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Activity of motor cortex neurons during backward locomotion

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Zelenin PV, Deliagina TG, Orlovsky GN, Karayannidou A, Stout EE, Sirota MG, Beloozerova IN. Activity of motor cortex neurons during backward locomotion. *J Neurophysiol* 105: 2698–2714, 2011. First published March 23, 2011; doi:10.1152/jn.00120.2011.—Forward walking (FW) and backward walking (BW) are two important forms of locomotion in quadrupeds. Participation of the motor cortex in the control of FW has been intensively studied, whereas cortical activity during BW has never been investigated. The aim of this study was to analyze locomotion-related activity of the motor cortex during BW and compare it with that during FW. For this purpose, we recorded activity of individual neurons in the cat during BW and FW. We found that the discharge frequency in almost all neurons was modulated in the rhythm of stepping during both FW and BW. However, the modulation patterns during BW and FW were different in 80% of neurons. To determine the source of modulating influences (forelimb controllers vs. hindlimb controllers), the neurons were recorded not only during quadrupedal locomotion but also during bipedal locomotion (with either forelimbs or hindlimbs walking), and their modulation patterns were compared. We found that during BW (like during FW), modulation in some neurons was determined by inputs from limb controllers of only one girdle, whereas the other neurons received inputs from both girdles. The combinations of inputs could depend on the direction of locomotion. Most often (in 51% of forelimb-related neurons and in 34% of the hindlimb-related neurons), the neurons received inputs only from their own girdle when this girdle was leading and from both girdles when this girdle was trailing. This reconfiguration of inputs suggests flexibility of the functional roles of individual cortical neurons during different forms of locomotion.

pyramidal tract neurons; limb controllers; cat

THERE ARE TWO IMPORTANT FORMS of locomotion in quadrupeds—forward walking (FW) and backward walking (BW). It was suggested that the functional organization of the control system for both forms of locomotion is basically similar (for a review, see Orlovsky et al. 1999). In brief, stepping movements of each of the limbs are generated by a spinal mechanism (the limb controller) that includes the central pattern generator (CPG) and the sensory feedback from the limb. The limb controllers interact to assure a common rhythm of stepping and proper phase relations between the stepping limbs.

Although the patterns of stepping limb movements in these two forms of locomotion differ considerably from one another (Buford et al. 1990), it was suggested that they are generated by the same limb controllers, subjected to only minor modifications (Buford and Smith 1990). This view was later supported by the finding that in spinal cats, the same electrical stimulation of the spinal cord could evoke either FW or BW, depending on the external conditions, i.e., on the direction of

treadmill movement (Gerasimenko et al. 2008; Musienko et al. 2007).

In quadrupeds, the spinal-limb controllers are activated and modulated by supraspinal commands transmitted through several descending pathways: reticulospinal, corticospinal, and others (Orlovsky et al. 1999). Apparently, supraspinal commands are also responsible for voluntary elicitation of the two forms of activity of the limb controllers, underlying FW and BW. In quadrupeds, supraspinal commands have been intensively studied for forward locomotion (see e.g., Orlovsky et al. 1999) and never studied for backward locomotion. However, descending commands controlling forward and backward locomotion were recently studied in the lamprey, a lower vertebrate. Recording the activity of reticulospinal neurons during forward and backward locomotion revealed several groups of these neurons with different activity patterns, suggesting their different functions, including activation of the locomotor CPG in the forward or backward mode (Zelenin 2011).

In higher vertebrates, one of the centers involved in control of locomotion is the motor cortex. The activity of its neurons [including pyramidal tract neurons (PTNs)] during FW has been analyzed in considerable details. It was found that first, the neurons are active during locomotion, and their firing frequency is modulated in the rhythm of stepping (Armstrong and Drew 1984a,b; Beloozerova and Sirota 1985, 1993a,b; Drew 1993). Second, the modulation is caused by influences from limb controllers, and inputs from several controllers may contribute to modulation of an individual neuron and collectively determine the phase and amplitude of modulation (Zelenin et al. 2011). Third, the depth of frequency modulation and occasionally, the mean firing frequency of the neurons considerably increase during walking on uneven terrain, which requires modifications of the locomotor movements on the basis of visual information (Beloozerova and Sirota 1988, 1993a; Drew 1988, 1991).

In contrast to FW, the motor cortex activity during BW has never been studied. This is unfortunate, because as a presumably more complex and less-exercised form of locomotion, BW might require stronger involvement of the motor cortex. Also, comparison of the motor cortex neuronal activity during two different forms of locomotion might shed more light on the specific functions of cortical neurons during locomotion. The present study is the first one devoted to the analysis of motor cortex activity during BW and to comparison of this activity with that during FW.

For characterization of the locomotion-related activity of the motor cortex, we recorded individual motor cortex neurons both during FW and during BW. We found that individual neurons were active and rhythmically modulated during BW,

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suggesting involvement of the motor cortex in the control of this form of locomotion.

To reveal the sources of rhythmical modulation of the neurons, we used the previously developed method (Zelenin et al. 2011) and recorded individual neurons, not only during quadrupedal locomotion but also during bipedal locomotion. We found that limb controllers of both girdles contributed to modulation in the majority of neurons. In almost one-half of all neurons, the combination of inputs from the two girdles was different during BW and during FW. Such dependence of inputs on the direction of locomotion suggests flexibility of the functional roles of individual cortical neurons during locomotion.

A brief account of this study was published in abstract form (Zelenin et al. 2010).

MATERIALS AND METHODS

Recordings were obtained from two adult cats, one male and one female. Activity of neurons during FW has been described in our previous publication (Zelenin et al. 2011). The present paper focuses on their activity during BW and on comparison of the activity during BW and FW. Some of the methods of data collection and analysis have been described earlier (Beloozerova et al. 2005; Karayannidou et al. 2008; Prilutsky et al. 2005; Zelenin et al. 2011) and will be reported briefly here. All experiments were conducted at Barrow Neurological Institute (Phoenix, AZ) in accordance with NIH guidelines and with the approval of the Barrow Neurological Institute Animal Care and Use Committee.

Surgical Procedures

Surgery was performed under isoflurane anesthesia using aseptic procedures. Bipolar electromyography (EMG) electrodes (flexible Teflon-insulated, stainless-steel wires) were implanted bilaterally into two forelimb muscles—*Musculus brachialis* (Bra; elbow flexor) and *M. triceps brachii* (Tric; elbow extensor)—and into four hindlimb muscles—*M. tibialis anterior* (Tib; ankle flexor), *M. gastrocnemius lateralis* (Gast; ankle extensor), *M. vastus lateralis* (Vast; knee extensor), and *M. gluteus medius* (Glut; hip extensor and abductor).

The skin and fascia were removed from the dorsal surface of the skull. At 10 points around the circumference of the head, stainless steel screws were screwed into the skull and connected together with a wire; the screw heads and the wire were then inserted into a plastic cast to form a circular base. Later, while searching for neurons before locomotion tests, awake cats were rigidly held by this base. The base was also used for fixation of connectors, a miniature microdrive, and a preamplifier, as well as contacts for stimulating electrodes and a protective cap.

A portion of the skull and dura above the left motor cortex was removed. The motor cortex was identified by the surface features and photographed (see Fig. 2A). The aperture was then covered by a plastic plate with many small holes filled with wax. The plate was fastened to the surrounding bone. Two, 26-gauge hypodermic guide tubes were implanted vertically above the medullary pyramids at the Horsley-Clarke coordinates (P10, L0.5 and P10, L1.5) at the depth of V0 for subsequent insertion of stimulating electrodes into the pyramidal tract.

Identification of Cortical Motor Area

Experiments were initiated after several days of recovery. The animal was positioned on a table equipped with a foam rubber pad and a head-restraining device. After the cat rested on this pad for several minutes, the base attached to the skull during surgery was fastened to

the head-restraining frame so that the resting position of the head was approximated. This procedure minimized stress on the neck while the head was immobilized. Over several days, a number of sessions of increasing duration were used to accustom the cat to the head restraint.

The motor cortex was mapped using multiple-unit recording and microstimulation techniques. Areas of fore- and hindlimb representations were delineated based on somatosensory receptive fields and evoked movements. A detailed description of the area of recording was given earlier (Beloozerova et al. 2005). In brief, the area immediately adjacent to and inside the lateral one-half of the cruciate sulcus in the cat is considered to be the motor cortex. This is based on a considerable body of data obtained by means of inactivation, stimulation, and recording techniques (Armstrong and Drew 1985; Beloozerova and Sirota 1993a; Drew 1993; Nieoullon and Rispal-Padel 1976; Vicario et al. 1983), as well as on histological considerations (Ghosh 1997; Myasnikov et al. 1997). Microelectrode entry points on the cortical surface are schematically shown (see Fig. 2A). Histological verification of recording sites within the forelimb and hindlimb representations of the motor cortex has been provided in the previous publication (Zelenin et al. 2011).

Cell Recording and Identification

Neuronal activity was recorded extracellularly from the left motor cortex using commercially available tungsten varnish-insulated electrodes (Frederick Haer & Co., Bowdoin, ME). The custom-made microdrive (5×5×30 mm, 2.5 g) was permanently fastened to the base on the cat's head and used to advance the microelectrode. The impedance of the electrodes was 2–4 MΩ at 1,000 Hz. After the electrode reached the depth of the cortex, where the responses of neurons-to-limb movements could be clearly observed (presumably layer V), two, 200-μm platinum-iridium wires were slowly inserted and lowered into the medullary pyramid through the guide tubes (implanted during surgery). Pulses of graded intensity (0.2 ms duration, up to 0.5 mA) were delivered through this bipolar electrode. The wires were fixed at the position that was most effective in eliciting antidromic responses in neurons of the motor cortex and served as the pyramidal tract-stimulating electrode for all following experiments. The criterion for identification of antidromic responses was the test for collision of spikes (see Fig. 2B) (Bishop et al. 1962; Fuller and Schlag 1976). The waveform analysis was used to discriminate and identify the spikes of a single neuron using the Power1401/Spike2 system (Cambridge Electronics Design, Cambridge, UK) waveform-matching algorithm. All encountered neurons were tested for antidromic activation before, during, and after each locomotor test, using identical current pulses and criterion. The neurons with a stable response latency and spike shape, which satisfied the collision test, were considered PTNs. The somatic-receptive fields of neurons were examined in resting animals under conditions of head restraint. Stimulation was produced by palpation of muscle bellies, tendons, etc., as well as by passive movements of joints.

The PTNs and nonidentified neurons had similar parameters of activity and were present in all functional groups (see RESULTS). That is why the data for all neurons are usually shown together. Detailed analysis of differences between activity of PTNs and nonidentified neurons needs further investigation. As all neurons were recorded from layer 5 of the motor cortex, some of the nonidentified neurons could be PTNs, but antidromic stimulation failed to activate their axons.

Locomotor Tests

During search for the neurons, the animal was sitting with its head fixed to the stationary frame. After a neuron was found and identified, the head was freed, and the animal was positioned on the belt of the treadmill (Fig. 1A). The belt gradually attained the speed of 0.5 m/s, maintained it for 1–1.5 min, and then slowly stopped. Cats were

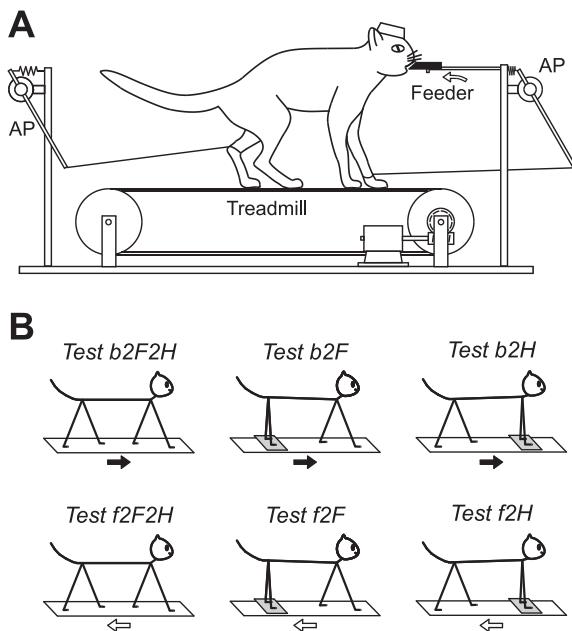


Fig. 1. Experimental design. *A*: the cat was walking on the moving treadmill belt backward or forward. The body configuration during backward walking (BW) is schematically shown. The anterior-posterior (AP) position of the limbs during stepping was recorded by mechanical sensors. *B*: different locomotor tests were used: the BW with all 4 limbs (*Test b2F2H*), with forelimbs only (*Test b2F*), with hindlimbs only (*Test b2H*), the forward walking (FW) with all 4 limbs (*Test f2F2H*), with forelimbs only (*Test f2F*), or with hindlimbs only (*Test f2H*). The black and white arrows indicate the direction of the treadmill movement during BW and FW, correspondingly. During all tests, the cat was continuously licking food from the feeder (black bar in *A*).

trained to perform different locomotor tasks (see the six forms of locomotion below) and were rewarded by a paste-like food, continuously ejected from a feeder (Karayannidou et al. 2008). The feeder (a plastic tube of 18 mm outer diameter and 6 mm inner diameter) was always positioned in front of the cat at a height of 21–23 cm (Fig. 1A). It took a few weeks for the cats to get acquainted with walking on the treadmill. After this training period, they were easily engaged in all locomotion tasks. The cats maintained a stable position in relation to the treadmill, which allowed them to hold the mouth against the feeder and to keep licking food during walking (Karayannidou et al. 2009).

Six forms of locomotion were tested; they included quadrupedal and bipedal walking both backward and forward (Fig. 1B): *Test b2F2H*: all four limbs walk backward; *Test b2F*: the forelimbs walk backward, while the hindlimbs stand on a stationary platform; *Test b2H*: the hindlimbs walk backward, while the forelimbs stand; *Test f2F2H*: all four limbs walk forward; *Test f2F*: the forelimbs walk forward, while the hindlimbs stand; *Test f2H*: the hindlimbs walk forward, while the forelimbs stand. Between the tests, a cat was standing and licking food. These periods lasted for 5–10 s.

Four mechanical sensors monitored the anterior-posterior (AP) position of limbs during walking (Karayannidou et al. 2008); two of the sensors (attached to the left forelimb and the left hindlimb) are shown in Fig. 1A. In selected experiments, limb movements were also monitored using the Visualeyez System (Real-Time 3D Motion Capture and Analysis System, PhoeniX Technologies, Burnaby, B.C., Canada). It detects positions of light-emitting photodiodes in 3D space and makes calculations of various kinematical parameters. The photodiodes were attached to the skin projections of the main limb joints, either on the right forelimb or on the right hindlimb (see Fig. 4). The frequency of frame sampling was 200 Hz. In some trials, cats' movements were also videotaped (30 frames/s). The movies were used for preliminary overview of the data and for drawing of the outline of the body during locomotion (Fig. 1A).

Data Collection and Processing

Signals from the microelectrode preamplifier, from EMG preamplifiers, as well as those from the position sensors, were amplified and filtered (300–10,000 Hz band-pass for neurons and 30–2,000 Hz band-pass for EMG and sensors) using a CyberAmp 380 (Axon Instruments, Union City, CA) amplifier, digitized with a sampling frequency of 30 kHz (microelectrode), 3 kHz (EMGs), and 400 Hz (sensors), displayed on the screen, and recorded to the disc of a computer using data-acquisition software (Power1401/Spike2, Cambridge Electronics Design). After digitization, the EMG signals were rectified and smoothed by filters with a time constant of 50 ms. An example of untreated data recording is shown in Fig. 2C.

All neurons were examined during quadrupedal walking backward (*Test b2F2H*) and forward (*Test f2F2H*). Most of them were also examined in four other tasks with bipedal walking. The activity of neurons was typically modulated in the rhythm of stepping movements (Fig. 2C). To characterize this modulation, the phase histogram of neuronal activity in the step cycle was created. Because of some variability in the duration and structure of step cycles within a test and between the tests (see RESULTS), we divided the step cycle in four periods and normalized them separately. These periods for *Tests 2F2H*, *2F*, and *2H* are shown in Fig. 3A–F: (1) the right-limb swing; (2) the early right-limb stance ending when the left limb begins swing; (3) the right limb midstance while the left limb is in swing; (4) the late right-limb stance starting when the left limb touches ground. Each of the four periods was normalized to one-quarter of the cycle. Such normalization ensured that muscular and neuronal activity during a definite phase (swing or stance) in one test was compared with activity during the same phase in all other tests or when these characteristics were compared in different steps within the same test. The range of phase values for the first period was from 0 to 0.25; for the second period, from 0.25 to 0.5; for the third period, from 0.5 to 0.75; and for the fourth period, from 0.75 to 1 (see Figs. 5, 6, 9, and 10).

The spike time sequence was converted to instantaneous rate vs. time and then to instantaneous rate vs. phase (250 points in each of the four periods of the cycle). The dependence of the instantaneous rate on the phase was averaged over all steps of a given test. Then, the histogram was smoothed (sliding window, 50 bins). Examples of the resulting histograms are shown (see Figs. 10 and 11). Phase histograms of the same type were also created for joint angles (see Fig. 5) and rectified EMG signals (see Fig. 6).

To evaluate the depth and the phase of step-related modulation of neuronal activity, we used the best two-level rectangular fit for instantaneous frequency within the step cycle; the *upper level* was defined as a “burst” and the *lower level* as an “interburst period” (see Fig. 10) (Karayannidou et al. 2009). The activity of neurons was considered modulated if the burst frequency was significantly different from the interburst frequency (*t*-test, $P < 0.05$). For the modulated neurons, the coefficient of frequency modulation (K_{mod}) was calculated using a formula $K_{mod} = (f_B - f_{IB})/f_B$, where f_B and f_{IB} are the burst and interburst frequencies, respectively. The middle of the burst was taken for the preferred phase of the neuronal activity Φ_{pref} . We also calculated the mean frequency of the neuronal activity f_M (see Fig. 10).

To evaluate the degree of similarity between modulation patterns of the same neuron in two different tests, we used an ordinary method of correlation analysis (see, e.g., Zar 1974) but calculated the coefficient of correlation (CC) not between two random variables but between two functions (phase histograms) obtained in these tests (Zelenin et al. 2011). This analysis reveals covariations of the two functions, i.e., parallel changes of the instantaneous discharge frequency within the cycle, while dismissing differences between f_M and depths of modulation. A few examples of neuronal discharges recorded in different tests, as well as the results of their comparison (the values of CC), are shown (see Fig. 10). We also used such analysis for comparison of phase profiles of angles in individual joints of the fore- and hindlimb

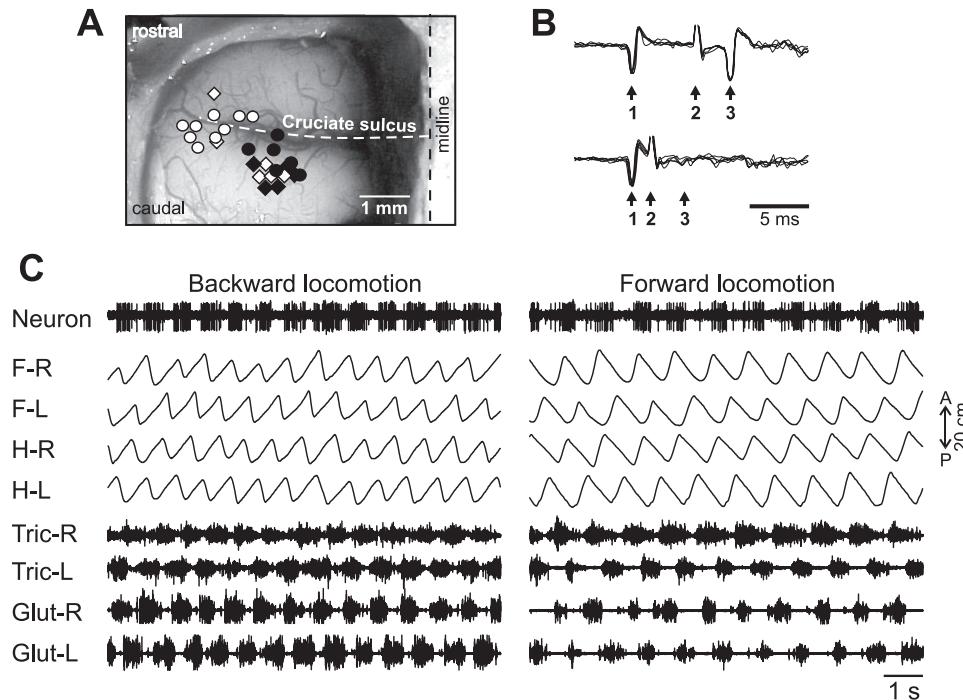


Fig. 2. Identification and recording of motor cortex neurons. **A:** areas of recording within representations of the fore- and hindlimb in the left motor cortex. Microelectrode entry points are combined from cat 1 (circles) and cat 2 (diamonds) and shown on a photograph of cat 2 cortex by white symbols (forelimb-related neurons in the track) or black symbols (hindlimb-related neurons in the track). **B:** collision test determines whether a response of a neuron is antidiromic. **Top trace:** a pyramidal tract neuron (PTN) spontaneously discharges (arrow 1), and pyramidal tract is stimulated ~6 ms later (arrow 2). PTN responds with latency ~3 ms (arrow 3). **Bottom trace:** a PTN spontaneously discharges (arrow 1), and pyramidal tract is stimulated ~1 ms later (arrow 2). The PTN does not respond (arrow 3), because in 1 ms, its spontaneous spike was still en route to the pyramidal tract, and thus collision/nullification of spontaneous and evoked spikes occurred. **C:** an example of the recording of a neuron activity along with movements of 4 limbs (F-R, right forelimb; F-L, left forelimb; H-R, right hindlimb; H-L, left hindlimb) and 4 electromyograms (EMGs; Tric-R, right triceps; Tric-L, left triceps; Glut-R, right gluteus; Glut-L, left gluteus) during quadrupedal BW and FW.

during two different tests (see Fig. 5), as well as for comparison of EMG patterns of the same muscle in two different tests (see Fig. 6).

To build the phase histograms in the tests in which the neuron's "own" girdle did not walk, we used movements of the other girdle for reference. In *Test b2F2H* and *Test f2F2H*, for each neuron, we built two phase histograms, using either the forelimb movements or the hindlimb movements for reference. For example, for a hindlimb-area neuron, we used movements of the forelimbs to define swing and stance phases in *Test b2F* and *Test b2F2H* and movements of the hindlimbs in *Test b2H* and (once more) in *Test b2F2H*. To calculate CC in *Test b2F*, the histogram for *Test b2F2H* in the forelimb cycle was used. To calculate CC for *Test b2H*, we used the histogram for *Test b2F2H* in the hindlimb cycle. Data from *Tests f2F2H*, *f2F*, and *f2H* were processed in the same way.

All quantitative data that characterize populations are presented as the mean \pm SD. Statistical comparisons were made using *t*-test, with the significance level $P = 0.05$.

Histological Procedures

At the termination of experiments, cats were deeply anaesthetized with pentobarbital sodium. Several reference lesions were made in the region of motor cortex from which neurons were sampled. Cats were then perfused with isotonic saline, followed by a 10% formalin solution. Frozen brain sections of 50- μ m thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet. The position of stimulation electrodes in the medullary pyramids was verified by observation of electrode track gliosis. The positions of recording tracks in the motor cortex were estimated in relation to the reference lesions.

RESULTS

Kinematics and EMG Patterns in Different Locomotor Tasks

In the present study, analyses of kinematics and EMG had two goals: 1) to reveal similarities and distinctions in the motor coordination between the two forms of quadrupedal locomotion—FW and BW—and 2) to provide evidence that stepping

movements during quadrupedal and bipedal BW are largely similar, as was previously demonstrated for FW (Zelenin et al. 2011). Such similarity allows the use of the correlation analysis for revealing the limb controllers responsible for modulation of the neurons (Zelenin et al. 2011).

Kinematics. Fig. 3, *A* and *B*, gives examples of stepping movements during quadrupedal BW (*A*) and FW (*B*). As one can see from the traces of the AP foot position (A↔P), the cyclic limb movements during BW and FW were very different: the limb moved forward in the stance phase and backward in the swing phase during BW, and these directions were opposite during FW. During both BW and FW, there was a phase-shift of one-half of the cycle between the two limbs of each girdle and about one-quarter of the cycle between the ipsilateral fore- and hindlimbs. These patterns of BW and FW were similar to those described by other authors (Buford et al. 1990; Rasmussen et al. 1978). In the tests with bipedal BW (Fig. 3, *C* and *E*), AP movements in the swing and stance phases were opposite compared with bipedal FW (Fig. 3, *D* and *F*), similarly to their reversal in the quadrupedal tests (Fig. 3, *A* and *B*).

We have found that the average cycle duration was ~1 s across all tasks except *Test b2F* and *Test b2H*, during which the cycle duration was shorter (Fig. 3*G*). The backward locomotion tended to be somewhat more variable, as one can see from the larger SD for the BW tests (Fig. 3*G*). The average phase-shift between the right and left limbs was about one-half of a cycle (Fig. 3*H*). The structure of the cycle (a relative duration of the periods 1–4) was rather different in different tests. The swing phase had a tendency to occupy less than one-quarter of a cycle during BW but more than one-quarter of a cycle during FW. Also, the phase duration of the swing during bipedal walking was longer than during quadrupedal walking for both FW and BW.

For a detailed analysis of kinematical patterns in different locomotor tasks, we used the VisualEyez System (PhoeniX

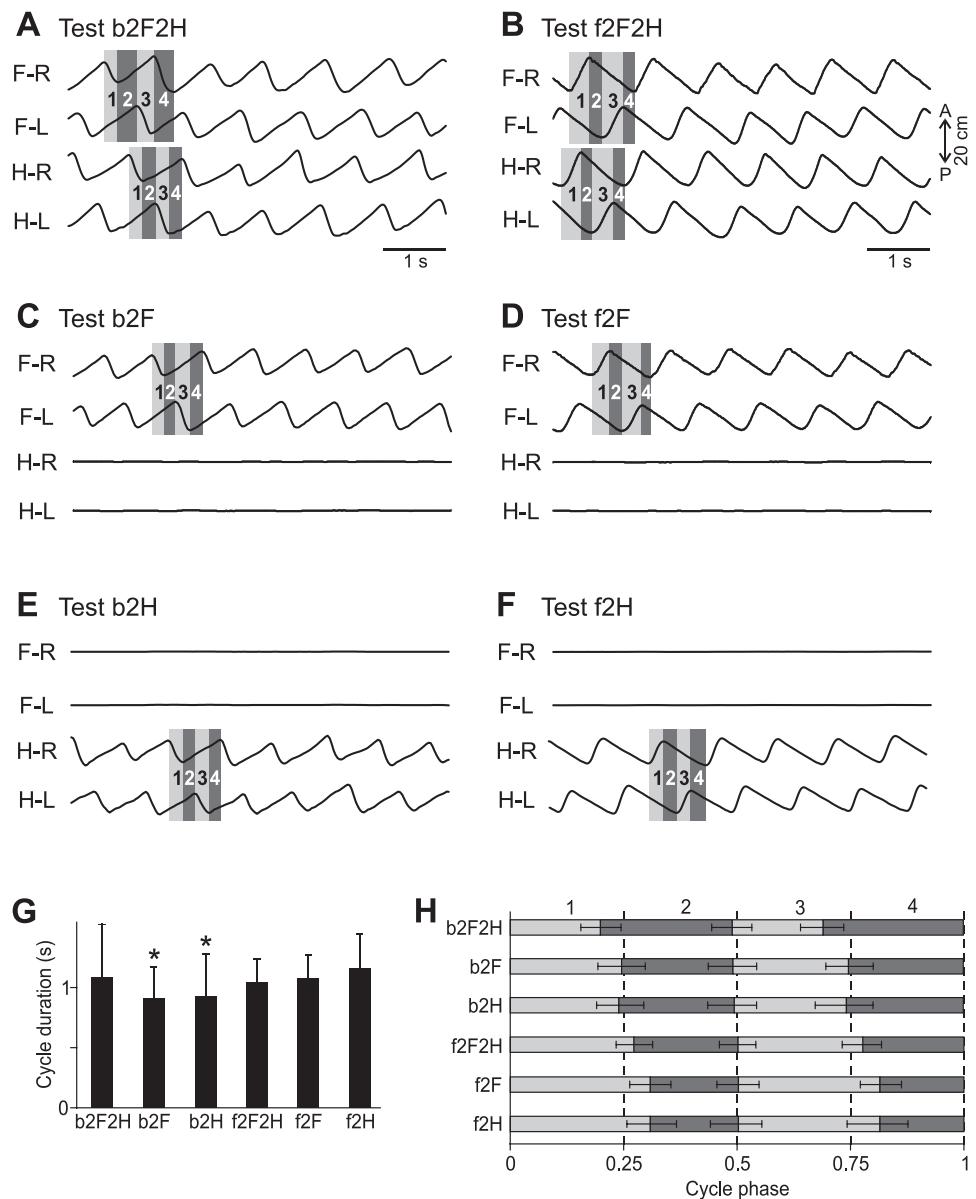


Fig. 3. General characteristics of different tests. *A–F*: limb movements in different tests. Four periods were recognized in each locomotor cycle for each girdle: the right-limb swing (1), the period after the right-limb swing but before the left-limb swing (2), the right limb midstance lasting while the left limb is in swing (3), the period after the left-limb swing but before the right-limb swing (4). *G*: the average cycle duration in different tests (mean \pm SD); *significant difference relative to *Test b2F2H* is indicated. *H*: the cycle structure, i.e., the parts of the cycle occupied by each of the 4 periods in different tests, averaged over all trials of each test (mean \pm SD). Note that the periods were close but not equal to one-quarter of the cycle (swing periods were shorter than one-quarter during BW and longer than one-quarter during FW). Each of the periods was normalized to one-quarter of the cycle in the further analysis. The swing of left or right limb (periods 1 and 3) are colored light gray, the remaining periods (2 and 4) are colored dark gray.

Technologies; see MATERIALS AND METHODS, Locomotor Tests section), which recorded limb position at sequential points of the step cycle and calculated joint angles at these points.

Figure 4 shows representative stick diagrams of the right hindlimb (*A*) and the right forelimb (*B*) obtained in different BW tests, separately for the swing and stance phases of the step. The stick diagrams for walking limbs during bipedal BW (*Tests b2F* and *b2H*) were similar to those during quadrupedal BW (*Test b2F2H*). In those tests in which a limb was standing, its movements were very small. One can also see that the stick diagrams during FW were very different from those during BW.

Figure 5 shows averaged angular movements in the main joints of the right forelimb (*A*) and of the right hindlimb (*B*) of one cat during quadrupedal and bipedal BW, as well as during quadrupedal FW. To characterize similarity of the angle profiles in the quadrupedal BW test (*b2F2H*) and in the bipedal BW tests, we calculated the CC between the two functions (see MATERIALS AND METHODS, Data Collection and Processing sec-

tion). In the example shown in Fig. 5, the CC values are indicated in each panel. We found that in all analyzed cases (two cats), when a limb was walking, CC was in the range of 0.7–1.0, thus indicating that the joint angle trajectory of the walking limb was similar in the quadrupedal and bipedal tests. By contrast, the kinematics of the standing limbs was completely different from that in *Test b2F2H* because of the absence of AP movements (CC was <0.5 in all analyzed cases). The angular trajectories during FW could be completely dissimilar from those during BW (e.g., for hip CC = −0.9 in Fig. 5*B*) or rather similar (e.g., for elbow CC = 0.72 in Fig. 5*A*). This confirmed the earlier findings of Buford et al. (1990) for the hindlimb joints and extended them to the forelimb joints.

We did not perform any detailed analysis of the animal's posture during BW. It can only be noted that the BW posture was different from the FW posture, as well as from the BW posture observed by other authors (Bufford et al. 1990). A typical body configuration during BW is shown in Fig. 1*A*. In

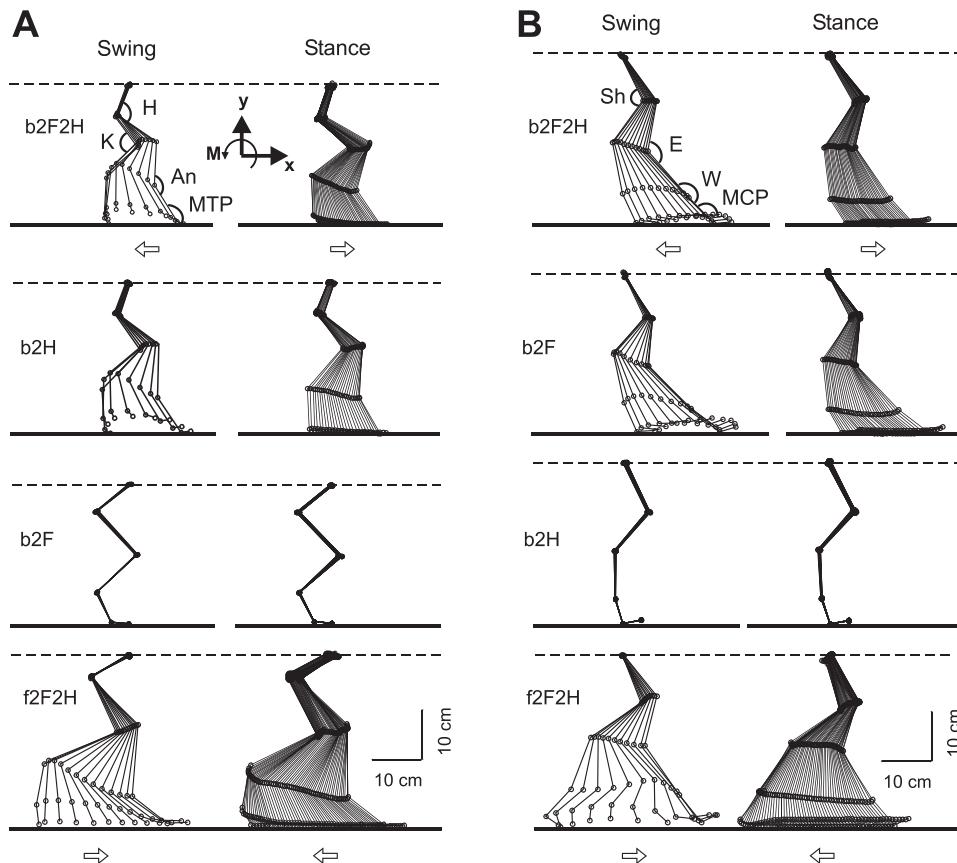


Fig. 4. Limb movements in different tests. *A*: configuration of the right hindlimb in different tests in which the limb was walking (*b2F2H*, *b2H*, and *f2F2H*) and in which the limb was standing (*b2F*). *B*: configuration of the right forelimb in different tests in which the limb was walking (*b2F2H*, *b2F*, and *f2F2H*) and in which the limb was standing (*b2H*). Superposition of stick diagrams was recorded by the Visualeyez System (PhoeniX Technologies, Burnaby, B.C., Canada) sequentially during swing and stance phase, respectively, with a time interval of 30 ms. *H*, hip; *K*, knee; *An*, ankle; *MTP*, metatarsophalangeal; *Sh*, shoulder; *E*, elbow; *W*, wrist; *MCP*, metacarpophalangeal joints.

our experiments, the walking animal was continuously licking food from a feeder and for this purpose, maintained the same head position during both FW and BW. The back was not elevated, and the hindquarters were usually lower than the forequarters (Fig. 4).

To conclude, the kinematical data demonstrated that during bipedal locomotion, the phasic signals coming from the standing limbs were either absent or very different from those during quadrupedal locomotion. By contrast, the signals from the controllers of the walking limb were similar during quadrupedal and bipedal locomotion, and therefore, they most probably produced similar modulation in the neurons. These data also suggest that somatosensory signals coming to the neurons during quadrupedal locomotion were strongly dependent on the direction of walking.

EMG patterns. In this study, we analyzed the EMG activity during quadrupedal and bipedal BW and compared this activity with the EMG activity during FW (the latter was analyzed earlier; see Zelenin et al. 2011). Figure 6 shows averaged EMGs from the muscles of the right fore- and hindlimbs recorded in one cat. During quadrupedal BW (*Test b2F2H*), all EMGs were profoundly modulated. The flexors [Bra-right fore- and hindlimbs (R) and Tib-R] were active during transition from the stance phase of the step to the swing phase (the end of period 4 and the beginning of period 1), whereas the extensors (Tric-R, Gast-R, Glut-R, and Vast-R) were active, mainly during the stance phase (periods 2–4). The patterns of hindlimb EMGs observed during quadrupedal BW in the present study were similar to those described previously (Buford and Smith 1990).

The EMG patterns in the tests with bipedal BW depended on the limb function. If a limb was involved in locomotion and performed stepping movements, the activity pattern of its muscles was similar to that of quadrupedal BW. Compare, for example, activity of Tib-R in *Test b2H* with that in *Test b2F2H* or activity of Tric-R in *Test b2F* with that in *Test b2F2H*. If a limb were standing, the EMGs in this limb were often not modulated (e.g., Fig. 6, Bra-R in *Test b2H*; Tib-R in *Test b2F*), or modulation was weaker and/or had substantially different phasing compared with *Test b2F2H* (e.g., Fig. 6, Gast-R, Glut-R, and Vast-R in *Test b2F*). The variability in EMGs was small in all tests.

To characterize the similarity of EMG profiles in a given bipedal test and in the quadrupedal test, we calculated the CC between the two curves (see MATERIALS AND METHODS, Data Collection and Processing section). The CC values are indicated in each panel of Fig. 6. For all cases, when a limb was walking, the modulation pattern of its muscles was similar to that in the quadrupedal test. The CC was as high as 0.98 and not lower than 0.77. The same was true for the other cases in all cats when we compared EMG patterns during bipedal and quadrupedal walking: the CC for a muscle involved in walking was in the range from 0.70 to 1 (most often, from 0.9 to 1). In contrast, the CC calculated for the bipedal tests in which the limb was standing, varied from −0.13 to 0.33 (Fig. 6, light gray histograms). The poor similarity was always observed for the EMGs in a standing limb: the CC was in the range from −0.8 to 0.5 (most often, from −0.2 to 0.2). One can thus conclude that during BW, the EMG patterns in quadrupedal and bipedal tests are similar if the limb is walking in both tests

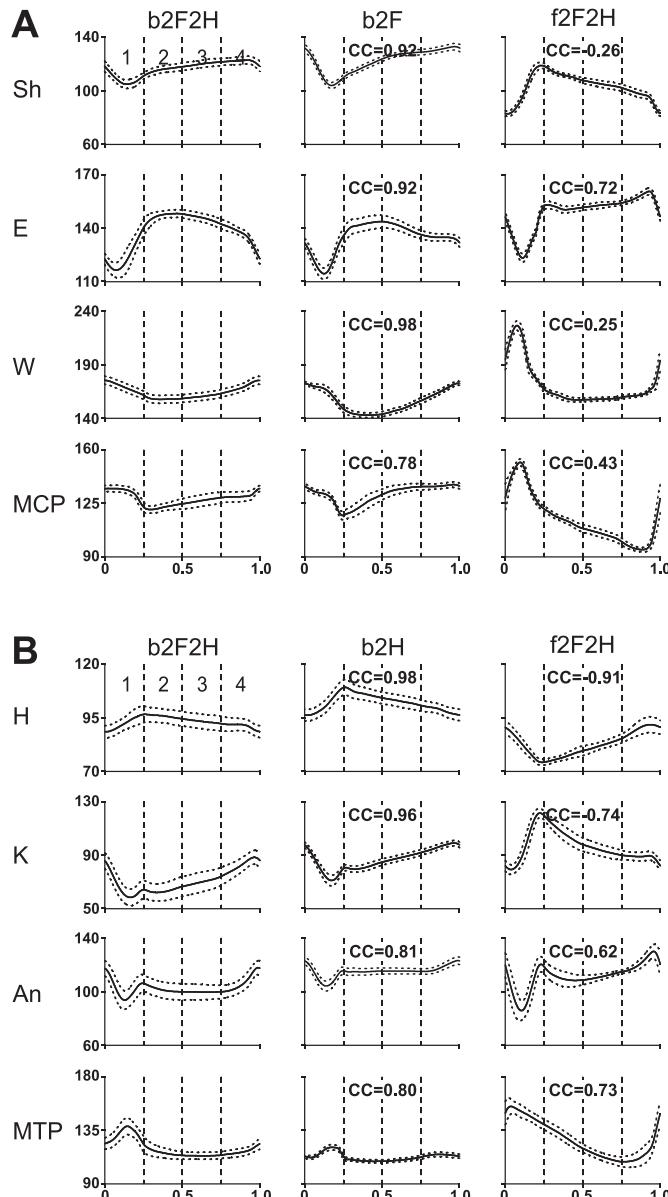


Fig. 5. Joint movements in different tests. Averaged joint angles in different tests for 4 joints of the forelimb (**A**) and the hindlimb (**B**) in one cat. Phase histograms were obtained for each of the periods (1–4) of the normalized locomotor cycle separately. Averaging was performed for 15–40 step cycles in each test; SD is shown by interrupted line. Ordinate: degrees. The coefficients of correlation (CC) of the joint angle pattern in a given bipedal test to the pattern in *Test b2F2H* are indicated.

and dissimilar if the limb is standing in the bipedal test. A similar conclusion was made for FW (Zelenin et al. 2011).

We compared the EMG profiles during quadrupedal BW (*Test b2F2H*) and during FW (*Test f2F2H*). For most muscles, they were rather similar (although not identical), with CC values in the range from 0.6 to 1 (Fig. 6, white histograms). This confirmed the earlier conclusion of Buford and Smith (1990) for the hindlimb muscles and extended it to the forelimb muscles. However, the EMG activity of the forelimb flexor (Bra) was different during FW and BW. This muscle belongs to a small group of muscles with the EMG profiles, depending on the direction of locomotion (Pratt et al. 1996). One can expect that other muscles of the forelimbs, which were not

recorded in this study, have patterns of activity dependent on the direction of walking.

General Characteristics of Neuronal Activity in Different Locomotor Tasks

Altogether, 93 neurons were recorded from the left motor cortex in two cats, including 43 forelimb-related neurons and 50 hindlimb-related neurons. All of these neurons were modulated during quadrupedal locomotion, either backward or forward or in both directions. Forelimb-related neurons most often responded to movements in the shoulder joint, fewer were activated by the wrist or toes movements, and several responded to movements in the elbow. In the hindlimb-related group, neurons were most often activated by movements in the hip or ankle joint, and many others responded to movements of toes or tapping on the sole. Receptive fields of several neurons included knee or encompassed the entire hindlimb. The majority of both forelimb-related and hindlimb-related neurons (73% and 68%, respectively) was identified as PTNs. The following characteristics of activity of the neurons were calculated in each of the locomotor tests.

Mean frequency. Figure 7, *A–D*, shows the f_M in bipedal BW tests (*b2F* and *b2H*) plotted vs. the f_M in the quadrupedal BW test (*b2F2H*), separately for the fore- and hindlimb-related neurons. In these plots, the data points are concentrated along the diagonal, indicating that f_M was mostly similar in the quadrupedal and bipedal locomotion. Correspondingly, no significant difference was found for the population f_M (Fig. 7, *G* and *H*).

Comparison of the f_M of the neurons during quadrupedal FW and quadrupedal BW revealed a slightly higher degree of variability (Fig. 7, *E* and *F*). Thus individual neurons can be preferably activated depending on the direction of locomotion. However, the population mean of f_M was not dependent on the direction of locomotion (compare the black columns with the white ones in Fig. 7, *G* and *H*). This finding shows that BW is not associated with a general increase or decrease of cortical activity compared with FW.

Coefficient of modulation. The majority of neurons that were modulated during the quadrupedal BW test (*b2F2H*) was also modulated during both bipedal BW tests (*b2F* and *b2H*). However, the percentage of modulated neurons and the depth of their modulation differed in different tests. Figure 8, *A–D*, shows the K_{mod} in the bipedal BW tests plotted vs. K_{mod} in the quadrupedal BW test, separately for the forelimb and hindlimb neurons (K_{mod} of nonmodulated neurons is set equal to 0). The data points are scattered over the plot, indicating that the depth of modulation could change differently in different neurons. There was, however, a clear tendency for K_{mod} to be smaller in those bipedal tests, in which the corresponding contralateral limb was standing (Fig. 8, *B* and *C*) compared with *Test b2F2H*. The value of K_{mod} was, on average, smaller during these tests (Fig. 8, *G* and *H*), compared with *Test b2F2H*. By contrast, K_{mod} was close to that in the quadrupedal test in those bipedal tests in which the limb was walking (Fig. 8, *G* and *H*).

These findings suggest that like during FW (Zelenin et al. 2011), during BW, the influences from the corresponding contralateral limb represent one, but not the only source for modulation of neuronal activity in the motor cortex.

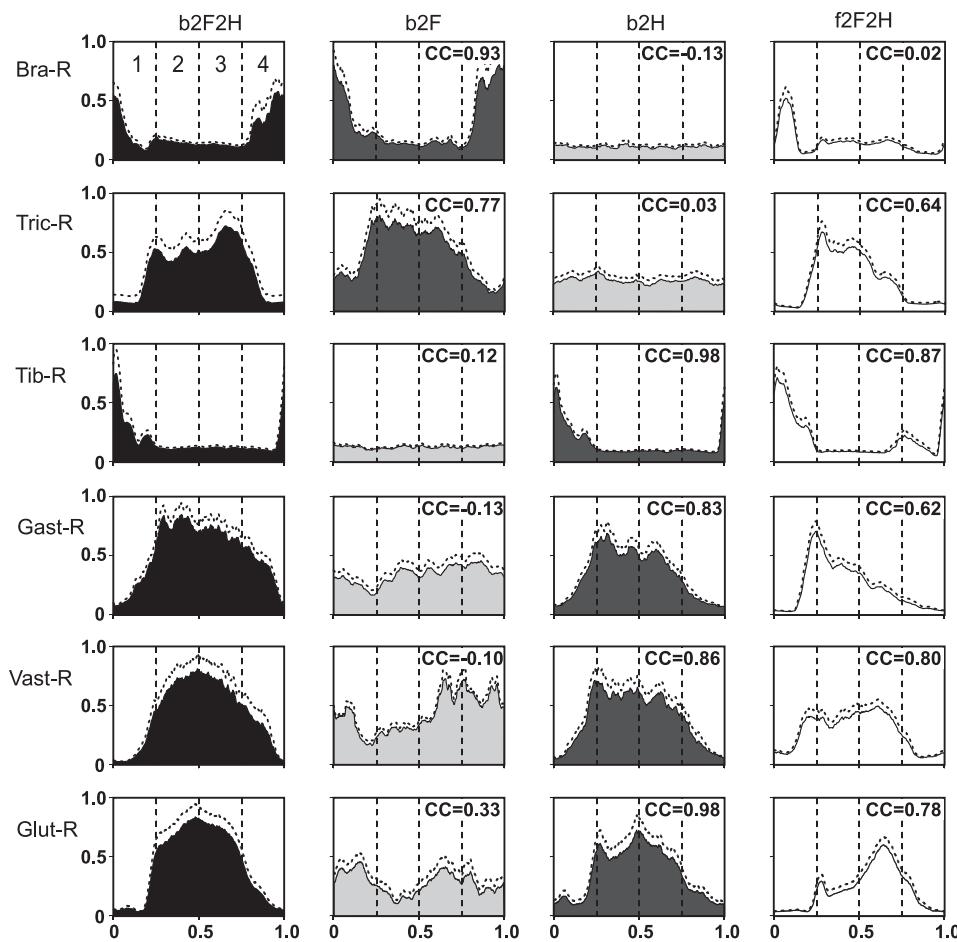


Fig. 6. EMG patterns in different tests. There are shown averaged EMGs in different tests for 6 muscles of the right fore- and hindlimbs: Bra-R, brachialis (elbow flexor); Tric-R, triceps (elbow extensor); Tib-R, tibialis (ankle flexor); Gast-R, gastrocnemius (ankle extensor); Vast-R, vastus lateralis (knee extensor); and Glut-R, gluteus medius (hip extensor and abductor). Phase histograms were obtained for each of the periods (1–4) of the normalized locomotor cycle in one cat. Averaging was performed for 15–30 locomotor cycles in each test. SD is indicated by interrupted line. Ordinate: arbitrary units (the same for all tests). The histograms for the quadrupedal BW (Test b2F2H) are black; for the bipedal BW tests, in which a limb containing the muscle was walking, dark gray; for the bipedal BW tests, in which the limb was standing, light gray; and for the quadrupedal FW (Test f2F2H), white. The CC of the EMG pattern in a given bipedal test to the pattern in Test b2F2H is indicated.

We compared the value of K_{mod} during BW with that during FW (Fig. 8, E and F). The modulation was slightly weaker during FW. Several neurons well-modulated during BW were not modulated at all during FW. The weaker modulation during FW can be seen also in the population averages (Fig. 8, G and H). The effect was statistically significant for the hindlimb neurons. These facts may suggest that BW is more demanding in terms of cortical control than FW (see DISCUSSION).

Preferred phase. During quadrupedal BW, the preferred phases of different neurons were almost evenly distributed over the step cycle, as shown in Fig. 9 for the forelimb (A) and hindlimb (B) populations of neurons. During bipedal BW, if modulation did not disappear, the preferred phases could change. Figure 9, C–F, shows the histograms of the difference in the preferred phase between the bipedal and the quadrupedal (b2F2H) backward locomotion tests. For most neurons, there was no shift of the preferred phase in those tests in which the contralateral limb corresponding to the neuron was walking (Fig. 9, C and F). By contrast, the shifts were much larger and exhibited by many more neurons in those tests in which that limb was standing (Fig. 9, D and E). The distribution of shifts in these tests was flat (χ^2 -test, $P > 0.05$). Thus when the corresponding contralateral limb is not walking, and the normal phasic influences from this limb are absent, the modulation pattern in the majority of the neurons is usually different from the normal one.

Preferred phase during FW (Test f2F2H) was usually also different from that during BW (Fig. 9, G and H). For only 21%

of forelimb and 36% of hindlimb neurons, there was no shift of the preferred phase. The phase-shifts, however, were not evenly distributed (χ^2 -test, $P < 0.05$).

Comparison of Modulation Patterns of Neurons in Different Locomotor Tasks

To reveal the contribution of different limb controllers to modulation of the neurons, correlation analysis of the patterns of modulation in different locomotor tests was performed. An example of such analysis is presented in Fig. 10. A forelimb-related neuron was strongly modulated during quadrupedal BW, the burst of activity mainly occupying the first half of the locomotor cycle (Fig. 10A). A similar pattern of activity was observed during bipedal BW, in which only the fore girdle was involved (Test b2F, Fig. 10B). The CC was as high as 0.92. In contrast, during bipedal BW, in which the fore girdle was standing, the neuron's activity was practically not modulated (Test b2H, Fig. 10D). The CC was correspondingly low—0.45. A similar situation could be seen during quadrupedal and bipedal forward locomotion. The activity pattern during Test f2F2H (Fig. 10E) was similar to the pattern during Test f2F (Fig. 10F), whereas activity during Test f2H was almost not modulated (Fig. 10H) and dissimilar from the quadrupedal locomotion (CC = 0.44). Based on many similar examples, as well as results of our previous study (Zelenin et al. 2011), we chose the CC values larger than +0.6 as indicative of similarity of the two modulation patterns.

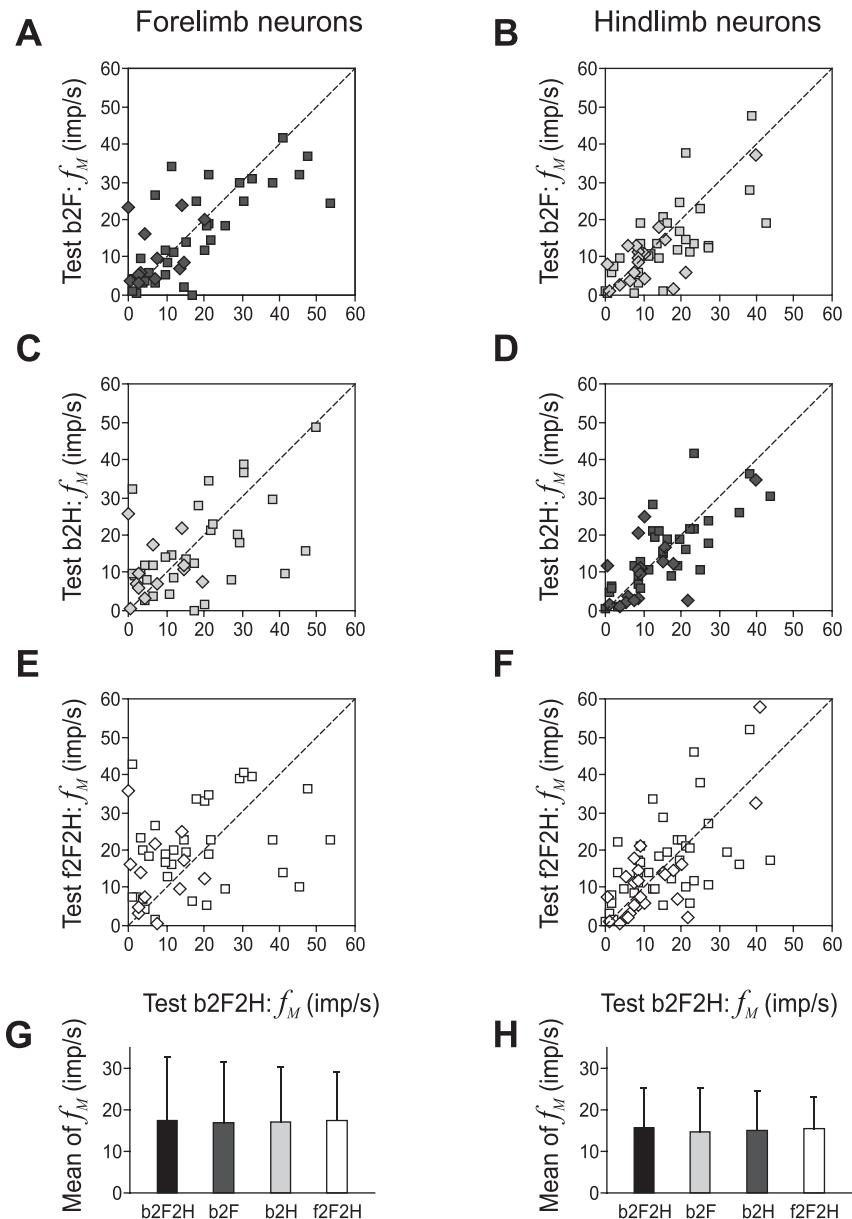


Fig. 7. Mean discharge frequency of neurons in different tests. *A–D*: in the scatter diagrams, the mean frequency of individual neurons (f_M) during bipedal BW (*Tests b2F* and *b2H*) is plotted against f_M during quadrupedal BW (*Test b2F2H*) for the forelimb neurons (*A* and *C*) and hindlimb neurons (*B* and *D*). *E* and *F*: the f_M during quadrupedal FW (*Test f2F2H*) is plotted against f_M during quadrupedal BW (*Test b2F2H*) for the forelimb and hindlimb neurons, respectively. Squares indicate the data points for PTNs; diamonds, for nonidentified neurons. *G* and *H*: the population average (\pm SD) of the f_M (Mean of f_M) in different tests for the forelimb and hindlimb population, respectively. The averages were calculated together for PTNs and nonidentified neurons. The columns for *Test b2F2H* are black; the symbols and columns for the tests in which the own limb was walking are dark gray; the symbols and columns for the tests in which the own limb was standing are light gray; and the columns for *Test f2F2H* are white.

Classification of neurons. The results of the correlation analysis are summarized in Fig. 11 for the forelimb neurons (*A* and *C*) and for the hindlimb neurons (*B* and *D*). In these scatter diagrams, one point represents one neuron. In Fig. 11*A*, we compared BW with different girdles for forelimb neurons. In this diagram, the abscissa of each point is CC in the comparison of the activity during BW with the fore girdle with that in quadrupedal BW (*b2F,b2F2H*), and the ordinate is CC in the comparison of the activity when the fore girdle was standing with that in quadrupedal BW (*b2H,b2F2H*). The CC varied within a wide range. The interrupted lines at CC = 0.6 divide the plot area into four parts (0, 1, 2, and 3) and all neurons into four corresponding groups, b0–b3 (indicated by different colors in Fig. 11*A*; *b*, backward).

In group b1, the pattern of modulation in *Test b2F2H* was similar to that in *Test b2F* (walking of the fore girdle) but dissimilar from that in *Test b2H* (walking of the hind girdle). In group b2, the pattern in *Test b2F2H* was similar to those in both *Test b2F* and *Test b2H*; i.e., it was similar during BW of

any girdle. In group b0, the pattern in *Test b2F2H* was not similar to the pattern in either *Test b2F* or *Test b2H*. Finally, no neurons were found in group b3, with the pattern in *Test b2F2H* dissimilar from that in *Test b2F* (BW of the fore girdle) but similar to that in *Test b2H* (BW of the other girdle).

In Fig. 11*C*, we compared quadrupedal FW with bipedal FW for the same forelimb neurons that were shown in Fig. 11*A*. Here, the abscissa of each point is CC in the comparison (*f2F,f2F2H*), and the ordinate is CC in the comparison (*f2H,f2F2H*). To keep track of neurons from the groups shown in Fig. 11*A*, they are indicated by the same colors. As in Fig. 11*A*, the lines CC = 0.6 divide the plot area into four parts (0, 1, 2, and 3) and the neurons of each group b0, b1, b2 (as defined in Fig. 11*A*) into the corresponding subgroups (b0–f0, b0–f1, b0–f2, b1–f0, b1–f1, b1–f2, b2–f0, b2–f1, b2–f2; *f*, forward).

Table 1 summarizes the results of these comparisons for forelimb neurons. It shows the number of neurons in each subgroup. For each subgroup, the tests with CC > 0.6 are indicated, as well as the corresponding primary sources of

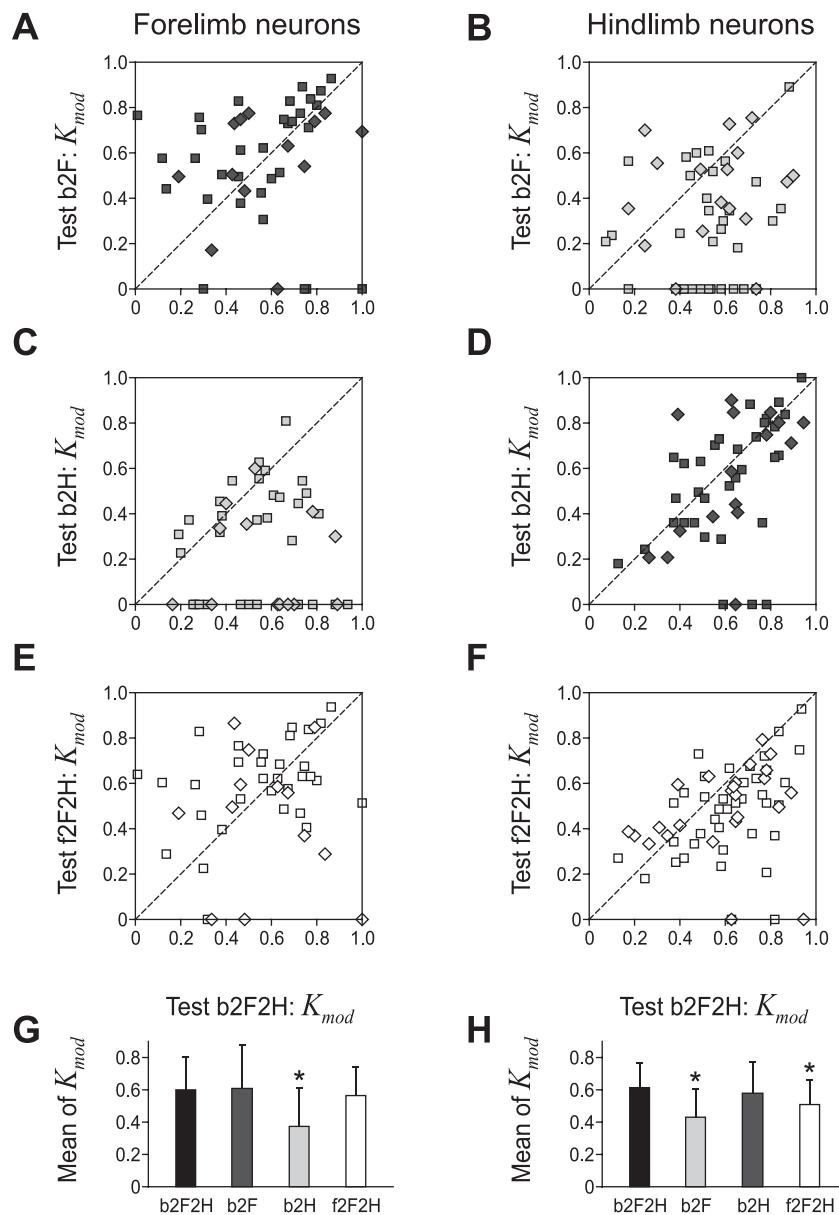


Fig. 8. Coefficient of frequency modulation (K_{mod}) of neurons in different tests. A–D: in the scatter diagrams, the K_{mod} of individual neurons during bipedal BW (Tests b2F and b2H) is plotted against K_{mod} during quadrupedal BW (Test b2F2H) for the forelimb neurons (A and C) and hindlimb neurons (B and D). E and F: the K_{mod} during quadrupedal FW (Test f2F2H) is plotted against K_{mod} during quadrupedal BW (Test b2F2H) for the forelimb and hindlimb neurons, respectively. G and H: the population average ($\pm SD$) of the K_{mod} (Mean of K_{mod}) in different tests for the forelimb population of neurons (G) and for the hindlimb population (H). *Statistically significant change of the population average relative to Test b2F2H. Other designations are as in Fig. 7.

the step-related modulation in the quadrupedal test (b2F2H or f2F2H).

Subgroup b1–f1 had CC > 0.6 in the comparisons (b2F, b2F2H) and (f2F, f2F2H). Since the only limbs walking in both tests were the forelimbs, the source of modulation in these neurons was the forelimb girdle.

Subgroup b1–f2 had CC > 0.6 in the comparisons (b2F, b2F2H), (f2F, f2F2H), and (f2H, f2F2H) but not in (b2H, b2F2H). This suggests that during FW, input from either of the girdles was sufficient for driving these neurons, whereas during backward locomotion, the main source of modulation was the own forelimb girdle.

Subgroup b1–f0 had CC > 0.6 only in the comparison (b2F, b2F2H), suggesting that during FW, modulation of these neurons requires inputs from controllers of both girdles, whereas during BW, the main source of modulation is the own forelimb girdle.

Subgroup b2–f1 had CC > 0.6 in the comparisons (b2F, b2F2H), (b2H, b2F2H), and (f2F, f2F2H) but not in

(f2H, f2F2H), suggesting that during BW, input from either of the girdles is sufficient for driving these neurons, whereas during forward locomotion, the main source of modulation is the own forelimb girdle.

Subgroup b2–f2 had CC > 0.6 in all comparisons (b2F, b2F2H), (b2H, b2F2H), (f2F, f2F2H), and (f2H, f2F2H). This suggests that during locomotion in any direction, input from either of the girdles is sufficient for driving these neurons.

Subgroup b2–f0 had CC > 0.6 in the comparisons (b2F, b2F2H) and (b2H, b2F2H), suggesting that during BW, input from controllers of any girdle is sufficient for driving these neurons, whereas during FW, modulation of these neurons requires inputs from controllers of both girdles.

Subgroup b0–f1 had CC > 0.6 only in the comparison (f2F, f2F2H), suggesting that inputs from the controllers of both girdles are necessary for normal modulation of these neurons during BW, whereas during FW, the modulation is mainly due to input from the own forelimb girdle.

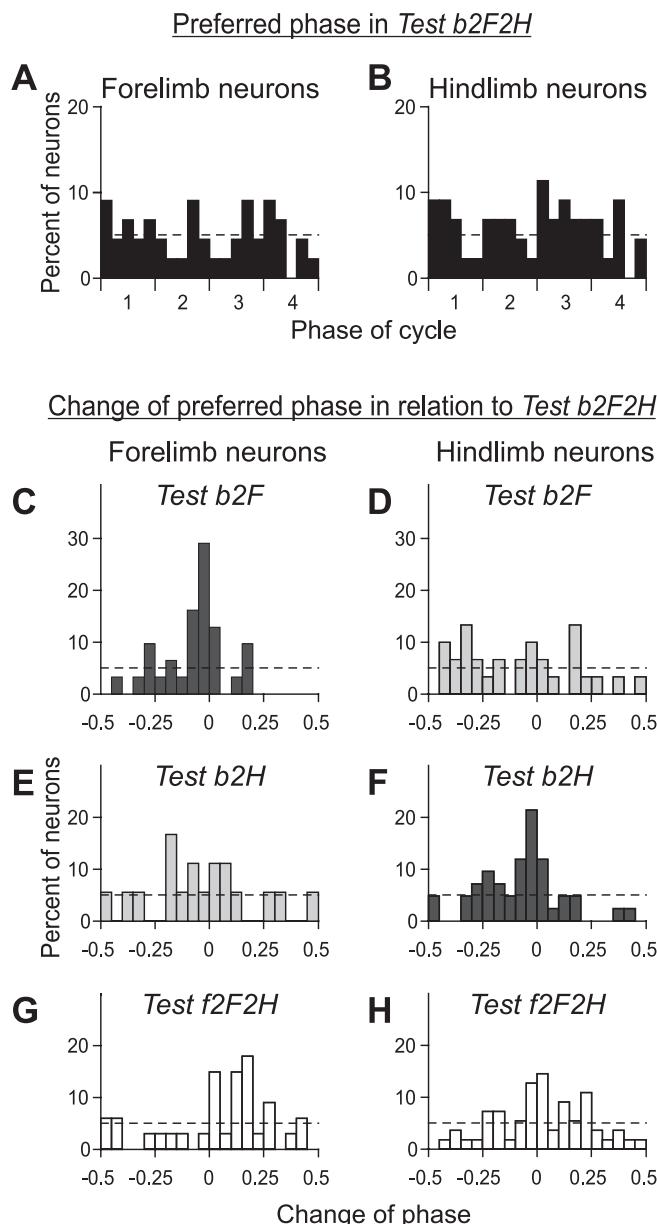


Fig. 9. Changes of preferred phase of neurons in different tests relative to quadrupedal backward locomotion. The histograms in *A* and *B* show the distribution of preferred phases of forelimb neurons (*A*) and hindlimb neurons (*B*) in *Test b2F2H*. The four periods (1–4) of the normalized locomotor cycle are indicated (see Fig. 3). The hatched line shows the level of random distribution of preferred phases. The histograms in *C*, *E*, and *G* and *D*, *F*, and *H* show the algebraic difference between the preferred phases of individual neurons in a given test and in *Test b2F2H* for the forelimb and hindlimb neurons, respectively. Histogram colors are the same as in Fig. 7. The hatched lines in *C*–*H* indicate the level of random distribution of the phase differences. The χ^2 -test showed that for the bipedal tests, in which the own limb was standing (*D* and *E*), the distribution was random ($P > 0.05$).

Subgroup b0–f2 had CC > 0.6 in the comparisons (*f2F,f2F2H*) and (*f2H,f2F2H*). It seems most likely that input from the controllers of each girdle is sufficient for driving these neurons during FW, whereas inputs from both girdles are necessary during BW.

Subgroup b0–f0 had CC > 0.6 in none of the comparisons, suggesting that inputs from all girdles are necessary

for normal modulation of these neurons during quadrupedal locomotion in any direction.

A similar analysis was performed for the hindlimb neurons. Its results are presented in Fig. 11, *B* and *D*, and summarized in Table 2. As one can see from Tables 1 and 2, nine subgroups of forelimb neurons and eight subgroups of hindlimb neurons have been found in the present study. The subgroups differed in the combination of inputs from different girdles and in the relative contribution of these inputs to the periodic modulation of neurons.

Functional groups. Modulation patterns of forelimb neurons from subgroup b0–f1 were apparently determined by input from both girdles during BW and by input only from the fore girdle during FW. The same combination of inputs determined modulation patterns of neurons from subgroup b2–f1. That is why we united these subgroups into one functional group, F3. On the same ground, we united subgroups b1–f0 and b1–f2 of the hindlimb neurons into one functional group, H3—modulating inputs come from both girdles when the own (hind) girdle leads, and the inputs come from both girdles when the own girdle trails.

In a similar fashion, we united the subgroups of neurons receiving inputs from identical combinations of limb controllers (irrespective of their relative contribution) into the functional groups (see Fig. 13). In total, there were four functional groups of forelimb neurons (F1–F4; see Fig. 13*A*) and four groups of hindlimb neurons (H1–H4; see Fig. 13*B*). Black bars at the side of the columns show the proportion of antidromically activated neurons in each neuronal group, correspondingly. PTNs constituted 73% of recorded forelimb neurons and 68% of recorded hindlimb neurons and were proportionally presented in each of the functional groups.

Neurons with persistent patterns. To reveal neurons that have the same sources of modulation independent of the direction of locomotion, we compared the modulation patterns during quadrupedal walking in different directions (*Test b2F2H* and *Test f2F2H*). We found nine forelimb neurons and 17 hindlimb neurons for which the CC in the pair (*b2F2H,f2F2H*) was higher than 0.6. An example of such a neuron is presented in Fig. 12, *A*–*F*. The neuron was active preferably during swing phase, independent of the direction of locomotion (Fig. 12, *A* and *D*), and the CC was as high as 0.95. A higher activity during swing was observed in six out of nine forelimb neurons and in seven out of 17 hindlimb neurons. This was different from the uniform distribution of preferred phases in the whole population (Fig. 9, *A* and *B*). We determined the source of the step-related modulation of the neurons with the modulation pattern independent of the direction of locomotion. It turned out that the majority of them (19/26) belonged to subgroup b1–f1 (groups F1 and H1 in Fig. 13, *C* and *D*), that is, their modulation was determined mainly by input from the corresponding girdle, independent of the direction of locomotion. The neuron whose activity is shown in Fig. 12, *A*–*F*, belonged to this subgroup.

DISCUSSION

The motor cortex does not contribute substantially to the generation of simple locomotion in quadrupeds, since its fast inactivation does not prevent this behavior to occur (Belozerova and Sirota 1993a,b). Nevertheless, the activity

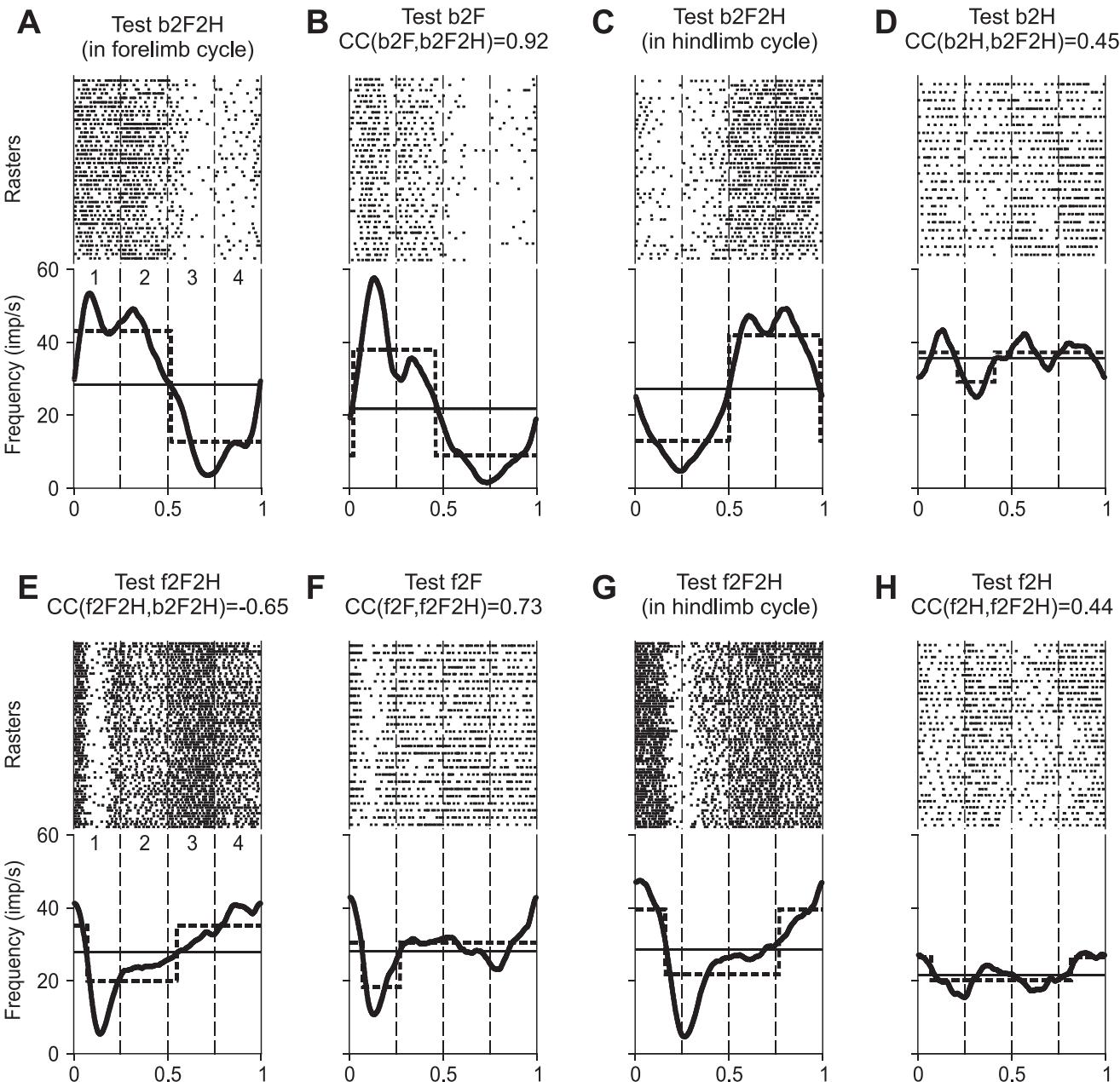
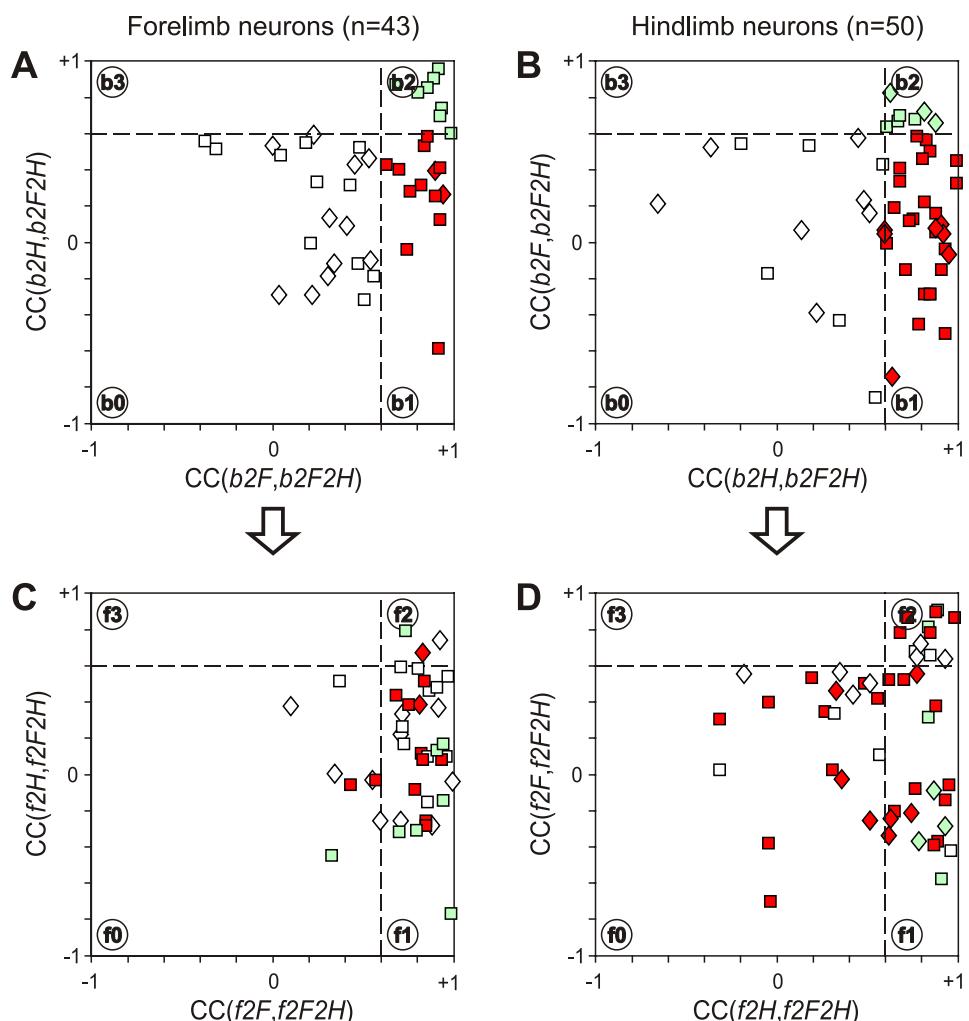


Fig. 10. Comparison of modulation patterns of individual neurons in different tests. For each test, the rasters and the histograms (thick line) of activity of a forelimb-projecting neuron are shown. Cycle periods (1–4) were taken from forelimb movements (*A*, *B*, *E*, and *F*) or hindlimb movements (*C*, *D*, *G*, and *H*). The hatched lines show the best two-level rectangular approximations of the histograms with the burst period (*upper level*) and interburst period (*lower level*). The thin lines give the mean spiking frequency in a test. The test type and the CC between the phase histograms obtained in a given test and a corresponding quadrupedal test are indicated.

of neurons of the motor cortex is modulated in the rhythm of stepping movements, suggesting their involvement in the control of some aspects of this form of motor behavior (see, e.g., Armstrong and Drew 1984a,b; Beloozerova and Sirota 1985, 1993a,b; Drew 1993; Widajewicz et al. 1994). Recently, it was shown that during simple, unobstructed locomotion, this modulation is caused by signals coming from the spinal mechanisms generating stepping movements of individual limbs (limb controllers), and different combinations of limb controllers can affect an individual motor cortex neuron and contribute to its modulation (Zelenin et al. 2011).

These results were obtained for the most common form of locomotion in quadrupeds—FW. In the present study, we analyzed the activity of cortical neurons during a different form of locomotion—BW—which is occasionally used by the animals, e.g., for moving (backing) away from a dangerous object. Under natural conditions, the episodes of BW are usually short, but the cats can be trained to walk backward on the treadmill for longer periods of time (Buford et al. 1990). Our study has shown for the first time that during BW (as during FW), the activity of neurons of the motor cortex is modulated in the rhythm of stepping. We addressed a number of questions regarding the activity of these neurons during BW.

Fig. 11. Similarities and distinctions in modulation patterns of neurons in different tests. In the scatter diagrams, the x and y values of each point show the CC for individual forelimb neurons (*A* and *C*) and hindlimb neurons (*B* and *D*). *A*: abscissa is CC of tests (*b2F,b2F2H*), and ordinate is CC of tests (*b2H,b2F2H*). *B*: abscissa is CC of tests (*b2H,b2F2H*), and ordinate is CC of tests (*b2F,b2F2H*). *C*: abscissa is CC of tests (*f2F,f2F2H*), and ordinate is CC of tests (*f2H,f2F2H*). *D*: abscissa is CC of tests (*f2H,f2F2H*), and ordinate is CC of tests (*f2F,f2F2H*). Similarity between the activity patterns was considered significant for $CC > 0.6$. *A* and *B*: two interrupted lines at $CC = 0.6$ divide each plot area into 4 parts (*b0–b3*, indicated in circles) and all neurons into corresponding groups. White symbols indicate the neurons with $CC > 0.6$ in none of the tests (area *b0*). Green symbols indicate the neurons with $CC > 0.6$ in both tests (area *b2*). Red symbols indicate the neurons with $CC > 0.6$ only in *Test b2F* (*A*) or only in *Test b2H* (*B*; area *b1*). The color for each point in plots *C* and *D* was taken from plots *A* and *B*, respectively. This allows for further divisions of each group of neurons into 4 subgroups (*f0–f3*) based on their coordinates on the *C* and *D* plots. Data for the neurons that were identified as PTNs are shown with squares; data for unidentified neurons are shown with diamonds. All revealed subgroups of neurons contained both PTNs and unidentified neurons. 0 in *b0/f0*, bipedal BW/FW with neither girdle was similar to the quadrupedal test; 1 in *b1/f1*, only bipedal BW/FW with the own girdle was similar to the quadrupedal test; 2 in *b2/f2*, both types of bipedal BW/FW were similar to the control.



First, we compared the mean level of activity (f_M) of individual neurons during two forms of locomotion, FW and BW. We found that the two frequencies were highly variable across the population, and some neurons were more active during FW, whereas others were more active during BW (Fig. 7, *C* and *G*). However, the population mean of f_M was not dependent on the form of locomotion (Fig. 7, *D* and *H*). This finding shows that

BW is not associated with a general increase or decrease of cortical activity compared with FW. This finding suggests that due to repetitive training, the motor task of BW in our studies was automated to the extent similar to FW and thus required similar involvement of the motor cortex.

Second, we compared the values of the K_{mod} in individual neurons during FW and BW. We found that K_{mod} was highly

Table 1. Classification of forelimb neurons ($n = 43$)

Groups and Subgroups	Number of Neurons*	Percent of Neurons*	Tests with $CC > 0.6$ Relative to 2F2H	Presumed Sources of Modulation†
b1-f1	10 (9)	23 (30)	<i>b2F,f2F</i>	<i>F</i> during FW and BW
b1-f2	1 (0)	2 (0)	<i>b2F,f2F,f2H</i>	<i>F</i> during BW, <i>F</i> and <i>H</i> during FW
b1-f0	2 (2)	5 (7)	<i>b2F</i>	<i>F</i> during BW, <i>F</i> and <i>H</i> during FW
b2-f1	6 (6)	14 (20)	<i>b2F,b2H,f2F</i>	<i>F</i> and <i>H</i> during BW, <i>F</i> during FW
b2-f2	1 (1)	2 (3)	<i>b2F,b2H,f2F,f2H</i>	<i>F</i> and <i>H</i> during FW and BW
b2-f0	1 (1)	2 (3)	<i>b2F,b2H</i>	<i>F</i> and <i>H</i> during FW and BW
b0-f1	16 (10)	37 (33)	<i>f2F</i>	<i>F</i> and <i>H</i> during BW, <i>F</i> during FW
b0-f2	1 (0)	2 (0)	<i>f2F,f2H</i>	<i>F</i> and <i>H</i> during FW and BW
b0-f0	5 (1)	12 (3)	—	<i>F</i> and <i>H</i> during FW and BW

*In parentheses, number or percentage of antidromically identified neurons is indicated; †*F*, input from forelimb girdle; *H*, input from hindlimb girdle. CC, coefficient of correlation; FW, forward walking; BW, backward walking; 2F2H, all four limbs walk; *b2F*, the forelimbs walk backward, while the hindlimbs stand on a stationary platform; *b2H*, the hindlimbs walk backward, while the forelimbs stand; *b0*, backward bipedal walking with neither girdle was similar to the quadrupedal test; *b1*, only backward bipedal walking with the own girdle was similar to the quadrupedal test; *b2*, both types of backward bipedal walking were similar to the control; *f0*, forward bipedal walking with neither girdle was similar to the quadrupedal test; *f1*, only forward bipedal walking with the own girdle was similar to the quadrupedal test; *f2*, both types of forward bipedal walking were similar to the control.

Table 2. Classification of hindlimb neurons ($n = 50$)

Groups and Subgroups	Number of Neurons*	Percent of Neurons*	Tests with CC >0.6 Relative to 2F2H	Presumed Sources of Modulation†
b1-f1	13 (9)	26 (27)	<i>b2H,f2H</i>	<i>H</i> during FW and BW
b1-f2	5 (5)	10 (15)	<i>b2H,f2F,f2H</i>	<i>H</i> during BW, <i>F</i> and <i>H</i> during FW
b1-f0	12 (9)	24 (26)	<i>b2H</i>	<i>H</i> during BW, <i>F</i> and <i>H</i> during FW
b2-f1	5 (2)	10 (6)	<i>b2F,b2H,f2H</i>	<i>F</i> and <i>H</i> during BW, <i>H</i> during FW
b2-f2	2 (2)	4 (6)	<i>b2F,b2H,f2F,f2H</i>	<i>F</i> and <i>H</i> during FW and BW
b0-f1	1 (1)	2 (3)	<i>f2H</i>	<i>F</i> and <i>H</i> during BW, <i>H</i> during FW
b0-f2	5 (3)	10 (9)	<i>f2F,f2H</i>	<i>F</i> and <i>H</i> during FW and BW
b0-f0	7 (3)	14 (9)	—	<i>F</i> and <i>H</i> during FW and BW

*In parentheses, number or percentage of antidromically identified neurons is indicated; †*F*, input from forelimb girdle; *H*, input from hindlimb girdle.

variable, and some neurons were deeper modulated during FW, whereas others were deeper modulated during BW (Fig. 8, C and G). On the average, however, the modulation was slightly deeper during BW, and some neurons well modulated during BW were not modulated during FW. The deeper modulation

during BW can also be seen in the population averages, especially in the hindlimb neurons (Fig. 8, D and H). These findings suggest that in the hindlimbs, BW is associated with more intensive cortical participation than FW. This can be explained by the different functional roles of the hindlimbs in

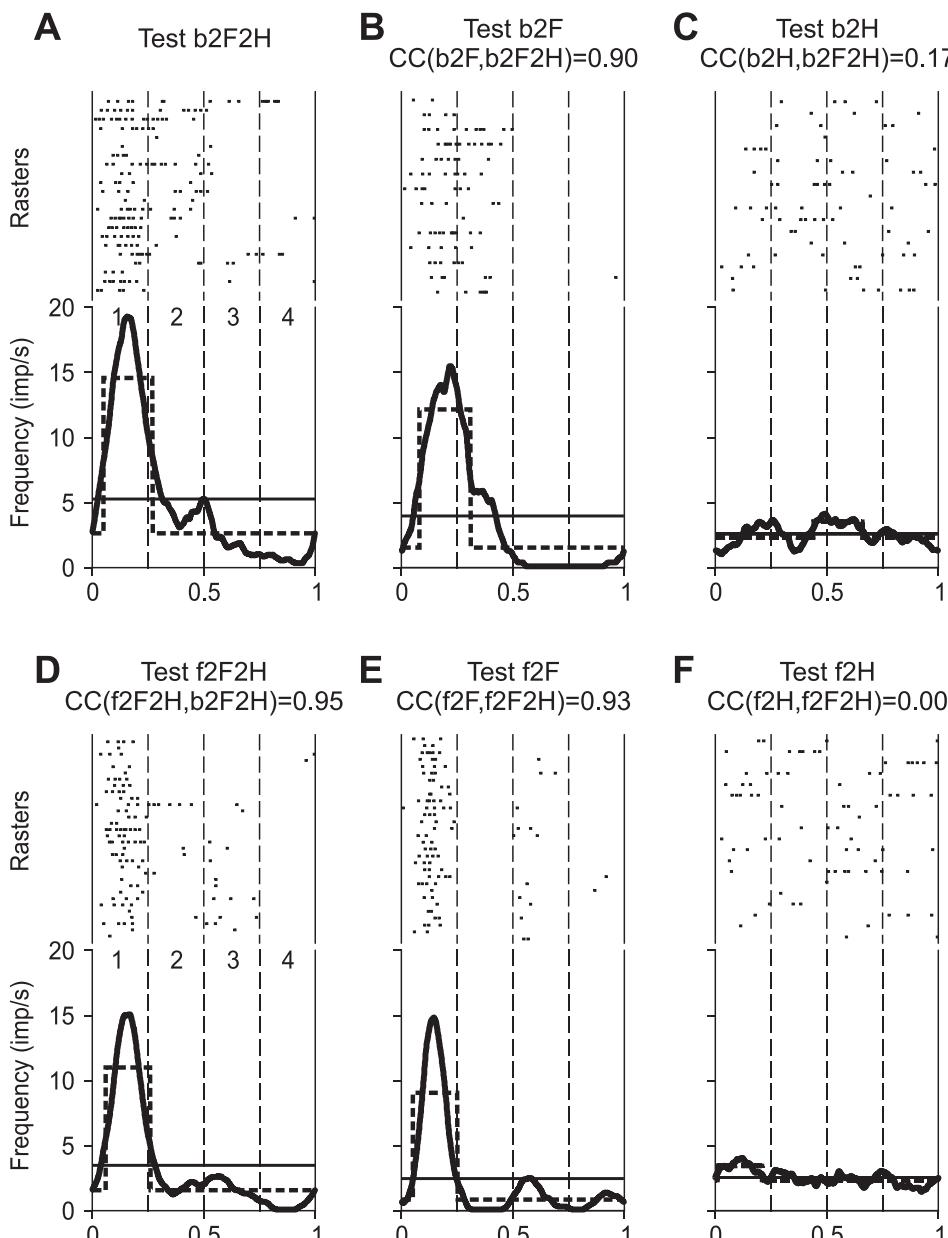


Fig. 12. Neuron with modulation patterns independent of the direction of locomotion. The rasters and the histograms of activity of forelimb-projecting neurons are shown. All designations are the same as in Fig. 10.

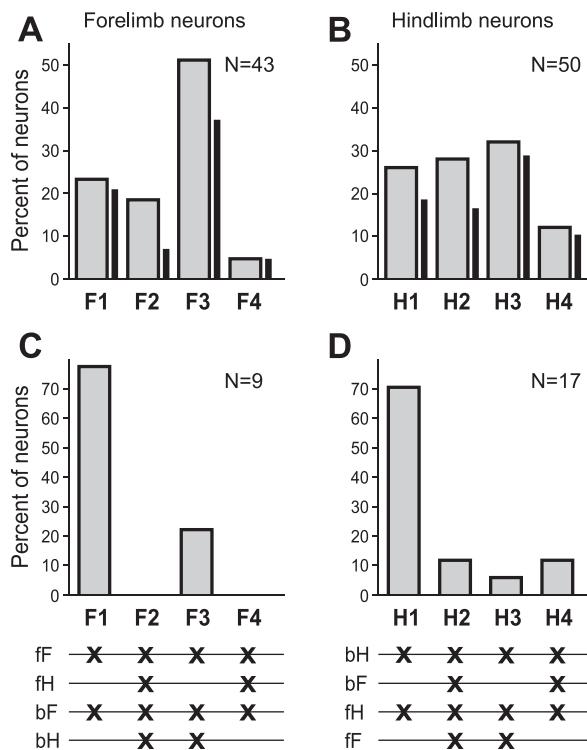


Fig. 13. Sources of modulation of motor cortex neurons. The bottom panels with crosses show the combinations of inputs from limb controllers of different girdles influencing forelimb and hindlimb populations of motor cortex neurons, respectively. Each cross indicates input to the neurons of left motor cortex from the corresponding girdle: fF, inputs from forelimbs during FW; fH, inputs from hindlimbs during FW; bF, inputs from forelimbs during BW; bH, inputs from hindlimbs during BW. *A* and *B*: the height of the bars indicates the percentage of left motor cortex neurons with a given combination of inputs. Four functional groups of forelimb neurons (F1–F4) and four functional groups of hindlimb neurons (H1–H4) were found. For each group of neurons, the proportion of antidromically identified neurons (PTNs) is shown by the thick vertical line near each bar. *C* and *D*: the height of the bars indicates the percentages of neurons having the modulation patterns independent of the direction of locomotion (these neurons were considered in Fig. 12).

the two forms of locomotion; these limbs are leading during BW and trailing during FW.

Third, we analyzed the origin of rhythmical modulation of the neurons. To reveal the limb controllers responsible for this modulation, we used the previously developed method (Zelenin et al. 2011) and recorded activity of the neurons, not only during quadrupedal locomotion but also during bipedal locomotion (when either the forelimbs or the hindlimbs were walking). Then, the modulation patterns of each neuron, observed in different tests, were compared using a correlation analysis. To use this method, we verified that the motor patterns of stepping in bipedal and quadrupedal BW were essentially similar, as was previously shown for bipedal and quadrupedal FW (Zelenin et al. 2011). Such similarity was demonstrated by comparing limb kinematics and EMG patterns of several muscles in *Test b2F2H* with those in *Tests b2F* and in *Test b2H* (Figs. 4–6).

We found that the modulating signals to individual neurons during BW come either from the limb controllers of the own girdle or from the limb controllers of both girdles. Similar results, i.e., convergence of influences from different limb controllers upon individual neurons, were previously obtained for FW (Zelenin et al. 2011). This conclusion relates both to

the neurons responding antidromically (projecting PTNs) and to nonidentified ones (Fig. 13).

A limb controller can send to the motor cortex two types of signals; i.e., the “efference copy” signals about activity of the CPG and the somatosensory signals about the limb position and movement (Orlovsky et al. 1999). The contribution of each of these two types of signals to the modulation of neurons of the motor cortex remains unclear.

A considerable difference in the modulation pattern between FW and BW can be due to a number of factors: 1) the changed kinematics; 2) the changed signals from those muscles, whose activity strongly depends on the direction of locomotion (e.g., *M. brachialis*, Fig. 6; see also Pratt et al. 1996; Trank and Smith 1996); and 3) the changed activity of CPG interneurons, reflected in the changed efference copy signals.

Fourth, we addressed the question of whether the same or different combinations of limb controllers contribute to the modulation of individual neurons in the two forms of locomotion—FW and BW. We found that in almost one-half of the neurons, the combinations of modulating inputs during BW and during FW were different. Most often (in 51% of forelimb neurons and in 34% of the hindlimb neurons), the neurons received inputs only from their own girdle when this girdle was leading, but they received inputs from both girdles when the own girdle was trailing. This change of modulating inputs well corresponds to the change of limb functions accompanying the reversal of the direction of locomotion. Different functional roles of the fore- and hindlimbs during FW in quadrupeds have been demonstrated previously (e.g., Prilutsky et al. 2005; Shik and Orlovsky 1965; von Holst 1938), and Eilam and Shefer (1992) showed a change of the functional roles of the fore- and hindlimbs during BW compared with FW.

Reconfiguration of modulating inputs in many neurons with the reversal of the direction of locomotion suggests different functional roles of these neurons in the two forms of locomotion. A different functional role of motor cortex neurons in different motor tasks was previously suggested based on the comparisons of their activity during locomotion, balancing, and scratching (Beloozerova et al. 2006), as well as during different postural tasks (Beloozerova et al. 2005). This was also a finding in some studies in primates (for review, see Scott 2008).

Fifth, to reveal a possible role of corticospinal commands, one can compare activity of cortical neurons and EMG activity in the two forms of locomotion—FW and BW. As was shown earlier (Buford et al. 1990; Pratt et al. 1996; Trank and Smith 1996) and confirmed in the present study (Fig. 6), the EMG patterns during BW and FW in a large proportion of muscles are rather similar, whereas in other muscles, they differ considerably. One of the possible functions of motor cortex neurons could be controlling the activity of specific muscles or muscle synergies (see, e.g., Drew et al. 2008). Since during FW and during BW, many muscles have similar patterns of activity, one could expect that the patterns of modulation of many cortical neurons (controlling particular muscle synergies) will also be similar during FW and during BW. However, that was the case for only about 25% of the neurons recorded in this study. For the vast majority of neurons, their modulation patterns during FW and during BW were different. These findings can be explained in several ways. 1) It may be that many of the muscles, which were not recorded in this study, have their activity patterns strongly dependent on the direction of locomotion. 2) Alternatively, the corticospinal commands (en-

coded as modulation of cortical neurons) may be addressed to different limb muscles during different behaviors. 3) Also, the commands may be sent to the same limb muscles, but the timing of the commands (phase of locomotor cycle) may be different for different behaviors (different directions of locomotion).

The absence of any simple correlation between the activity of many cortical neurons on one hand and the variables characterizing the motor pattern on the other hand is a common finding in many studies on the motor cortex (see e.g., Beloozerova et al. 2010; Drew 1993; Kakei et al. 2003; Karayannidou et al. 2008, 2009). It seems likely that these neurons do not directly participate in the production of motor output. In the locomotor system, their possible function may be to contribute to the activation and reconfiguration of the spinal locomotor networks controlling different forms of locomotion. Such function seems to be necessary when one considers the enormous variety of modifications of the basic locomotor pattern accessible for animals and humans. Among other possible functions, not related directly to the production of motor output, the neurons could participate in the modulation of afferent signal transmission to different motor centers. The step-related modulation of cortical neurons during regular locomotion may determine the phase of their response during voluntary gait modifications (Beloozerova and Sirota 1988, 1993a,b; Drew 1988, 1993).

To conclude, this study presented the first-ever information on the activity of motor cortex neurons during backward locomotion. Together with our previous study of their activity during forward locomotion (Zelenin et al. 2011), this study is an important step toward understanding the functional role of the motor cortex in the control of locomotion.

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DISCLOSURES

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

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