons, the onset of the swing phase of this limb was taken as the beginning of the step cycle. For hindlimb-related neurons, the beginning of the 16th bin of the forelimb cycle, which corresponds to the beginning of the swing phase of the right hindlimb, was taken as the onset of the hindlimb stride cycle. For each neuron, a distribution of spike activity in the stride cycle was generated for each walking condition (e.g., Fig. 2, D and F). Phase activity distributions were smoothed by recalculating the values of the bins according to the equation \( F_n = 0.25F_{n-1} + 0.5F_n + 0.25F_{n+1} \), where \( F_n \) is the bin’s original value. The first bin in the cycle was considered to follow the last bin, and the last bin was considered to precede the first bin. The “depth” of modulation, \( dM \), was calculated as \( dM = (N_{max} - N_{min})N \times 100\% \), where \( N_{max} \) and \( N_{min} \) are the number of spikes in the maximal and minimal bin in the activity distribution and \( N \) is the total number of spikes in the activity distribution. Neurons with \( dM > 4\% \) were judged to be stride related. This was based on an analysis of fluctuations in the activity of neurons in the resting animal (Efron and Tibshirani 1993; Marlinski et al. 2012). In stride-related neurons, the period of elevated firing (PEF) was defined as the portion of the cycle in which the activity level exceeds the minimal activity by 25% of the difference between the maximal and minimal frequencies in the neuronal discharge distribution (Fig. 2, D and F). PEFs were smoothed by removing all one-bin peaks and troughs (a total of 0.4% of bins were altered throughout the database). The “preferred phase” of discharge of each neuron with a single PEF was assessed with circular statistics (Batshelet 1981; Belozerova et al. 2003a; Drew and Doucet 1991; Fisher 1993).

The following parameters were calculated for each recorded neuron: mean discharge frequency, depth of modulation \( dM \), number of PEFs, duration of PEF (s), and, for neurons with a single PEF per cycle, the preferred cycle phase. For populations of neurons the following parameters were calculated: proportion of neurons at their PEF during different phases of the locomotion cycle, distribution of discharge frequency over the cycle, range of coefficients of modulation, and average widths of PEFs. The difference in all parameters of the activity of individual neurons and populations of neurons between unconstrained and wide-stance walking was determined.

**Statistical Analysis**

The effects of walking condition on parameters of walking strides, time-normalized mechanical variables, mean EMG activities, discharge rate of individual neurons, and mean parameters of activity of groups of neurons were tested with Student’s paired \( t \)-test. When comparing depth of frequency modulation \( dM \) of individual neurons, their preferred phases, and duration of PEF, differences equal to or greater than 2%, 10%, and 20%, respectively, were considered significant. These criteria were established based on the results of a bootstrapping analysis (Efron and Tibshirani 1993; Stout and Belozerova 2013), which compared differences in discharge rates between various reshufflings of strides of the same locomotion task. Unless noted otherwise, for all mean values the standard deviation (SD) is given. When data were categorical, a nonparametric Fisher’s two-tailed test was used. The significance level was set at 0.05.

**RESULTS**

Overall, 241 strides of wide-stance and unconstrained walking from five cats were included for analysis of mechanics and 952 strides of wide-stance and unconstrained walking from seven cats were included for analysis of EMGs. The activity of 121 neurons from the motor cortex of two cats was recorded and analyzed for both locomotor tasks, each neuron over a range of 23–186 strides of each locomotion task.

**Mechanics of Walking**

**General stride parameters.** The average stance width measured as the distance between centers of the two paws of the same girdle was significantly larger during wide-stance walking than during unconstrained locomotion (12.5 ± 0.6 vs. 2.7 ± 1.3 cm and 13.1 ± 0.5 vs. 2.5 ± 1.1 cm for fore- and hindlimbs, respectively, \( P < 0.05 \)). Stride length tended to be shorter during wide-stance walking than during the unconstrained condition for the forelimbs (41.3 ± 2.5 cm vs. 43.7 ± 3.3 cm, \( P = 0.053 \)) and was significantly shortened for the hindlimbs (41.2 ± 2.4 cm vs. 44.7 ± 2.9 cm, \( P < 0.05 \)). The mean forward speed during wide-stance walking tended to be slower than during unconstrained walking, but the difference was not significant (0.54 ± 0.09 m/s vs. 0.61 ± 0.05 m/s, \( P = 0.06 \)). Similarly, stance, swing, and stride cycle times during wide-stance walking tended to be longer compared with unconstrained walking; however, the differences did not reach the significance level (\( P > 0.05 \); Fig. 3A). Duty factor for the hindlimbs, but not for the forelimbs, was, however, slightly but statistically significantly higher during wide-stance.
walking compared with the unconstrained condition \((P < 0.05; \text{Fig. 3A}).\)

The general pattern of limb support seen during wide-stance walking was similar to that during unconstrained locomotion exhibiting pattern 2-3-2-3-2-3-2-3, where the numbers indicate how many limbs are supporting the body during eight phases of the stride (4 limbs \(\times 2\) swing-stance phases; Fig. 3, B and C). While the overall limb support pattern and individual limbs’ stride timing parameters during wide-stance and unconstrained walking were very similar, the interlimb coordination as expressed in the duration of phases of two-legged and three-legged support was significantly different (Table 2). During the cycle of wide-stance walking, the cats spent more time in three-legged support phases and less time in two-legged support phases than during unconstrained walking. During walking with wide stance cats supported their body with three legs during 43.8% of the cycle time, whereas they only used 29.2% of the cycle in such support during unconstrained walking (Table 2). Conversely, only 56.2% of the wide-stance walking cycle cats spent in two-legged support as opposed to 70.8% during unconstrained walking, a difference of 14.6% (Table 2).

The duration of the three-legged support phases was statistically longer in wide-stance walking than in unconstrained walking \((P < 0.05)\), whereas the duration of the two-legged support phases tended to be shorter in wide-stance walking; however, this difference reached significance only in one of four double-support phases (Table 2).

The number and position of the limbs in contact with the ground determined the BoS area for each stride phase. During wide-stance walking, in three-legged support phases \((\text{phases 2, 4, 6, and 8})\) the BoS area was several times larger than during unconstrained walking \((P < 0.05; \text{Table 2, Fig. 4E}).\) In addition, during diagonal double support with a forelimb and contralateral hindlimb, BoS was larger during wide-stance walking \((P < 0.05; \text{Table 2, Fig. 4E}).\)

Margins of stability during unconstrained and wide-stance walking. Indexes of static (ISS) and dynamic (IDS) stability followed different patterns during the gait cycle and were closely tied to the BoS area and the support phases of the cycle (Figs. 3 and 4, Table 3) for both unconstrained and wide-stance walking. During three-legged support phases formed by two forelimbs and one hindlimb \((\text{phases 4 and 8})\), the cats were statically stable: ISS was significantly greater than zero during both unconstrained and wide-stance walking. This stability index was significantly higher for wide-stance walking than for unconstrained walking \((P < 0.05; \text{Fig. 4});\) indicating greater margins of static stability for wide-stance walking during phases 4 and 8. During two-legged support in phases 1 and 5 (ipsilateral support by 1 forelimb and 1 hindlimb; Fig. 3, B and C), the cats were statically and dynamically unstable during wide-stance walking (both ISS and IDS stability indexes were <0, \(P < 0.05)\), whereas during unconstrained walking the cats were statically stable during these periods (ISS was \(\geq 0, P > 0.05).\)

During phases 2 and 6 (3-legged support; 1 forelimb and 2 hindlimbs, Fig. 3, B and C), ISS became positive for both wide-stance and unconstrained walking for substantial parts of these phases, indicating greater margins of static stability (Fig. 4D). During these phases, total IDS increased slightly; however, it remained negative, including its two components IDS\(_{s}\) and IDS\(_{d}\), of which only IDS\(_{s}\) was significantly less than zero.
Throughout the entire duration of the phases, indicating dynamic instability of the body in the frontal plane (Fig. 4, A–C). During support phases 3 and 7 (2-legged support; diagonal fore- and hindlimb; Fig. 3, B and C), ISS during wide-stance walking was either positive or not significantly different from zero, i.e., the animals on average were statically stable in these phases (Fig. 4D). Total IDS, however, decreased and was significantly less than zero during both wide-stance and unconstrained walking (Fig. 4A). This dynamic instability was caused by substantial instability in the sagittal plane for both wide-stance and unconstrained walking conditions (IDSs < 0, P < 0.05) and by instability in the frontal plane (IDSf, P < 0.05), which was significantly larger for wide-stance than for unconstrained walking (P < 0.05; Figs. 4, B and C).

Considering the entire gait cycle, cats were dynamically unstable during phases 3 and 7 of unconstrained walking and during at least some portion of phases 1, 2, 5, 6, and 7 of wide-stance walking. During unconstrained walking, the dynamically unstable phases were primarily due to the XCoM progressing ahead of the base of support in phases 3 and 7 (Fig. 3C). In phases 1, 2, 5, and 6 of wide-stance walking, the major contributing factor to the instability was IDSf. During phases 3 and 7, both sagittal and frontal components were negative and contributed to the total dynamic instability.

Both static and dynamic instability depend on CoM position with respect to BoS. The left-right displacement of the CoM during wide-stance walking was significantly greater than during unconstrained walking: the displacement range was close to 4.0 cm for wide-stance and <1.0 cm for unconstrained walking (Fig. 5A). Also, the CoM acceleration had substantially greater peak values in left and right directions during wide-stance walking than during unconstrained walking (P < 0.05; Fig. 5B). The vertical position and acceleration of the CoM were similar between the walking conditions (not shown).

Kinetics of wide-stance walking. The ground reaction forces determined the displacement and acceleration of the CoM during walking. While the patterns of the vertical and anterior-posterior ground reaction forces were generally similar between unconstrained and wide-stance walking (Fig. 6, A–D), there were several notable differences. During wide-stance walking, the forelimbs exerted smaller vertical forces during the first half of stance (P < 0.05; Fig. 6A) and both the fore- and hindlimbs had smaller braking forces in the anterior-posterior direction than during unconstrained walking (P < 0.05; Fig. 6, C and D). Especially large differences between wide-stance and unconstrained walking were observed in the medial-lateral forces: the peak lateral forces were ~10 times greater during wide-stance walking for both fore- and hindlimbs (P < 0.05; Figs. 6, E and F).

During wide-stance walking, the frontal plane muscle moment acting at the shoulder tended to abduct the forelimb, but its magnitude was reduced compared with the abduction moment during unconstrained walking (P < 0.05; Fig. 6G). The moment acting about the hip during wide-stance walking followed a pattern similar to unconstrained walking, with a decreased abduction moment in early stance and increased moment in the second half of the stance phase (P < 0.05; Fig. 6H).

Other mechanical characteristics. During swing phase, the peak of paw vertical displacement (swing height) was significantly higher for wide-stance walking compared with unconstrained walking (Fig. 7, A and B) in all limbs. During wide-stance walking the fore- and hindlimbs underwent medial circumduction evident from medial displacements of the paws, whereas during unconstrained walking the cats performed lateral circumduction (Fig. 7, C and D). The hind- and forelimbs were significantly abducted during wide-stance walking, i.e., the hip and shoulder frontal angles were >90° (Fig. 1A and Fig. 7, E and F). During unconstrained walking, on the other hand, the hind- and forelimbs in the frontal planes were adducted in stance and most of swing (Fig. 7, E and F).

The vertical position of the head-neck segment during wide-stance walking was ~1 cm lower than during unconstrained walking. The head-neck segment was also rotated ~10° more toward the ground during wide-stance walking (Fig. 5, C and D). Both the head-neck vertical position and orientation during wide-stance walking were significantly different from those of unconstrained walking for the entire walking cycle duration.

Summary of Differences in Walking Mechanics Between Wide-Stance and Unconstrained Walking

Walking with wide-between-paw distance in stance had stride parameters (stride length, durations of stride, stance, and swing) and limb support pattern (2-3-2-3-2-3-2-3) generally similar to those during unconstrained walking. However, stance width, BoS area during three-legged support, lateral CoM displacement and acceleration, fore- and hindlimb abduction angles, and lateral ground reaction forces were much greater during wide-stance walking. In both walking conditions, the cat was dynamically unstable in the sagittal plane during the end of swing of each forelimb; however, in the frontal plane walking with wide stance was much more dynamically unstable than unconstrained walking. The head-neck
Values indicate instability.

Unconstrained locomotion, and thick line shows wide-stance walking. Negative

IDS averaged across cats and shown as means

A

stance walking as functions of the normalized cycle time of the right forelimb.

Fig. 4. Base of support and stability indexes during unconstrained and wide-stance walking. A: IDS averaged across cats and shown as means ± SD. Thin line indicates unconstrained locomotion, and thick line shows wide-stance walking. Negative values indicate instability. B: IDSs. C: IDSs. D: ISS. E: base of support area. Gray areas indicate 2-legged support phases and are shown for unconstrained walking only. For A–D, top horizontal line indicates where in the stride the shown variables are statistically different between wide-stance and unconstrained locomotion. Middle and bottom red lines indicate where in the stride variables for wide-stance and unconstrained walking were significantly different from zero, respectively. For E, horizontal bar indicates significant difference in support area between wide-stance and unconstrained walking.

Table 3. Characteristics of base of support area and dynamic stability of unconstrained and wide-stance walking

<table>
<thead>
<tr>
<th>Characteristic of Walking Stability</th>
<th>Unconstrained</th>
<th>Wide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal IDS, cm</td>
<td>1.63 ± 0.30</td>
<td>5.29 ± 0.69*</td>
</tr>
<tr>
<td>Minimal IDS, cm</td>
<td>−6.53 ± 1.50</td>
<td>−8.46 ± 1.91*</td>
</tr>
<tr>
<td>Peak lateral CoM acceleration, cm/s²</td>
<td>−59.80 ± 14.62</td>
<td>−174.79 ± 32.60*</td>
</tr>
<tr>
<td>Max BoS area, cm²</td>
<td>146.5 ± 23.5</td>
<td>355.57 ± 19.61*</td>
</tr>
<tr>
<td>Min BoS area, cm²</td>
<td>31.7 ± 1.5</td>
<td>39.51 ± 2.73*</td>
</tr>
</tbody>
</table>

Values are means ± SD. IDS, index of dynamic stability; CoM, center of mass. *Significant difference between unconstrained and wide-stance walking.

segment was lowered and rotated closer to the ground during wide-stance walking.

Muscle Activity During Wide-Stance Walking

EMG activity was recorded from 13 muscles of the hindlimb and 3 muscles of the forelimb during wide and unconstrained walking. Examples of rectified, low-pass-filtered EMG activity of selected muscles averaged over a number of strides for each locomotion task are shown in Fig. 8, A–F.

The mean EMG activity of the three hindlimb extensors, MG, AF, and BFA, as well as the majority of hindlimb flexors, TA, SAM, IL, and BFP (bursts BFP1 and BFP2), was greater during wide-stance than unconstrained walking (P < 0.05; Fig. 8G). The activity of some other hindlimb muscles, SO, LG, VM, and VL, and RF, was lower or not significantly different during wide-stance than unconstrained walking (P > 0.05). The triceps surae heads (SO and LG vs. MG) showed distinct changes in activity and timing between the walking conditions. The SO and LG had significantly lower mean activity during wide-stance compared with unconstrained walking, whereas the MG mean activity was higher (Fig. 8G). The burst duration of SO, MG, GLM, and, occasionally, VL was longer during wide-stance walking. The mean RF activity was indistinguishable between the two walking conditions; however, this muscle had significantly longer burst duration during wide-stance walking (P < 0.05; Fig. 8H). Both bursts of BFP showed a significantly larger mean activity during wide-stance walking but no difference in burst duration between wide-stance and unconstrained conditions.

Forelimb EMG activity was recorded from TRL (elbow extensor), EDC (flexor of wrist and digits), and FCU (wrist plantar flexor). The mean activity of all three forelimb muscles tested was lower during wide-stance than during unconstrained walking (Fig. 8, A–C and G). EMG bursts in these muscles, however, started earlier in the cycle of wide-stance walking (by 5–10%); thus the duration of activity bursts in all these muscles was slightly but significantly longer during this walking task (Fig. 8, A–C and H).

Characteristics of Neurons

Neuronal data were collected from a total of 40 tracks through the motor cortex of two cats (23 tracks in cat Zv and 17 tracks in cat Mc; Fig. 2A). These data were considered together to analyze the activity of 121 neurons (66 from cat Zv and 55 from cat Mc). Sixty-eight neurons were recorded from the motor cortex forelimb representation and 53 from the
representation of the hindlimb. On the basis of cytoarchitectural features, it was determined that all neurons were located in layer V of motor cortex area 4y. One hundred and six cells responded to stimulation of the pyramidal tract (61 from in layer V of motor cortex area 4). Sensory receptive fields of all neurons were located on the contralateral (right) side of the body, and all but three were excitatory. From neurons responding to the forelimb, 13 were activated by passive movements of the shoulder, 7 responded to movements in the elbow joint, and 18 were activated by movements of the wrist or palpation of the paw. In addition, 30 neurons responded to movement in two joints [either both shoulder and elbow (n = 4) or both elbow and wrist (n = 7)] or had a receptive field spanning the entire forelimb (n = 19). Neurons activated by a passive movement in a joint typically preferred one or two directions. One-third (n = 5) of shoulder-related cells responded to abduction or abduction and extension of the shoulder, while others responded to extension (n = 3), flexion (n = 2), or adduction (n = 1) of the joint. From elbow-related cells, three neurons responded to flexion of the joint. From wrist-related neurons, three responded to wrist plantar flexion while three were activated by dorsal flexion. The remaining forelimb-related cells did not respond to a select movement direction, only to general movements in the joint or limb.

From neurons responding to stimulation of the hindlimb, 10 were activated by passive movements of the hip, 4 responded to movement of the knee or a touch to the skin on the back of the knee joint, and 7 were activated by movements of the ankle or palpation of the paw. In addition, 24 neurons responded to movement in two joints [either both hip and knee (n = 6) or both knee and ankle (n = 4)] or had a receptive field spanning the entire hindlimb (n = 11). One-half (n = 5) of hip-related cells preferentially responded to flexion, some in conjunction with abduction; two more cells responded to either abduction or adduction of the hip. From ankle-related neurons, three responded to flexion and one to extension.

An example of activity of a neuron during unconstrained and wide-stance walking is shown in Fig. 2B along with activities of selected muscles. This neuron was recorded from the motor cortex forelimb representation region and was responsive to passive movements in elbow and wrist joints (in Fig. 2A the microelectrode track in which this neuron was recorded is indicated with a star). The raster plots in Fig. 2, C and E, show the activity of the neuron across 50 strides during unconstrained (Fig. 2C) and wide-stance (Fig. 2E) walking. The activity is summed in Fig. 2, D and F, which show corresponding distributions of firing rate across the step cycle; the period of elevated firing (PEF; see definition in METHODS) is indicated by a black horizontal bar. One can see that the discharge of the neuron was modulated in the rhythm of strides during both locomotion tasks. However, during unconstrained walking there were two PEFs: a large one during the swing phase and a smaller one during the midstance phase, while the activity of the neuron in the beginning and the third quarter of the stance phase was very low. During wide-stance walking, the neuron’s activity at the end of the stance phase and throughout the swing phase was substantially higher than during unconstrained walking but was entirely absent during the first three quarters of the stance phase. It had only one PEF during this task.

**Activity of Neurons During Unconstrained Locomotion**

**Forelimb-related neurons.** The mean activity of the forelimb-related population during unconstrained walking was 15.2 ± 1.3 spikes/s (mean ± SE). The discharge rate of 97% (66/68) of cells was modulated in the rhythm of strides: it was
higher in one phase of the stride and lower in another phase. The majority of neurons (70%, 46/66) exhibited a single PEF, while 26% (17/66) had two PEFs and three cells had three. The activity of neurons in the one-PEF group was more modulated while 26% (17/66) had two PEFs and three cells had three. The majority of neurons (70%, 46/66) exhibited a single PEF, discharging at 17 ± 0.9 vs. 14.1 1 ± 3 spikes/s, respectively (P < 0.05; Fig. 9A2).

Neurons with receptive fields related to different joints differed in their stride-related activity. Shoulder-related cells as a group were relatively steadily active over the cycle, at ~15 spikes/s (Fig. 10, A2 and A4). In contrast, elbow-related neurons more often were active during the stance phase, and the whole elbow-related population was more active during stance than swing, discharging at 18.4 ± 2.1 vs. 13.2 ± 2.0 spikes/s, respectively (P < 0.05; Fig. 10B). The wrist/paw-related group was active largely in the opposite phase compared with elbow-related cells (Fig. 10C). In this group, there were more neurons with preferred phases during swing than stance phase (P < 0.05; Fig. 10, C1 and C3) and they were much more active (Fig. 10C2), which resulted in the activity of the wrist/paw population being sharply modulated with a peak around 20 spikes/s during the swing phase and a depression to ~10 spikes/s during stance (Fig. 10C4).

**Hindlimb-related neurons.** The mean activity of hindlimb-related neurons (this group also included 8 cells with receptive fields spanning over both the fore- and hindlimb) during unconstrained walking was 13.3 ± 1.3 spikes/s (mean ± SE), which was similar to that of forelimb-related neurons. The discharge rate of 98% (52/53) of hindlimb-related cells was modulated in the rhythm of strides. However, unlike the forelimb-related neurons, a quarter of which discharged more than one PEF per stride, 88% (46/52) of neurons in the hindlimb-related population had only one PEF. The average duration of the PEF across all hindlimb-related neurons was 59 ± 18%, and PEFs of different neurons were evenly distributed across the cycle (Fig. 9B1). Because of a long duration of PEFs and their even phase distribution, PEFs of different neurons overlapped, and 50–60% of neurons were simultaneously active in all phases of the stride (Fig. 9B3). Similar to forelimb-related neurons, neurons with receptive fields related to different joints differed in their stride-related activity. All but one hip-related neuron discharged preferentially during the early stance phase (Fig. 11, A1 and A3), and the whole hip-related group was more active during the stance than swing phase, on average at 14 ± 2.3 spikes/s (P < 0.05; Fig. 11, A2 and A4). In contrast, ankle/paw-related neurons had a preference to discharge during mid-late stance, and their group activity was then 50% higher at 20.7 ± 1.9 spikes/s (P < 0.05, Fig. 11, C2 and C4). The group of knee-related neurons also discharged more intensively during the late stance phase (Fig. 11, B2 and B4), but PEFs of individual knee-related neurons were evenly distributed over the cycle (Fig. 11, B1 and B3).

**Abduction and extension- vs. adduction and flexion-related neurons.** The group of nine neurons that in resting conditions were activated by either abduction and/or extension of the shoulder (n = 8) or hip (n = 1) were active during unconstrained walking fairly steadily over the stride cycle, producing between 10 and 15 spikes/s during most phases (Fig. 12A). In contrast, the group of six neurons that at rest were excited by either adduction and/or flexion of the shoulder (n = 3) or hip (n = 3) had a preference to discharge during the end of
the stance phase, where they generated over 20 spikes/s, while firing only 14.4 spikes/s on average during the swing and transition from the swing to stance phases ($P < 0.05$; Fig. 12B).

**Activity of Neurons During Walking with Wide Stance**

**Forelimb-related neurons.** The activity of 82% (56/68) of forelimb-related neurons was different during walking with wide stance compared with the unconstrained condition.

The mean discharge rate was different in 26% (18/68) of neurons: it was higher in 10 cells by 25–330% and lower in 8 by 20–50% ($P < 0.05$; Fig. 13A). The depth of frequency modulation was different in 42% (27/65) of stride-related neurons. During wide-stance walking, it was higher in 15 cells by 49 ± 22% and lower in 12 neurons by 30 ± 9% (Fig. 13B). The duration of the PEF was different in 14% (9/65) of neurons: during wide-stance walking, it was shorter in five and longer in four cells by 25–45% of the cycle. Despite these differences in the activity of individual neurons, the average discharge rate, depth of modulation, and duration of PEF for the entire forelimb-related population were similar between unconstrained and wide-stance walking because a roughly similar number of neurons changed the above firing parameters in opposite ways (Fig. 9, A and C).

The situation was different, however, in respect to the number of PEFs and preferred activity phases. Seventeen of twenty (85%) neurons that had two or three PEFs per cycle during unconstrained walking discharged only one PEF during wide-stance walking. This typically was because the activity in one or two of the PEFs during unconstrained walking decreased during walking with wide paw separation so that these activity periods did not qualify as PEFs anymore ($n = 11$; e.g., Fig. 2, C–F). All but 1 of these 11 neurons, similarly to the neuron shown in Fig. 2, lost their stance-phase PEF, thereby concentrating the activity within the swing phase during wide-stance walking. On the other hand, only three neurons that had one PEF during unconstrained walking acquired an additional PEF during walking with wide paw separation. As a result of these changes in the number of PEFs, during wide-stance walking there were fewer two-PEF and more one-PEF neurons than during the unconstrained condition (Fig. 9, A1 and B1 vs. C1 and D1; $P < 0.05$). The few multi-PEF neurons that were still present during wide-stance walking discharged during the transition between swing and stance phases.

In the population of neurons that had one PEF during both walking conditions, 40% (17/43) changed the preferred activity phase by 10–15% of the stride cycle (Fig. 13C). They nearly always kept it within the same general phase of the stride (swing or stance, open areas in Fig. 13C) but typically discharged it earlier in the cycle during walking with a wide between-paw distance (dots below the diagonal in Fig. 13C; Fisher’s 2-tailed test, $P < 0.05$). Considering phases of dy-
namic stability and instability as described above and illustrated in Fig. 4, 6 neurons kept their PEFs to a stable phase while 10 shifted them to an unstable phase during wide-stance walking.

Overall, the majority of neurons (65%, 44/68) had only one or two parameters of their activity different between the two walking conditions. The activity of some cells (15%, 10/68), however, differed in three or four parameters.

Neurons with receptive fields related to different joints responded differently to wide-stance walking. The shoulder-related cell group became slightly less active during the stance phase, thus focusing its activity more on the swing phase (Fig. 10, A and D4). This was exclusively due to changes in the depth of modulation and preferred phase of activity of individual neurons, as all cells in this group had similar average discharge rates between the two walking conditions. In contrast, 30–40% of neurons in the elbow- and wrist/paw-related groups discharged at different mean rates during the two walking tasks. Many neurons in the elbow-related group also shifted their activity to the swing phase, so the entire elbow-related population became more active during swing than stance phase (Fig. 10, B and E). Because of opposing responses of individual neurons within the wrist/paw population, there was little change in this population output between the walking tasks (Fig. 10, C4 and F4).

Hindlimb-related neurons. Similar to the forelimb-related group, the activity of 83% (44/53) of hindlimb-related neurons was different during walking with wide stance compared with the unconstrained condition. The mean discharge rate was different in 43% (23/53) of neurons: it was higher in 11 cells by 70–300% and lower in 12 by 27 ± 88% (P < 0.05; Fig. 13D). This was similar to the activity differences in the forelimb-related population. The depth of frequency modulation, however, was different in a larger proportion of hindlimb-related neurons than it was in the forelimb-related group [68% (36/53), P < 0.05]. During wide-stance walking, it was higher in 26 cells by 57 ± 6% and lower in 10 neurons by 30 ± 4% (Fig. 13E). There was also a larger proportion of cells increasing rather than decreasing the depth of modulation than was observed in the forelimb-related group (P < 0.05). The duration of the PEF was different in 19% (10/53) of hindlimb-related neurons. During wide-stance walking, it was shorter in eight and longer in two cells by 25–60% of the cycle. Similar to the forelimb-related group, however, the average discharge rate, depth of modulation, and duration of PEF for the entire hindlimb-related population remained unchanged between the conditions because here too compatible numbers of neurons changed these activity parameters in opposite directions (Fig. 9, B and D).

In 49% (24/49) of the hindlimb-related neurons, preferred activity phases were different by 10–45% of the stride cycle between the two walking conditions (Fig. 13F). And, similar to the forelimb-related cells, hindlimb-related neurons typically discharged earlier in the cycle during walking with wide paw separation (P < 0.05). Unlike the forelimb-related group, however, in which the majority of neurons kept their activity to the same general phase of the stride (swing or stance) during both locomotion tasks, 10 of 24 hindlimb-related neurons had...
different preferred phase during the two tasks, switching their activity between the stance and swing phases. And 8 of these 10 neurons had a PEF in the stance phase during unconstrained walking but in the swing phase during wide-stance walking. In respect to phases of dynamic stability and instability (Fig. 4), the behavior of hindlimb-related cells was similar to that of the forelimb-related cells: they typically also either kept their activity in a stable stride phase during both locomotion tasks ($n = 10$) or became preferentially active during an unstable period of walking with wide stance ($n = 9$).

The great majority of neurons (49%, 26/53) had only one or two parameters of their activity different between walking conditions. The activity of some neurons (32%, 17/53), however, differed in three or four parameters. There was a larger proportion of neurons responding to the wide-stance walking condition by a change in multiple parameters of activity compared with the forelimb-related population ($P < 0.05$).

Neurons with receptive fields related to different joints responded differently to walking with wide paw separation. The hip-related cell group, similar to the shoulder-related group, displayed a slightly lower activity during the stance phase, thus moving the focus of activity to the swing-to-stance transition period (Fig. 11, A4 and D4). Unlike the shoulder-related neurons, however, all of which had the same average discharge rate during the two walking tasks, almost all hip-related neurons had different mean activity between the tasks, typically along with different depth of modulation and/or preferred activity phase. The knee-related population responded similarly. In contrast, the ankle/paw-related group was rather unresponsive. All seven ankle/paw-related neurons had similar discharge rates between the two walking conditions, and four did not show any activity differences at all.

Abduction and extension vs. adduction and flexion-related neurons. The group of neurons that at rest were activated by either abduction and/or extension of the shoulder or hip during walking with wide stance developed a preference to discharge during the end of the swing phase ($P < 0.05$; Fig. 12C4). The group of neurons that at rest were excited by either adduction and/or flexion of the shoulder or hip changed their activity even more profoundly by nearly abandoning the end of the stance phase where they were preferentially active during unconstrained walking and shifting most of activity to the end of the swing and midstance phases ($P < 0.05$; Fig. 12D).

Summary of Differences in Neuronal Activity Between Wide-Stance and Unconstrained Walking

During wide-stance walking compared with the unconstrained condition, the activity of a great majority of motor cortex neurons, both forelimb and hindlimb related, was different. The largest proportions of neurons showed different depths of stride-related frequency modulation. In addition, in

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Fig. 9. Population characteristics of forelimb-related (A and C) and hindlimb-related (B and D) neurons during locomotion with unconstrained (A and B) and wide-stance (C and D) walking. A1 and C1: phase distribution of PEFs of all forelimb-related neurons during unconstrained (A1) and wide-stance (C1) walking. Each row represents PEF of 1 cell. A circular mark on a PEF denotes the preferred phase for cells with a single PEF per stride cycle. Neurons are rank-ordered so that those with PEFs earlier in the cycle are plotted at top of graph. Vertical dashed lines indicate end of swing and beginning of stance phase. A2 and C2: corresponding phase distributions of discharge frequencies. The average discharge frequency in each 1/20th portion of the cycle is color-coded according to the scale shown at bottom of figure. A3 and C3: proportion of active forelimb-related neurons (neurons in their PEF) in different phases of the step cycle during unconstrained (A3) and wide-stance (C3) walking. A4 and C4: mean discharge rate of forelimb-related neurons during unconstrained (A4) and wide-stance (C4) walking. Thin lines show SE. B1–4 and D1–4 show characteristics of hindlimb-related neurons during unconstrained (B1–4) and wide-stance (D1–4) walking.
differed in activity more often and to a somewhat greater extent than the forelimb-related neurons.

DISCUSSION

Static and Dynamic Stability of Quadrupedal Walking

While the whole body kinematics and kinetics of unconstrained cat walking observed in this study were similar to those previously reported (Beloozerova et al. 2010; Manter 1938; Misiaszek 2006; Prilutsky et al. 2005; Prilutsky and Klishko 2011), the analysis of body stability unexpectedly revealed that the cat is dynamically unstable in the sagittal plane during diagonal double-support phases just prior to paw

Fig. 10. Population characteristics of forelimb-related neurons with different somatosensory receptive fields during unconstrained and wide-stance walking. A and D: activity of neurons responsive to movements in the shoulder joint during unconstrained (A) and wide-stance (D) walking, A1 and D1: phase distribution of PEFs. A2 and D2: corresponding phase distribution of discharge frequencies. The average discharge frequency in each 1/20th portion of the cycle is color-coded according to the scale shown at bottom of figure. A3 and D3: proportion of active neurons (neurons in their PEFs) in different phases of the step cycle. A4 and D4: mean discharge rate. Thin lines show SE. Vertical dashed lines denote end of swing and beginning of stance phase. B and E: activity of neurons responsive to passive movement of the elbow joint or palpation of arm muscles. C and F: activity of neurons responsive to passive movement in the wrist joint or palpation of muscles on the forearm or paw.

Fig. 11. Population characteristics of hindlimb-related neurons with different somatosensory receptive fields during unconstrained and wide-stance walking. A and D: activity of neurons responsive to movements in the hip joint during unconstrained (A) and wide-stance (D) walking. B and E: activity of neurons responsive to passive movement of the knee joint. C and F: activity of neurons responsive to passive movement of the ankle joint or palpation of muscles in the paw. Designations are as in Fig. 10.
Our hypothesis that a wide between-paw distance during cat walking will aid body stability in the frontal plane, as reported in human studies (Hof et al. 2007; McAndrew Young and Dingwell 2012), has not been fully supported. Static and frontal dynamic stability during wide-stance walking did increase in three-legged support phases (Figs. 3 and 4, phases 2, 4, 6, and 8), which corresponded to similar findings for double-support phases in human walking. In contrast to human studies, increasing stance width led to static and frontal dynamic instability of cat locomotion during ipsilateral double-support phases (Fig. 4, C and D, phases 1 and 5), corresponding roughly to single-leg support phases in human gait. Thus the analysis of stability for wide-stance walking showed unexpectedly that static and dynamic stability depended on the area of BoS, which increased for three-legged support phases and decreased for two-legged support phases compared with unconstrained walking (Fig. 4, Tables 2 and 3). Margins of static stability increased during wide-stance walking for three-legged support, but the cat became statically unstable during two-legged support phases of wide-stance walking. Moreover, margins of dynamic stability increased during wide-stance compared with unconstrained walking in only two phases (phases 4 and 8 of 3-legged support). The cats altered the duration of support phases in wide-stance walking by making three-legged supports with larger margins of dynamic stability longer and two-legged supports (dynamically unstable phases) shorter than during unconstrained walking (Table 2). This change in duration of limb support phases appears appropriate for improving stability of walking with wide stance.

It should be kept in mind that our analysis of dynamic stability of locomotion (see METHODS) differs somewhat from that of Hof (Hof et al. 2005, 2007, 2010). Hof’s margins of dynamic stability are computed as the distance between XCoM and CoP. Since measuring CoP during quadrupedal walking was challenging because of the limited number of force plates we had available (n = 3), we chose to evaluate dynamic stability based on the extrapolated center of mass position XCoM with respect to BoS. Although our measure of dynamic stability does not guarantee body dynamic stability when XCoM is inside BoS, it does identify phases of dynamic instability in which XCoM crosses the boundary of BoS beyond which CoP can be shifted in front of XCoM only by making a step and placing a foot in a new position (see condition C, p. 3 in Hof et al. 2005).

Frontal-Plane Kinetics and Kinematics of Walking

The resultant muscle moments in the frontal plane at the hip and shoulder during stance of unconstrained as well as wide-stance walking in the cat were computed for the first time to our knowledge and thus cannot be compared with previously published data. For unconstrained walking, the resultant action of all muscles around the hip and shoulder joints was to abduct the fore- and hindlimb (Fig. 6, G and H). The direction of the muscle moments during stance was generally consistent with changes in the frontal shoulder and hip angles from adduction to abduction (Fig. 7, E and F; see also Misiaszek 2006). The shoulder and hip moments in the frontal plane were computed with Eq. 4, which neglects linear and angular acceleration of the leg segments. This was done because moments of inertia with respect to the anterior-posterior and longitudinal axes of the cat limb segments are not known and the limb accelerations during stance are relatively small.

Fig. 12. Population characteristics of neurons responsive to abduction or extension vs. adduction or flexion of a limb. A and C: activity of neurons responsive to abduction or extension of shoulder or hip during unconstrained (A) and wide-stance (C) walking. B and D: activity of neurons responsive to abduction or flexion of shoulder or hip. Designations are as in Fig. 10.
small (see Fig. 7, panel 3 in Prilutsky et al. 2005), especially in the medial-lateral direction, judging from the medial-lateral CoM acceleration (Fig. 5B) and ground reaction force (Fig. 6; see also Manter 1938). To examine the validity of the assumption of small acceleration, we computed the sagittal muscle moments at the ankle, knee, and hip during unconstrained walking using both methods, quasi-static equations assuming zero accelerations (see Eq. 4) and dynamic equations that include inertial terms (Manter 1938; Prilutsky et al. 2005). The inertial segment parameters for the second approach were obtained with the regression equations (Hoy and Zernicke 1985). The results of this comparison (Fig. 6F) demonstrated that during the stance phase the error caused by assuming zero limb accelerations was negligibly small.

Other kinetic and kinematic changes observed during wide-stance walking appear to be necessary to accommodate the increase in stance width. For example, the increased lateral forces exerted by the limbs on the ground (Fig. 6, E and F) and increased abduction moments at the hip joints later in the stance phase (Fig. 6H) during wide-stance walking explain a larger displacement of the CoM in the medial direction (Fig. 5A) and greater abduction angles at the shoulder and hip joints (Fig. 7, E and F).

Muscle Activity During Wide-Stance Walking

Activity of several muscles contributing to motion in the frontal plane was analyzed in this study. AF (hip adductor and extensor) and GLM (hip abductor and hip extensor) had higher mean EMG magnitude and burst duration during wide-stance walking (Fig. 8, G and H). This result was generally consistent with the abduction action of GLM necessary to exert higher hip abduction moment and lateral forces during wide-stance walking (Fig. 6, F and H) and with the fact that AF and GLM are coactive during stance phase of unconstrained walking (Miaszek 2006).

In addition, a number of studied limb muscles with primary actions in the sagittal plane may contribute to propelling the body in the medial direction during wide-stance walking. First, many hindlimb extensors can generate an abduction moment at the joint (Bolton and Miaszek 2012; Carrasco and English 1999; Lawrence et al. 1993). For example, among ankle extensors, MG is the strongest abductor at the ankle. Its maximal frontal-plane moment is ~40% of the maximal extensor moment, and the MG maximal frontal moment exceeds that of LG and SO by 2.5 and 5.5 times, respectively, because the MG tendon wraps around and inserts on the lateral aspect of calcaneus (Lawrence et al. 1993; Nichols et al. 1993). The increased MG mean EMG burst magnitude and duration during wide-stance walking compared with unconstrained walking (Fig. 8, G and H) is consistent with the ability of this muscle to contribute to abduction, which is necessary for exerting laterally directed forces on the ground during wide-stance walking (Fig. 6F). On the other hand, the other ankle extensors SO and LG had significantly lower mean activity during

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Fig. 13. Comparison of activity characteristics of individual neurons from forelimb (A–C) and hindlimb (D–F) representation in the motor cortex between unconstrained and wide-stance walking. A and D: mean discharge frequency averaged over the stride. B and E: depth of frequency modulation. C and F: preferred phase of the activity of neurons with a single PEF during both locomotion tasks. A–F: x-axis and y-axis of each point show the values of a characteristic of a neuron during locomotion with unconstrained and wide paw separation, respectively. Neurons whose characteristics were statistically different during locomotion in the 2 conditions (see METHODS) are shown with filled circles; nonsignificantly different values are shown with open circles.
wide-stance compared with unconstrained walking (Fig. 8G) in agreement with their smaller potential to produce abduction moment. Biceps femoris anterior and posterior muscles can contribute a modest abduction moment at hindlimb joints (Carrasco and English 1999), and in agreement with this mechanical action these muscles demonstrated a larger mean EMG magnitude (BFA, BFP1, BFP2) and burst duration (BFA) during wide-stance walking (Fig. 8, G and H).

The greater activity of hindlimb flexor muscles during wide-stance walking (TA, SAM, IP, and 2 bursts of BFP) was consistent with the higher vertical displacements of the hindpaw in this walking condition (Fig. 7, A and B).

The smaller mean EMG burst magnitude and only the slightly higher burst duration of the three studied forelimb muscles (TRL, EDC, and FCU; Fig. 8, G and H) during wide-stance walking suggest that these muscles do not play a major role in propelling the body in the frontal plane during this task.

Activity of Motor Cortex Neurons During Wide-Stance Walking

The main finding of this study regarding the activity of the motor cortex during wide-stance walking compared with the unconstrained condition is that the activity of a great majority of motor cortex layer V neurons in both forelimb- and hindlimb-related representations was different between these two walking conditions, with the largest proportions of neurons showing different depths of stride-related frequency modulation (42% in the forelimb- and 68% in the hindlimb-related groups) and the hindlimb-related cells showing differences in the activity more often and occasionally to a larger extent than forelimb-related cells (Fig. 13). Overall, the activity of both forelimb- and hindlimb-related populations was subtly but statistically significantly greater in the swing phase of the stride during wide-stance walking (Fig. 9, A4 and B4 vs. C4 and D4). These findings first of all suggest that, as we have shown previously for the task of walking on a laterally inclined surface (Karayannidou et al. 2009), the motor cortex participates in the control of limb configuration during locomotion. Moreover, as more neurons responded to the task of walking with wide stance than on the tilted surface, these data suggest that, in addition to the altered limb configuration, in the task of walking with a wide between-paw distance the motor cortex was also responding to the increased demand for lateral body stability in phases with $\text{IDS}_t < 0$ (Figs. 3C and 4C, phases 1, 3 and 5, 7).

Overall, the activity of the motor cortex during unconstrained locomotion observed in this study was consistent with previous reports (e.g., Armer et al. 2013; Beloozerova et al. 2003a; Beloozerova and Sirota 1993a, 1993b; Drew 1993; Fitzsimmons et al. 2009; Karayannidou et al. 2009; Prilutsky et al. 2005; Stout and Beloozerova 2012, 2013; Widajewicz et al. 1994). Namely, the discharge rate of nearly all neurons, 88% of which were identified as pyramid tract projecting neurons (PTNs), was modulated in the rhythm of strides, with different neurons active during different phases of the cycle, so that the entire populations of forelimb- and hindlimb-related neurons showed only a minor stride-related activity modulation (Fig. 9). The peak of the activity of the forelimb-related population occurred around the end of the swing phase (Fig. 9A), the period of body instability in the sagittal plane, in which a proper placement of forepaw in front of the extrapolated center of mass is critically important for maintaining stable locomotion (Fig. 4). Within the forelimb-related population, the neurons with receptive fields on the wrist, as a group, showed a stronger preference to be active during the swing phase (Fig. 10C). A similar finding was also reported in our other recent studies (Armer et al. 2013; Stout and Beloozerova 2012). In addition, we have previously found that, among all forelimb-related neurons, fast-conducting PTNs as a group have the highest preference to discharge during the swing phase (Stout and Beloozerova 2013). Thus one can suggest that wrist-related neurons, particularly the fast-conducting wrist-related PTNs, contribute to a proper placement of the forelimb and, consequently, to the stability of the body during locomotion. The mechanisms of such contribution are not well understood; they might involve synaptic gating in 1) descending and ascending pathways between the motor cortex and other brain areas and spinal cord, 2) spinal interneuron circuits comprising the locomotor rhythm generator and/or pattern formation networks, and 3) interneurons mediating proprioceptive input, etc. (Brownstone and Bui 2010; Drew et al. 2008; Markin et al. 2012; Stecina et al. 2013).

The activity of the hindlimb-related population within the database considered in this study was nearly evenly distributed over the cycle of unconstrained walking (Fig. 9B). This was not the case in the group of cells in our previous research (Armer et al. 2013) and in a study from the laboratory of Dr. Drew (Widajewicz et al. 1994): those hindlimb-related populations, similar to the forelimb-related one, showed a preference to fire during the end of swing phase, possibly because an accurate hindpaw placement on the ground beyond XCoM in the frontal plane provides lateral dynamic stability at the transition from double-support phase by ipsilateral fore- and hindlimbs to a three-legged support phase (Fig. 3B, phases 2 and 6).

When the cat was required to walk with a wide stance base, which in addition to altering the configuration of the limbs dramatically decreased margins of body stability during most of the stride cycle, especially in the frontal plane (Fig. 4C), and the cat combated this instability by prolonging the duration of the three-legged support phases and shortening the double-support phases (Table 2), the overwhelming majority of layer V neurons in both the fore- and hindlimb representations (82% and 83%, respectively) changed their activity compared with unconstrained walking (Fig. 13). This behavior was similar to the previously reported massive responses of these neurons to changes of postural adjustments during standing (Beloozerova et al. 2005) and accuracy demands on paw placements during locomotion (Beloozerova et al. 2010; Stout and Beloozerova 2012, 2013).

Among all characteristics of the neuronal discharge that we have analyzed, the depth of stride-related frequency modulation was the one that was different in the largest proportions of neurons (in 42% of the forelimb- and 68% of the hindlimb-related neurons) between unconstrained and wide-stance walking. We have previously found that motor cortical cells respond in this manner to change in accuracy demands during walking, and believe that an unusual placement of paws wide apart during wide-stance walking might have engaged the same “accuracy” mechanism. Indeed, previous results have shown that, when the accuracy of paw placement is required as during walking on a horizontal ladder or narrow pathway, the head-neck segment rotates closer to the ground and its height is
lowered (Beloozerova et al. 2010; Farrell et al. 2011). We have hypothesized that these kinematic postural adjustments facilitate acquisition of visual information about target location for paw placement. Here we observed that similar postural adjustments took place during wide-stance walking as well (Fig. 5, C and D), suggesting that the cats could have been selecting locations for paw placements during this locomotion task.

Many neurons that discharged one PEF per stride during both walking tasks changed the preferred phases of their activity in the stride cycle upon transition from unconstrained to wide-stance walking (40% in the forelimb- and 49% in the hindlimb-related populations). And although the changes were modest for most of them, especially in the forelimb-related population, constituting only 10–15% of the cycle, neurons tended to shift their activity in a single direction, namely, to a phase earlier in the cycle (Fig. 13, C and F). As a result, some neurons that discharged during the stance phase of unconstrained walking discharged during the swing phase of wide-stance walking, a change that was also observed in the activity of neurons that discharged during the stance phase of unconstrained walking. And although the changes were modest for most of them, especially in the forelimb-related hindlimb-related populations discharging slightly more intensively during the swing phase of wide-stance walking (Fig. 9). Neurons that responded to adduction or flexion of proximal joints at rest demonstrated more pronounced changes in the phase of their activity than other subpopulations (Fig. 12), probably contributing to the changes in the magnitude of the shoulder and hip frontal plane moments (Fig. 6, G and H). Overall, however, populations as a whole responded to walking with a wide between-paw distance to a much lesser degree than individual neurons. This is similar to observations made during other locomotion tasks, such as walking in the darkness or on rungs of a horizontal ladder (Armer et al. 2013; Stout and Beloozerova 2013). These results first of all may simply reflect the fact that, as we have suggested previously (Karayanidou et al. 2009), different neurons within such broadly defined groups as “forelimb-related” and “hindlimb-related” have different roles in control of locomotion overall and postural control during locomotion in particular. In addition, for control of posture, it has been previously suggested that the spinal postural networks may need a certain supraspinal drive to be operational (Delagiina et al. 2000, 2006). A similar concept could be extended to the cortical control of locomotion and, in addition to a relatively constant input signal to brain stem and spinal locomotor networks in the form of the mean discharge rate of motor cortex neuronal populations, may also include the average depth of modulation of the cortical signal and other parameters of the cortex activity.

It is clear that all four major descending systems, reticulo-, vestibulo-, rubro-, and cortico-spinal, contribute to the control of posture during locomotion. Indeed, the activity of neurons of the corresponding descending tracts is profoundly modulated with respect to phases of the stride (Orlovsky et al. 1999) and changes with the tilt of the support surface (Karayanidou et al. 2009; Matsuyama and Drew 2000; Zelenin et al. 2010). Chronically decerebrate animals can sit, stand, and walk, demonstrating that an essential part of neural mechanisms providing the control of posture in quadrupeds is located in the brain stem, cerebellum, and spinal cord (Bard and Macht 1958; Magnus 1924). Several recent reviews discuss the coordination between spinal and various supraspinal postural networks (e.g., Delagiina et al. 2007, 2008, 2012). The present study has demonstrated for the first time that during normal unconstrained quadrupedal cat walking there are periods of body dynamic instability, which are associated with increased activity of neurons in the motor cortex. When additional stability demands in the frontal plane are introduced, i.e., walking with a wide between-paw distance, the cat prolongs the time spent in three-leg support and shortens the double-support phases and cortical neurons strongly respond, typically by changes in the depth of their stride-related frequency modulation and shifting their activity in time toward the swing phase of the stride where most of the instability occurs.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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