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Pyramidal tract neurons receptive to different forelimb joints act differently during locomotion

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Stout EE, Beloozerova IN. Pyramidal tract neurons receptive to different forelimb joints act differently during locomotion. *J Neurophysiol* 107: 1890–1903, 2012. First published January 11, 2012; doi:10.1152/jn.00650.2011.—During locomotion, motor cortical neurons projecting to the pyramidal tract (PTNs) discharge in close relation to strides. How their discharges vary based on the part of the body they influence is not well understood. We addressed this question with regard to joints of the forelimb in the cat. During simple and ladder locomotion, we compared the activity of four groups of PTNs with somatosensory receptive fields involving different forelimb joints: *1*) 45 PTNs receptive to movements of shoulder, *2*) 30 PTNs receptive to movements of elbow, *3*) 40 PTNs receptive to movements of wrist, and *4*) 30 nonresponsive PTNs. In the motor cortex, a relationship exists between the location of the source of afferent input and the target for motor output. On the basis of this relationship, we inferred the forelimb joint that a PTN influences from its somatosensory receptive field. We found that different PTNs tended to discharge differently during locomotion. During simple locomotion shoulder-related PTNs were most active during late stance/early swing, and upon transition from simple to ladder locomotion they often increased activity and stride-related modulation while reducing discharge duration. Elbow-related PTNs were most active during late swing/early stance and typically did not change activity, modulation, or discharge duration on the ladder. Wrist-related PTNs were most active during swing and upon transition to the ladder often decreased activity and increased modulation while reducing discharge duration. These data suggest that during locomotion the motor cortex uses distinct mechanisms to control the shoulder, elbow, and wrist.

motor cortex; cat; accuracy; somatosensory

DURING LOCOMOTION, nearly all neurons that project to the pyramidal tract (pyramidal tract neurons, PTNs) discharge in close relation to strides (Armstrong and Drew 1984a, 1984b; Beloozerova and Sirota 1985). This stride-related modulation of activity is substantially enhanced when locomotion requires accurate stepping, e.g., while negotiating barriers or walking along a horizontal ladder (Beloozerova and Sirota 1993a; Beloozerova et al. 2010; Drew 1993; Marple-Horvat and Armstrong 1999; Sirota et al. 2005; Widajewicz et al. 1994). While a lesion to the motor cortex or its short-lasting inactivation does not disturb simple locomotion over a flat surface, it has devastating effect on complex locomotion tasks involving accurate paw positioning (Beloozerova and Sirota 1988, 1993a; Chambers and Liu 1957; Drew et al. 1996; Liddell and Phillips 1944; Trendelenburg 1911). Thus it appears that PTN activity during complex locomotion composes cortical commands for accurate foot placement. PTNs, however, exhibit diverse locomotion-related activity patterns, and the differences in their activity between simple and complex locomotion vary in magnitude and characteristics. The commands that PTNs transmit during locomotion are not uniform. How the different commands are channeled to spinal cord networks remains poorly understood. In a few previous studies, the activity of forelimb- and hindlimb-related PTNs were compared and it was found that while some quantitative differences exist, qualitatively, commands sent by PTNs to forelimbs and hindlimbs are quite similar (Karayannidou et al. 2009; Widajewicz et al. 1994; Zelenin et al. 2011). In this study we hypothesized that there are different spinal targets within each girdle's neuronal network that receive different signals from the motor cortex during locomotion. Specifically, we hypothesized that spinal networks related to different joints of the limb receive different commands from the motor cortex.

Indeed, segments of the limb differ in mechanical characteristics, such as dimensions and weight, and differ in their role during movements. Whereas displacement of a proximal segment greatly affects the kinematics and kinetics of more distal segments, the influence of a distal segment movement on the mechanical characteristics of proximal segments is much smaller. Many observations suggest that, during movements, different joints have different functions and are likely to be controlled in different manners. For example, during a reach and prehension task, motor cortex PTN postspike effects are both more numerous and more prominent on distal compared with proximal muscles (McKiernan et al. 1998). It has long been known that lesions to the pyramidal tract in primates destroy fine movements of the fingers and wrist, while the disturbances to movements in the proximal joints are much less severe (e.g., Lawrence and Kuypers 1968). In contrast, a poor control over the shoulder joint appears to be one of the signature deficits of cerebellar patients (Bastian et al. 2000). During locomotion, the angle of the hip is an important factor in determining initiation of the swing phase of the stride, while the positions of distal joints have no effect (Grillner and Rossignol 1978). In a study of postnatal development of the forelimb representation in the motor cortex in the cat, Chakrabarty and Martin (2000) found that the motor map develops in a proximal-to-distal sequence, with shoulder and elbow controls developing earlier than wrist and digit controls. Developmental differences in the controls for different forelimb joints have been reported in humans as well (e.g., Konczak and Dichgans 1997). Different controls for different forelimb joints have also been suggested on the basis of the results of biomechanical analyses. For example, Galloway and Koshland (2002) studied point-to-point whole arm movements in humans and found that movement dynamics differed greatly between the joints. On the basis of this and other biomechanics

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evidence, a "leading joint hypotheses" has been advanced (Dounskaia 2005), proposing that the joints of a limb play different roles in movement production according to their mechanical subordination in the joint linkage. It is not known, however, whether the motor cortex conveys differential controls to the spinal networks associated with different joints of a limb.

In this study, we addressed this question with regard to the forelimb. We took advantage of the fact that in the spinal cord most PTNs influence the same part of the limb that they receive somatosensory information from (Asanuma et al. 1968; Murphy et al. 1975; Rosen and Asanuma 1972; Sakata and Miyamoto 1968). Moreover, even though axons of individual PTNs from the forelimb representation of the motor cortex branch along several cervical and thoracic segments of the spinal cord (Shinoda et al. 1986), physiological experiments have shown that microstimulation in about half of sites within the forelimb motor cortex at 15 μA produces effects in only one or two muscles (Armstrong and Drew 1985a). Spike-triggered averaging of EMGs in primates showed that about half of PTNs influence motoneuron pools that innervate muscles working around a single joint of the limb (Buys et al. 1986; McKiernan et al. 1998). Thus, using the correspondence between the locations of the source of afferent input and the target of motor output, we inferred which part of the limb a PTN influences based on its somatosensory receptive field. We recorded the activity of individual PTNs from the motor cortex in chronically instrumented cats. We selected only PTNs that receive somatosensory input from only shoulder, only elbow, or only wrist and asked whether these PTNs act differently during locomotion. We tested two locomotion tasks: simple locomotion over a flat surface, a task that does not require participation of the motor cortex, and a complex locomotion task over the crosspieces of a horizontal ladder, a task that requires the activity of the motor cortex to be successful (Beloozerova and Sirota 1988, 1993a; Chambers and Liu 1957; Drew et al. 1996; Liddell and Phillips 1944; Trendelenburg 1911). We found that PTNs receptive to different forelimb joints—and thus likely influencing those different joints—tended to discharge differently during locomotion of both types and often adjusted their activity patterns between the two tasks in unique, stereotyped manners. We suggest that during locomotion the motor cortex, via subpopulations of PTNs with precisely targeted connections, uses distinct mechanisms to control the shoulder, elbow, and wrist.

A brief account of this study was published in abstract form (Stout and Beloozerova 2009).

METHODS

Recordings were obtained from eight adult cats, five males and three females (Table 1). Some data on the activity of the motor cortex in several of these cats have been included in previous publications (Beloozerova et al. 2010; Sirota et al. 2005); however, the selection of neurons for this study is unique. Methods of data collection and spike train analysis have been described previously (Beloozerova and Sirota 1993a; Beloozerova et al. 2010; Prilutsky et al. 2005) and are briefly reported below. All experiments were conducted in accordance with National Institutes of Health guidelines and with the approval of the Barrow Neurological Institute Animal Care and Use Committee.

Locomotion tasks. Two locomotion tasks were used: *1*) simple locomotion on a flat surface and *2*) complex locomotion over the crosspieces of a horizontal ladder (Fig. 1*A*). It has been demonstrated in several studies that simple locomotion does not require participation of the motor cortex, while complex locomotion does (Beloozerova and Sirota 1993a; Chambers and Liu 1957; Liddell and Phillips 1944; Trendelenburg 1911).

Positive reinforcement (food) was used to adapt cats to the experimental situation and to engage them in locomotion (Pryor 1975; Skinner 1938). A box 2.5 m long and 0.5 m wide served as the experimental chamber. A longitudinal wall divided the box into two corridors that cats passed through sequentially and repeatedly. In one of the corridors the floor was flat, while the other corridor contained a horizontal ladder (Fig. 1*A*). The crosspieces of the horizontal ladder were flat and 5 cm wide. The width of the crosspieces was chosen to slightly exceed the cat's mean paw length (3 cm), so that cats had full paw support on the crosspieces. Crosspieces were spaced 25 cm apart, that is, at half of the mean stride length observed in the chamber during locomotion on a flat floor at a self-selected pace (Beloozerova and Sirota 1993a; Beloozerova et al. 2010). After each round, food was dispensed into a feeding dish in one of the corners. Cats were trained, upon arrival, to stand in front of the feeding dish quietly on all four feet during a delay period of 4 s. During data analysis, 1 s in the middle of this period was considered as "standing."

Cats were accustomed to wearing a cotton jacket, a light backpack with connectors, and an electromechanical sensor on the paw for recording of swing and stance phases of stride. The floor in the chamber and the crosspieces of the ladder were covered with an electroconductive rubberized material. During locomotion the duration of the swing and stance phases of the forelimb contralateral to the side of recording in the motor cortex was monitored by measuring the electrical resistance between the electromechanical sensor and the floor (Sw/St trace in Fig. 3*A*) (see, e.g., Beloozerova and Sirota 1993a; Beloozerova et al. 2010).

Surgical procedures. After cats were trained, surgery was performed under isoflurane anesthesia with aseptic procedures. A portion of the skull and dura above the left motor cortex was removed. The area of the motor cortex was identified by the surface features and photographed (Fig. 2*A*). The aperture was then covered by a 1-mm-thick acrylic plate. The plate was preperforated with holes of 0.36-mm

Table 1. *PTNs recorded in different subjects*

Cat No.	Sex	Mass, kg	Shoulder Related	Elbow Related	Wrist Related	Nonresponsive	Total
1	Male	3.9	7	4	1	4	16
3	Female	3.0	2	1	1	2	6
4	Male	3.8	3	5	2	6	16
7	Female	2.7	13	5	10	6	34
8	Male	4.5	8	2	9	4	23
9	Male	3.9	5	8	5	4	22
11	Female	3.7	5	1	8	4	18
12	Male	4.0	2	4	4	0	10
Total (n = 8)			45	30	40	30	145

PTN, pyramidal tract neuron.

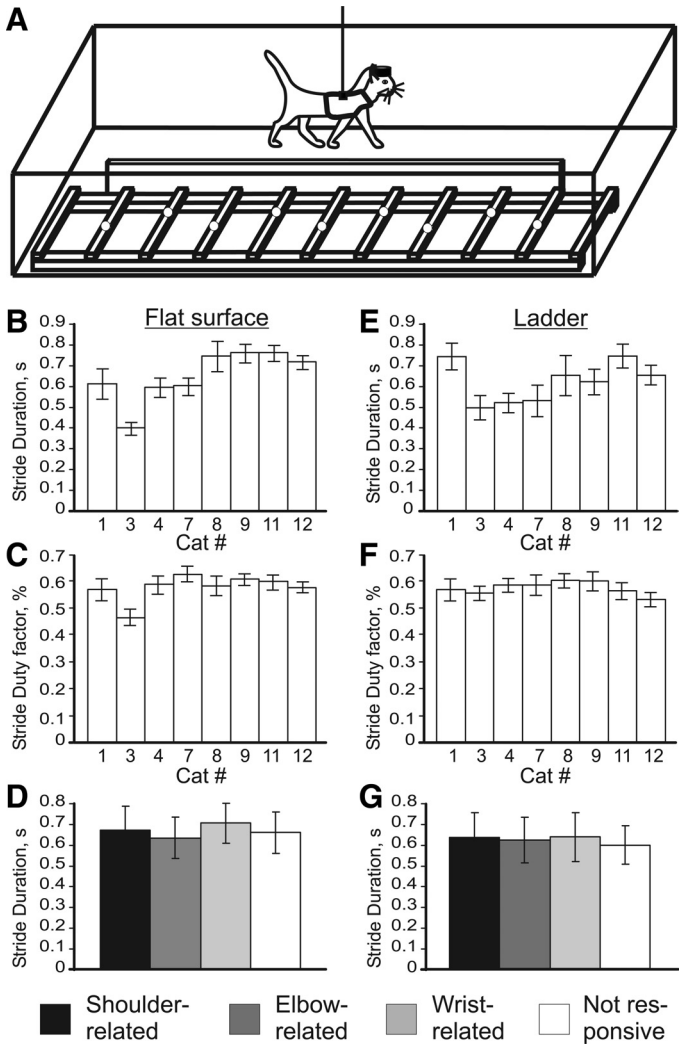

Fig. 1. Locomotion tasks. *A*: the experimental box was divided into 2 corridors. In 1 of the corridors the floor was flat, while the other corridor contained a horizontal ladder. White circles on the crosspieces of the ladders schematically show placements of cat forelimb paws. *B* and *E*: average durations of the step cycles in different cats during simple (*B*) and ladder (*E*) locomotion. *C* and *F*: average step duty factors (ratios of stance duration to cycle duration) in different cats during simple (*C*) and ladder (*F*) locomotion. *D* and *G*: average durations of the step cycles taken for the analysis of activity of different pyramidal tract neuron (PTN) groups during simple (*D*) and ladder (*G*) locomotion. In *B–G* vertical bars are SDs.

diameter spaced at 0.5 mm, and holes were filled with bone wax. Two 26-gauge hypodermic guide tubes were implanted vertically above the medullary pyramids with tips approximately at the Horsley-Clarke coordinates (P7.5, L0.5) and (P7.5, L1.5) and the depth of H0. They were later used for physiologically guided insertion of stimulating electrodes into the pyramidal tract (Prilutsky et al. 2005; Fig. 2*B*). These electrodes were used for identification of PTNs in the awake animal. A ring-shaped base was formed around all implants, and a plastic cap was used to protect them.

Cell recording and identification. Experiments were initiated after several days of recovery. Extracellular recordings were obtained with conventional tungsten varnish-insulated microelectrodes (120-μm OD, Frederick Haer) or platinum-tungsten quartz-insulated microelectrodes (40-μm OD; Reitboeck 1983). The impedance of both types of electrodes was 1–3 MΩ at 1,000 Hz. A custom-made lightweight (2.5 g) manual single-axis micromanipulator chronically mounted to the animal's skull was used to advance the microelectrode. Signals from the microelectrode were preamplified with a miniature custom-made preamplifier positioned on the cat's head and then further amplified with CyberAmp 380 (Axon Instruments). After amplification, signals were filtered (0.3- to 10-kHz band pass), displayed on a screen, fed to an audio monitor, and recorded to the hard disk of a computer by means of a data acquisition hardware and software package (Power-1401/Spike-2 System, Cambridge Electronic Design, Cambridge,

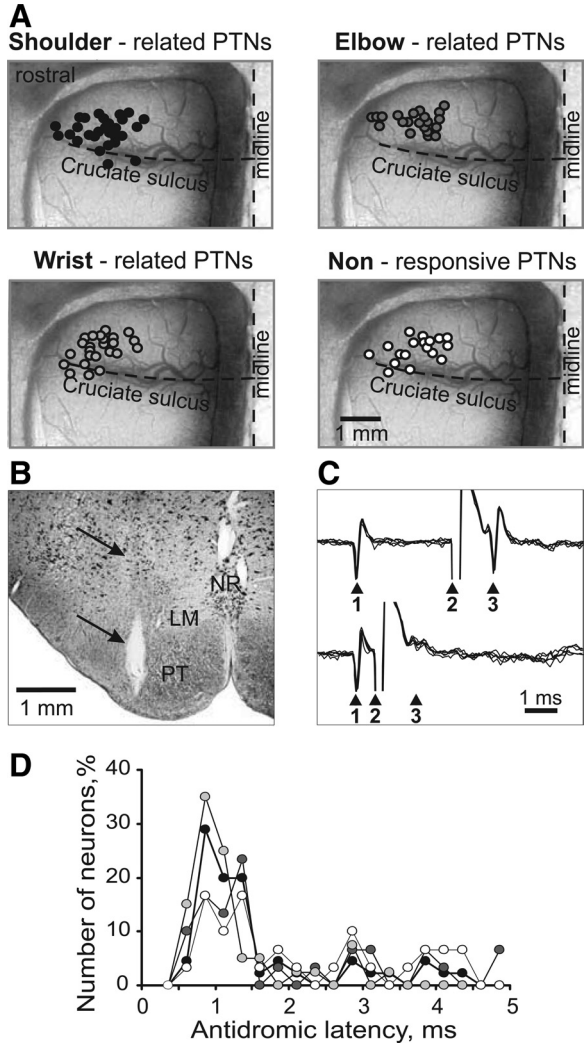

Fig. 2. Location of PTNs and their identification. *A*: area of recording in the forelimb representation of the left motor cortex. Microelectrode entry points into the cortex are combined from all cats and shown by circles on the photograph of *cat 9* cortex. Tracks where PTNs with shoulder-related, elbow-related, and wrist-related receptive fields and nonresponsive PTNs were recorded are shown by black, dark gray, light gray, and white circles, respectively. *B*: reference electrolytic lesion in the left pyramidal tract made with the stimulation electrode in *cat 8*. Gliosis surrounding the electrode track and the reference lesion are indicated by arrows. The electrode was positioned approximately at the Horsley-Clarke rostro-caudal coordinate of P7.5. LM, lemniscus medialis; NR, nucleus raphes; PT, pyramidal tract. Frontal 50-μm-thick section, cresyl violet stain. *C*: collision test determines whether PTN response is antidromic. *Top*: the PTN spontaneously discharges (*arrowhead 1*), and the pyramidal tract is stimulated 3 ms later (*arrowhead 2*). The PTN responds with latency of 1 ms (*arrowhead 3*). *Bottom*: the PTN spontaneously discharges (*arrowhead 1*), and the pyramidal tract is stimulated 0.7 ms later (*arrowhead 2*). The PTN does not respond (*arrowhead 3*) because in 0.7 ms its spontaneous spike was still en route to the site of stimulation in the pyramidal tract, and thus collision/nullification of spontaneous and evoked spikes occurred. *D*: distribution of latencies of antidromic responses to stimulation of the pyramidal tract of PTNs of different groups. Shoulder-related, elbow-related, wrist-related, and nonresponsive PTNs are denoted by black, dark gray, light gray, and white circles, respectively.

UK). An example recording from a PTN during locomotion is shown in Fig. 3*A*.

All encountered neurons were 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. The criterion for identification of antidromic responses was the test for collision of spikes (Bishop et al. 1962; Fuller and Schlag 1976); it is illustrated in Fig. 2*C*. Neurons were checked for antidromic activation before, during, and after testing during locomotion.

Receptive field classification. The somatic receptive fields of the PTNs were examined in animals sitting on a comport pad with their head restrained. Stimulation was produced by palpation of muscle bellies and tendons and by passive movements of joints. For any region found to consistently elicit action potentials, the extent of the receptive field was determined by listening to the audio monitor and determining the entire expanse that the cell was responsive to. PTNs responsive to passive movements of joints were assessed for directional preference. For this study, only neurons with the following somatosensory receptive fields were included in the analysis. *1*) The shoulder-related group included PTNs responsive only to passive movements in the shoulder joint and/or palpation of upper back, chest, or lower neck muscles. *2*) The elbow-related group included PTNs responsive only to passive movements in the elbow joint and/or palpation of upper arm muscles. *3*) The wrist-related group included PTNs responsive only to passive movements in the wrist joint and/or palpation of distal arm muscles and/or to stimulation of the palm or back of the paw. *4*) The nonresponsive group included neighboring PTNs that showed no somatosensory responses. PTNs that had receptive field spanning more than one forelimb segment, for example, those responsive to movements in both wrist and elbow joints, were not included in the analysis. Neurons responsive to movements of toes or claws were not included.

Processing of neuronal activity. From each run down a corridor, two or three strides made in the middle of the walkway were selected for the analysis. The onset of swing phase was taken as the beginning of the step cycle. The duration of each step cycle was divided into 20 equal bins, and a phase histogram of spike activity of the neuron in the cycle was generated and averaged over all selected cycles. The Rayleigh test for directionality was used to determine whether the activity of a neuron was modulated in relation to the step cycle (Batshelet 1981; Fisher 1993). If the activity of a neuron was judged to be step cycle related, the "depth" of modulation, dM, was calculated with the histogram. It was defined as $\mathrm{dM}\ (\%) = (N_{max} - N_{min})/N \times 100$, where N_{max} and N_{min} are the number of spikes in the maximal and the minimal histogram bin and N is the total number of spikes in the histogram. In addition, the portion of the cycle in which the activity level exceeded 25% of the difference between the maximal and minimal frequencies in the histogram was defined as a "period of elevated firing" or PEF (as illustrated in Fig. 3, *C* and *E*). The "preferred phase" of discharge of each neuron with a single PEF was assessed by circular statistics (Batshelet 1981; Fisher 1993; see also Beloozerova et al. 2003a; Sirota et al. 2005).

To determine what natural fluctuations exist in the locomotion-related discharge of individual neurons, we performed a comparison of neuronal activity between randomly selected sets of steps from the same locomotion task. For 75 PTNs, at least 2 sets of 25–40 steps for each task were selected, and >100 comparisons were made. For each neuron, mean discharge frequency, dM, preferred phase, and duration of PEF were calculated for each set of steps and compared. For each parameter, a 95% confidence interval for the difference was determined; it was, respectively, ±20%, ±20%, ±10% of the step cycle, and ±20% of the step cycle. Thus, when different tasks were compared, changes within this interval were considered to be due to natural fluctuations in neuronal locomotion-related activity while changes outside of this interval were considered, with 95% confidence, to be caused by differences in the locomotion tasks.

Parametric tests were used when possible to compare between groups. Unless noted otherwise, for all mean values the standard error of the mean (SE) is given. The discharge frequency and modulation of neurons during different tasks were compared with a paired-samples *t*-test, and comparisons across different groups of neurons were assessed with ANOVA. When data were categorical, a nonparametric χ^2-test was used.

Histological procedures. At the termination of experiments, cats were deeply anesthetized with pentobarbital sodium. Several reference lesions were made in the region of the motor cortex from which neurons were sampled. Cats were then 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. The positions of recording tracks in the motor cortex were estimated in relation to the reference lesions. The position of stimulation electrodes in the medullar pyramids was verified (Fig. 2*B*).

RESULTS

Characteristics of locomotion tasks. Cats walked between 10 and 100 (typically 20–40) times down each of the chamber's corridors during the recording of each individual PTN. From these runs, 25–150 strides (70 ± 30, mean ± SD) in the middle of each corridor (during walking on the flat surface or along the horizontal ladder) were selected for analysis. Four of the cats walked relatively quickly during simple locomotion (*cats 1*, *3*, *4*, and *7*), and four were relatively slow (*cats 8*, *9*, *11*, and *12*). Their average step durations were around 600 ms and 750 ms, respectively (Fig. 1*B*). This corresponded to a

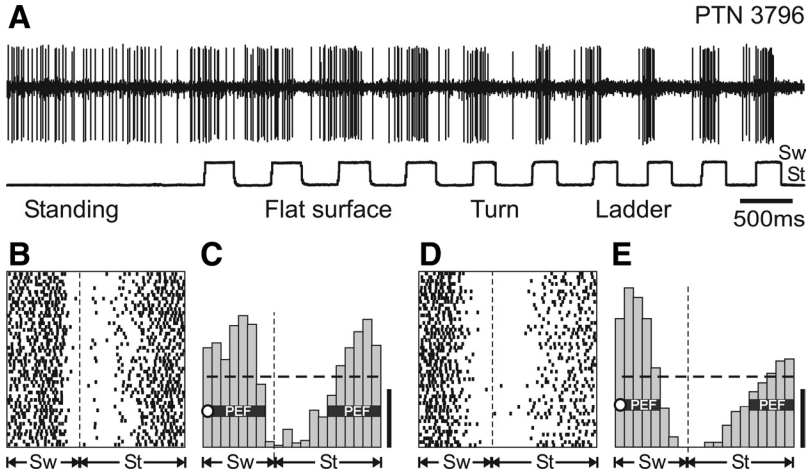

Fig. 3. Example of the typical activity of a PTN neuron. *A*: activity of the PTN neuron during standing and simple and ladder locomotion. *Bottom*: swing (Sw) and stance (St) phases of the step cycle of the right forelimb that is contralateral to the recording site in the cortex. *B* and *C*: activity of the same neuron during simple locomotion presented as a raster of 50 step cycles (*B*) and as a histogram (*C*). In the raster, the duration of step cycles is normalized to 100%. In the histogram, the interrupted line shows the level of activity during standing. The horizontal black bar shows the period of elevated firing (PEF), and the circle indicates the preferred phase (see definition in METHODS). *D* and *E*: activity of the same neuron during ladder locomotion presented as a raster (*D*) and as a histogram (*E*). In *C* and *E*, the vertical scale bar equals 20 imp/s.

walking speed of 0.7–0.8 m/s. The ratio of the stance duration to the cycle duration (the stride duty factor) varied only slightly between cats, however (Fig. 1*C*), and was 0.59 ± 0.05 (mean ± SD) on average. Because cats contributed fairly equally to each of the databases on PTNs with different receptive fields (Table 1), the average duration of steps chosen for PTNs of different groups was very close ($P > 0.05$, ANOVA; Fig. 1*D*), as was the duty factor ($P > 0.05$, ANOVA).

When walking along the ladder, four cats walked with nearly the same speed as on the flat surface, three were somewhat faster, and one was slower (Fig. 1*E*). The stride duty factor was 0.58 ± 0.04 (mean ± SD) on average, similar to simple locomotion, and was consistent across cats (Fig. 1*F*). Again, because cats contributed rather equally to the different PTN groups (Table 1), the average duration of steps included in the analyses of the activity of different groups was similar (Fig. 1*G*). The average durations of selected simple and ladder locomotion strides for shoulder-related, elbow-related, and nonresponsive PTNs were similar (Fig. 1, *D* and *G*). Strides selected for wrist-related PTNs were on average just slightly faster on the ladder than during simple locomotion.

The gait that cats used during locomotion both on the flat surface and along the ladder was a walk with the support formula of 2-3-2-3-2-3-2-3, indicating the number of limbs supporting the body during different phases of the step cycle (Hildebrand 1965). Details of the biomechanics and muscle activities of cats during walking on the flat surface and along the horizontal ladder in a similar experimental setup have been recently reported elsewhere (Beloozerova et al. 2010). Ladder locomotion is similar to simple locomotion in nearly all kinematic and EMG parameters; the few forelimb-related differences include a somewhat more bent-forward posture, a lower wrist flexion moment during stance, and a slightly enhanced activity of selected distal muscles during ladder locomotion.

Characteristics of neurons. The activity of 145 PTNs was included in the analysis. Of these, 45 responded exclusively to passive movements in the shoulder joint and/or palpation of upper back, chest, or lower neck muscles (shoulder-related group, Table 1). Thirty PTNs responded exclusively to passive movements in the elbow joint or palpation of upper arm muscles (elbow-related group, Table 1). Forty PTNs responded to passive movements in the wrist joint, palpation of the lower arm muscles, or stimulation of the palm or back of the paw (wrist-related group, Table 1). Finally, 30 PTNs had no receptive field (nonresponsive group, Table 1).

Of the 115 PTNs with receptive fields, most had some directional preference. Among shoulder-related PTNs, 33% (15/45) were preferentially receptive to flexion while 20% (11/45) were preferentially receptive to extension. The remaining 43% (19/45) were receptive to abduction or adduction of the joint or to palpation of the muscles on the back or chest. Among elbow-receptive PTNs, 37% (11/30) were preferentially receptive to flexion and 60% (18/30) were preferentially receptive to extension. Finally, among wrist-receptive PTNs, 42.5% (17/40) were receptive to ventral (plantar) flexion of the wrist while 32.5% (13/40) were receptive to its dorsal flexion. The remaining 25% (10/40) of the wrist-related PTNs were receptive to palpation of muscles on the forearm or paw, including two cells that additionally responded to cutaneous stimulation.

The vast majority of PTNs were recorded from the region of the motor cortex rostral to the cruciate sulcus. In Fig. 2*A*, color-coded dots overlaying the cortex schematically show microelectrode entry point into the cortex for tracks, in which PTNs of different groups were recorded during locomotion. There was extensive overlap between PTN groups.

The latencies of antidromic responses of different PTNs to pyramidal tract stimulation varied in the range of 0.4–5.0 ms (Fig. 2*D*). Estimated conduction velocities were between 5 and 80 m/s. Approximately three-fourths of neurons (107/145) responded at 2.0 ms or faster, conducting at 25 m/s or faster, and thus were "fast-conducting" PTNs (Bishop et al. 1953; Brookhart 1952; Takahashi 1965). In shoulder-, elbow-, and wrist-related and nonresponsive PTN groups, the proportions of fast- and slow-conducting neurons were similar (Fig. 2*D*).

An example of typical activity of a PTN during standing, as well as simple and ladder locomotion, is shown in Fig. 3. This PTN was nonresponsive to somatosensory stimulation. The PTN was steadily active during standing. Once locomotion began, the PTN's activity became modulated with respect to the step cycle. The neuron was highly active during most of the swing and the second half of the stance phase and less active during the end of the swing and the early stance phase. Upon transition from simple to ladder locomotion, the neuron's activity became even more strongly modulated. The neuron became even more active during the swing phase, while its activity during the stance phase decreased. The rasters in Fig. 3, *B* and *D*, show the activity of the neuron across 50 individual strides during simple (Fig. 3*B*) and ladder (Fig. 3*D*) locomotion. The pattern of activity was very consistent across strides of each locomotion task. The activity is summed in Fig. 3, *C* and *E*, showing a histogram of PTN firing rate across the step cycle during simple (Fig. 3*C*) and ladder (Fig. 3*E*) locomotion. The period of elevated firing (PEF; see definition in METHODS) is indicated by a black horizontal bar; it was contained within the swing and late stance phase of the step during both simple and ladder locomotion and was 15% of the cycle shorter during ladder locomotion. The preferred phase (indicated by a circle in Fig. 3, *C* and *E*) was in the very beginning of the swing phase during both locomotion tasks.

Activity during locomotion on flat surface. While the cat was standing, all PTNs were active. The average discharge rate was 13.0 ± 0.7 imp/s. The discharge rates of different PTN groups were similar (Fig. 4*A*). Upon transition from standing to walking, the average discharge rate of PTNs increased to 17.4 ± 0.9 imp/s ($P < 0.05$, *t*-test). Elbow-related PTNs were now, however, less active than either shoulder- or wrist-related PTNs ($P < 0.05$, ANOVA; Fig. 4*B*).

During locomotion, the discharge of 97% (141/145) of PTNs was modulated with respect to the stride: it was greater in one phase of the stride and smaller in another phase. Most PTNs (79%, 115/145) had one PEF, while 21% (29/141) had two PEFs per step cycle. The proportion of two-PEF cells was similar between groups of PTNs with different somatosensory receptive fields. The depth of modulation was also similar between the groups and was 10.2 ± 0.4% on average (Fig. 4*C*; 1-PEF and 2-PEF neurons were considered jointly). The duration of the PEF was similar as well and lasted between 55% and 60% of the cycle on average (Fig. 4*D*). PEFs and preferred phases of individual PTNs of all groups were distributed across the step cycle. However, this distribution was uneven and

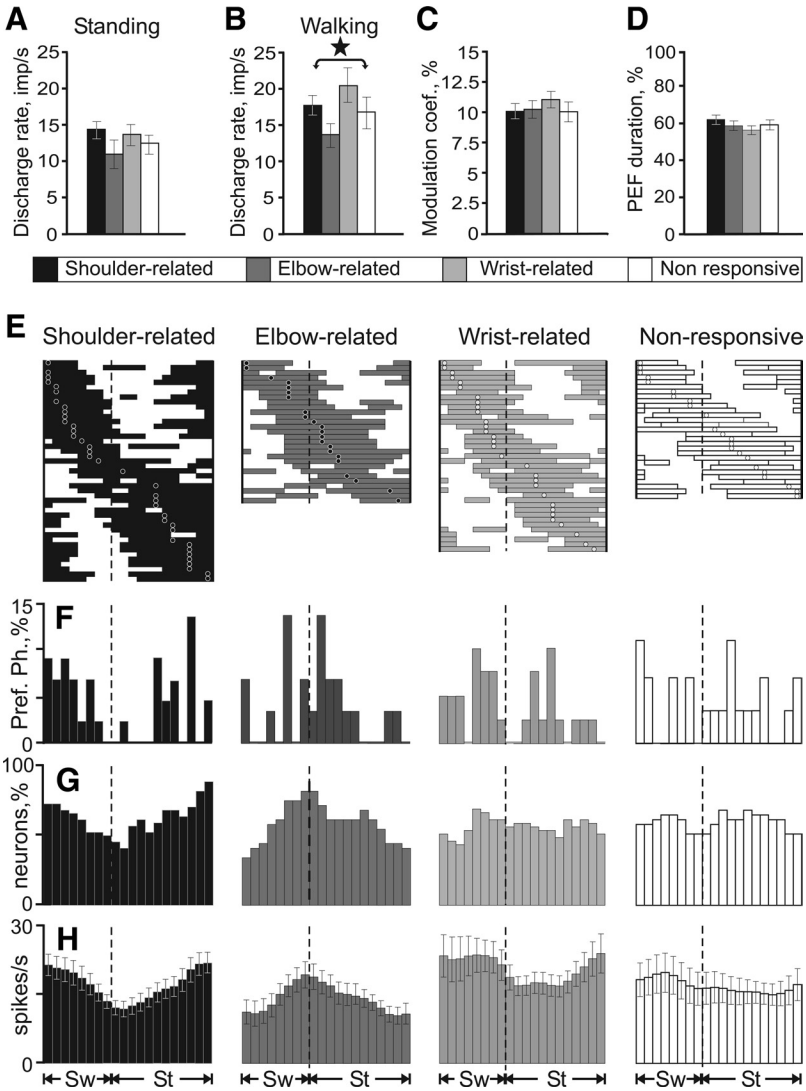

Fig. 4. Activity of PTNs with receptive fields involving different forelimb joints during simple locomotion. *A*: discharge rate during standing in different PTN groups. *B*: discharge rate during walking. *C*: depth of modulation. *D*: duration of the PEF. In *A–D*, error bars are SE and the star indicates significant differences in discharge rates during walking ($P < 0.05$, ANOVA). *E*: distribution of PEFs of individual PTNs in the step cycle. Each trace represents PEF of 1 PTN. Circles indicate preferred phase of each neuron. Neurons are rank ordered so that those whose preferred phase is earlier in the cycle are plotted at *top* of graph. *F*: distribution of preferred phases of neurons across the step cycle. *G*: proportion of cells active during the step cycle. The traces from *E* were summed into a histogram and normalized. *H*: phase histogram of the average firing rate of PTNs across the step cycle. Error bars are SE. *E–H*: Sw, swing phase; St, stance phase.

different between PTN groups. Shoulder-related PTNs were most often active during the late stance and early swing, and elbow-related PTNs were most often active during the late swing and early stance, while the periods of elevated activity of both wrist-related and nonresponsive neurons were distributed fairly equally throughout the step cycle (Fig. 4, *E–G*). In accordance with the phase distribution of PEFs and preferred phases, the mean discharge rate of the shoulder-related group was highest during the stance-to-swing transition, at 21.8 ± 2.0 imp/s, while the firing rate during the opposite phase was 13.4 ± 1.4 imp/s ($P < 0.05$, *t*-test; 8.4 imp/s difference) (Fig. 4*H*). The mean discharge rate of the elbow-related group was higher during the swing-to-stance transition period and was 17.4 ± 2.4 imp/s vs. 10.6 ± 2.1 imp/s during the stance-to-swing transition ($P < 0.05$, *t*-test; 6.8 imp/s difference) (Fig. 4*H*). In contrast, the average discharge rate of wrist-related and nonresponsive PTNs overall was around 20 and 17 imp/s, respectively, with only slight fluctuations (Fig. 4*H*).

Activity during locomotion on ladder. The ladder added accuracy requirements to the locomotion task. The cat was forced to constrain its paw placement during locomotion to the raised crosspieces of the ladder. It has been shown that the activity of the motor cortex is required to successfully perform this task (Beloozerova and Sirota 1988, 1993a; Chambers and Liu 1957; Liddell and Phillips 1944; Trendelenburg 1911). All PTNs that were tested during walking on the flat surface were also tested during complex locomotion along the ladder. Upon transition from simple to ladder locomotion, high proportions of PTNs in all groups, 27–42% depending on the group, increased their discharge on average by 99 ± 74%, while somewhat smaller proportions (15–40%) decreased it, on average by 43 ± 16% (SDs) (Fig. 5*A*). Thus the average rate of discharge across all PTN groups during complex locomotion was slightly higher than during simple locomotion (19.1 ± 1.0 vs. 17.4 ± 0.9 imp/s, $P < 0.05$, *t*-test). In addition, disproportional changes in the activity of different groups (relatively more neurons increased activity in shoulder and elbow-related groups, and more neurons decreased in the wrist-related group; Fig. 5*A*) led to more homogeneous discharge rates between groups during ladder compared with simple locomotion (see Figs. 7*A* and 4*A*). There were now no significant differences in the mean discharge rates of different groups of PTNs.

Substantial changes were also observed in the magnitude of frequency modulation (Fig. 5*B*). Half (51%) of shoulder-related PTNs and 40–45% of wrist-related and nonresponsive

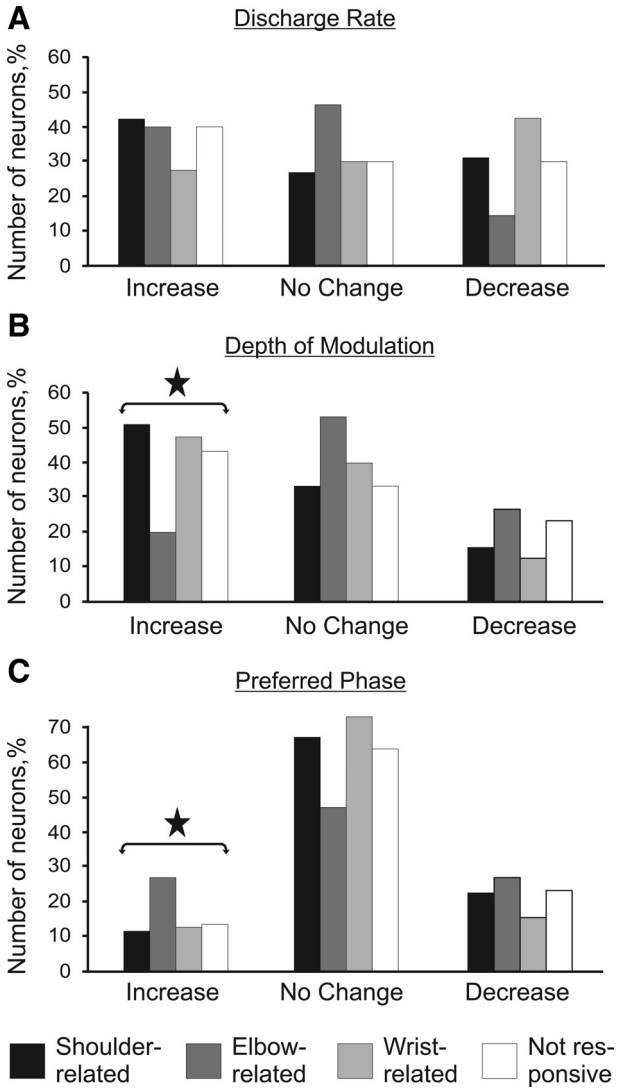

Fig. 5. Changes in mean discharge rate (*A*), depth of modulation (*B*), and preferred phase (*C*) of PTN populations observed upon transition from simple to ladder locomotion. Star indicates significant differences ($P < 0.05$, ANOVA).

cells showed increases in the depth of modulation on the ladder, on average by 62 ± 44% (mean ± SD). Decreases of modulation were also observed, but only half as frequently. Relatively fewer elbow-related PTNs changed the depth of modulation upon transition from simple to complex locomotion compared with shoulder- and wrist-related groups (Fig. 5*B*). These trends caused groups of PTNs with different somatosensory receptive fields to produce activity with more heterogeneous modulation depth during ladder locomotion compared with simple locomotion, with shoulder- and wrist-related PTNs having higher depths of modulation on average than elbow- and nonreceptive neurons ($P < 0.05$, ANOVA; see Fig. 7*B*).

The observed increases in the depth of modulation upon transition from simple and complex locomotion could be achieved by a variety of changes to neuronal activity patterns: *1*) an increase in firing rate during the PEF (additive increase in modulation), *2*) a decrease in the firing rate during the inter-PEF interval (subtractive increase in modulation), or *3*) a combination of both mechanisms. Purely additive or subtractive mechanisms accounted for the vast majority of changes to the depth of modulation, and only ~15% of changes were achieved by both mechanisms. PTNs of different groups tended to exhibit different mechanisms (Fig. 6). Only shoulder-related PTNs would often use a purely additive mechanism to increase modulation depth (Fig. 6*A*), while the purely subtractive mechanism, although seen in shoulder-related and nonreceptive PTNs, was most common for wrist-related PTNs (Fig. 6*B*). Additive modulation increase accounted for 33% (19/57) of all modulation increases, while subtractive modulation increase accounted for 54% (30/57).

Decreases in the depth of modulation were overall much less common. Elbow-related PTNs were the only group to decrease modulation in a purely subtractive manner in any significant numbers (Fig. 6*C*). However, additive decreases in modulation, achieved by a discharge rate increase during the inter-PEF interval, were comparatively common, and nonreceptive PTNs most often exhibited this change to their discharge patterns (Fig. 6*D*). Overall, subtractive modulation decrease accounted for 21% (6/28) of all decreases in modulation, while the additive mechanism accounted for 64% (18/28).

In addition to the activity and depth of modulation changes, modifications to the duration of the PEF were also observed upon transition from simple to complex locomotion. About one-third (31%) of shoulder-related PTNs and 33% of wrist-related PTNs decreased the duration of their PEF, on average by 43 ± 9% and 36 ± 9% (SDs), respectively. In contrast, elbow-related and nonresponsive PTNs tended not to change the duration of their PEF. As a result, during ladder locomotion, shoulder- and wrist-related PTNs had average PEF durations of 55 ± 2% and 51 ± 3% of the cycle, respectively, shorter than the averaged PEF duration of elbow-related PTNs, which was 63 ± 2% of the step cycle (*t*-test, $P < 0.05$).

The preferred phases of most PTNs were similar during simple and complex locomotion, with the exception of the elbow-related PTN group (Fig. 5*C*). Only 11–23% of shoulder-related, wrist-related, and unresponsive PTNs had preferred phase either earlier or later in the cycle during ladder locomotion compared with simple walking. The preferred phases moved from stance to swing phase slightly more often than from swing to stance. In contrast, in the elbow-related PTN group the preferred phases of half of neurons were different between the tasks.

Despite some changes in the preferred phases in a number of individual PTNs, the phasing preferences of PTN groups were largely similar during both tasks (compare Fig. 7, *D–G*, for ladder locomotion and Fig. 4, *E–H*, for simple locomotion). The strength of the phasing preference of shoulder-related PTNs remained unchanged: their mean discharge rate during stance-to-swing transition slightly rose to 24.4 ± 2.9 imp/s; however, the activity during the opposite phase also rose, reaching 16.1 ± 2.4 imp/s (Figs. 7*G* and 4*H*). Elbow-related PTNs still had a tendency to discharge more intensively during swing-to-stance transition, and the activity of nonresponsive PTNs was still distributed evenly throughout the cycle. In stark contrast to those groups, wrist-related PTNs developed a strong phase preference. Although during simple locomotion this group showed a slight tendency (not statistically significant) to discharge more intensively during swing, during ladder locomotion this preference became pronounced. The discharge

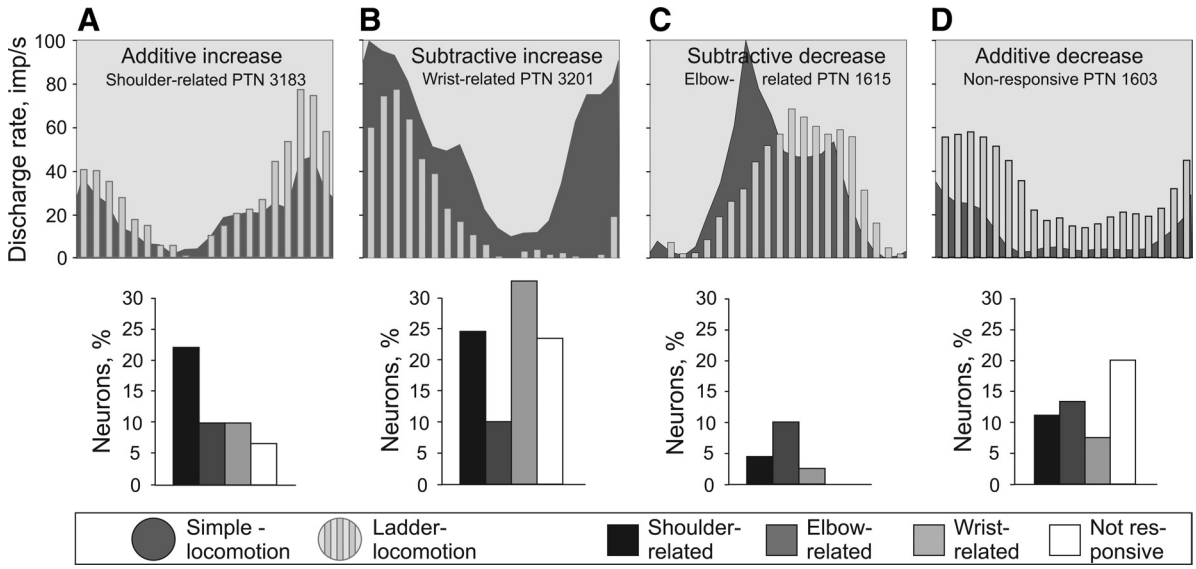

Fig. 6. Typical changes in the depth of modulation upon transition from simple to ladder locomotion. Area histograms show the activity of a typical PTN during simple locomotion. Bar histograms show activity of the same PTN during ladder locomotion. Bar graphs beneath the histograms show the proportion of neurons from each group exhibiting that type of modulation change. *A*: increase in depth of modulation by additive mechanism. *B*: increase in depth of modulation by subtractive mechanism. *C*: decrease in depth of modulation by subtractive mechanism. *D*: decrease in depth of modulation by additive mechanism.

during swing was now slightly higher, and, in addition, the discharge rate during stance substantially decreased (to 12.5 ± 1.7 imp/s from 17.5 ± 2.1 imp/s during simple locomotion; *t*-test, $P < 0.05$). Therefore, the difference in the discharge rate between swing and stance of the wrist-related PTNs was 14.6 imp/s during ladder locomotion.

To summarize, upon transition from simple to complex locomotion, different PTN groups changed their activity in distinct manners. Shoulder-related PTNs often increased their activity and depth of modulation while reducing their discharge duration and typically did not alter their preferred phase. As a group, they became slightly more active during stance-to-swing transition. Wrist-related PTNs often decreased their activity, increased depth of modulation while also reducing discharge duration, and typically did not change their preferred phase. As a group, they became more active during swing phase. Elbow-related PTNs most often did not change their activity, depth of modulation, or discharge duration but relatively often changed their preferred phase. Their group activity was distributed more evenly throughout the cycle during complex locomotion. Nonresponsive PTNs had mixed responses and had no preferred phase as a population.

Comparison of activity of PTNs responsive to flexion or extension of same joint. When we separated PTNs into groups that responded preferentially to either flexion or extension, we found that many of these groups exhibited distinct activity during simple locomotion (Fig. 8*A*). Wrist-related PTNs responsive to wrist dorsal ($n = 13$) or ventral ($n = 17$) flexion were dissimilar in all characteristics. PTNs responsive to the wrist ventral flexion were substantially more active than their counterparts. However, PTNs responsive to the wrist dorsal flexion were more strongly modulated, and their PEFs were shorter. Elbow-related PTNs that were responsive to extension ($n = 18$) had longer PEFs but were otherwise similar to elbow flexion-related PTNs ($n = 11$). Only shoulder-related PTNs that were responsive to flexion ($n = 11$) and extension ($n = 15$) were similar in all characteristics tested.

Many PTNs changed their discharge characteristics upon transition from simple to ladder locomotion (Figs. 5–7). PTNs responsive to flexion or extension of the same joint often altered activity in distinct manners (Fig. 8*B*). Shoulder-related PTNs that were responsive to extension of the shoulder changed both their average discharge rate and the depth of step-related modulation, and had a tendency to have a shorter PEF compared with simple locomotion. Their counterparts (those responsive to shoulder flexion) discharged similarly in both tasks. Elbow extension-related PTNs substantially increased their average activity, while elbow-flexion related PTNs did not; in contrast, only elbow flexion-related cells increased their average PEF duration. Wrist-related PTNs that were responsive to ventral flexion of the wrist decreased their average discharge rate and increased depth of step-related modulation compared with simple locomotion, while their counterparts showed no significant changes.

As a result, during complex locomotion there were fewer differences in the activity of PTNs responsive to flexion or extension of the same joint compared with simple locomotion (Fig. 8*A*). Wrist dorsal and ventral flexion-receptive PTNs became similar in all parameters, and the average duration of PEFs in the groups of elbow-receptive PTNs became similar. Although elbow extension-receptive PTNs became more active than elbow-flexion PTNs, this was the only observed group difference between any flexion- and extension-receptive pairing during complex locomotion. Shoulder-receptive PTNs remained similar in all parameters tested.

Relation of activity phasing and kinematics. During locomotion, each joint undergoes repeating phases of flexion and extension throughout the step cycle. We tested whether PTNs that respond to the movement of a joint in a single direction at rest would discharge in phase with that joint movement during locomotion or out of phase.

Figure 9*A* shows the distribution of PEFs of those neurons that were responsive exclusively to flexion of shoulder (Fig. 9*A*, *left*), elbow (Fig. 9*A*, *center*), or ventral flexion of wrist

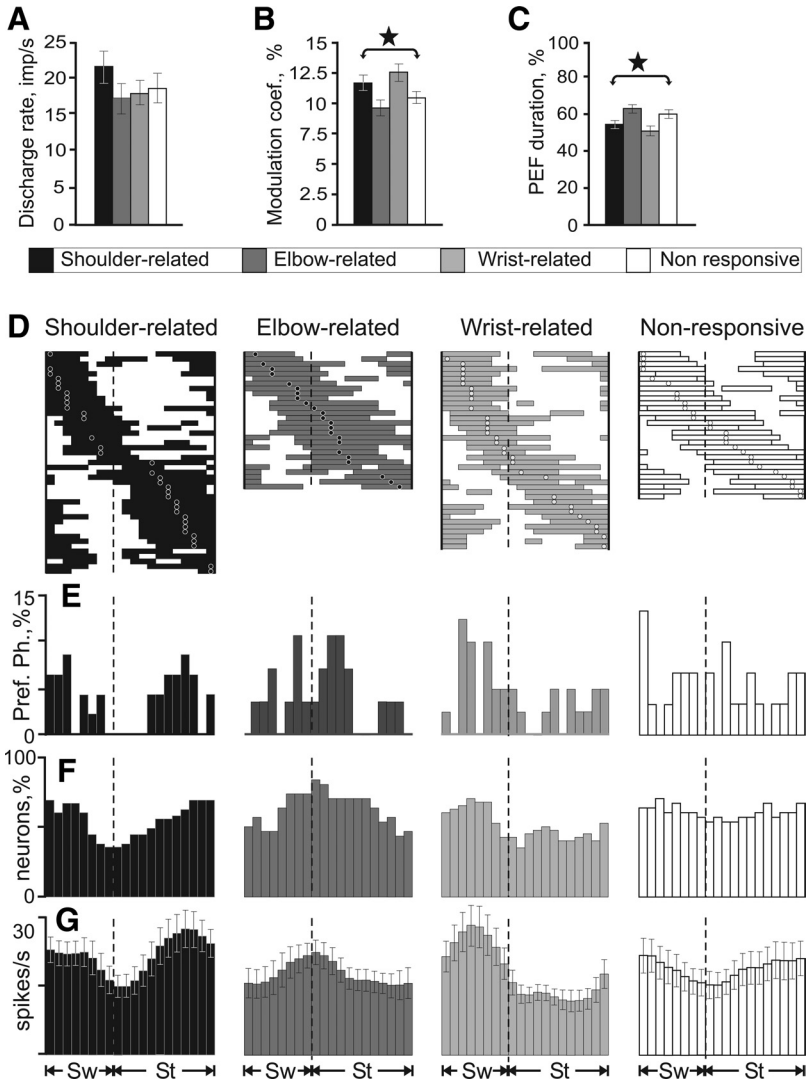

Fig. 7. Activity of PTNs with receptive fields involving different forelimb joints during ladder locomotion. *A*: discharge rate during walking. *B*: depth of modulation. *C*: duration of the PEF. In *A–C*, error bars are SE and stars indicate significant differences in values ($P < 0.05$, ANOVA). *D*: distribution of PEFs of individual PTNs in the step cycle. Each trace represents PEF of 1 PTN. Neurons are rank ordered so that those whose preferred phase is earlier in the cycle are plotted at *top* of graph. *E*: distribution of preferred phases of neurons across the step cycle. *F*: proportion of cells active during the step cycle. *G*: phase histogram of the average firing rate of PTNs across the step cycle. *E–G*: Sw, swing phase; St, stance phase.

(Fig. 9*A*, *right*). Angle movements of these joints, defined after Prilutsky and colleagues (2005), are shown in Fig. 9*C*. We found that shoulder flexion-responsive PTNs typically discharged in phase with flexion of the shoulder in both locomotion tasks (Fig. 9*B*). However, elbow-related PTNs most often discharged out of phase, and wrist ventral flexion-related PTNs had no preference (Fig. 9*B*). The same analysis applied to extension-receptive PTNs showed that shoulder- and wrist-related PTNs had no preference to discharge in or out of phase with their respective joint extension, while elbow-related PTNs preferred to discharge out of phase (Fig. 9, *D–F*).

DISCUSSION

The main finding of this study is that PTNs responsive to stimulation of different forelimb joints have different activity characteristics during locomotion, both simple and complex. While it might be tempting to suggest that these differences are due to differences in the PTNs' somatosensory receptive field characteristics, in fact, somatosensory information seems not to play a leading role in determining the locomotion-related discharges of most PTNs during either simple or complex locomotion. Indeed, neurons with similar receptive fields often discharge during quite different times of the locomotion cycle (Fig. 9; Armstrong and Drew 1984b). It has been shown that the locomotion-related responses of motor cortical neurons are only slightly affected by changes in the vigor of movements during up- and downslope walking, weight bearing, or alterations in speed (Armstrong and Drew 1984a; Beloozerova and Sirota 1993b)—changes that most certainly cause significant changes to proprioceptive afferentation. In regard to cutaneous input, Armstrong and Drew (1984b) have demonstrated that in motor cortex neurons with cutaneous receptive fields, including on the forefoot, the discharges during locomotion remained rhythmic and their phasing relative to the step cycle was unchanged when the response to mechanical stimulation in the receptive field was temporarily much reduced or abolished by local anesthesia of the skin. In the present study we found that the great majority of PTNs with direction-specific receptive fields did not show any particular preference to discharge in phase with stimulation of their receptive field during locomotion, and elbow-related PTNs even preferred to discharge out of phase (Fig. 9). Similarly poor relationships between phasing of task-related discharges and directional specificity of PTN resting receptive fields were reported in previous studies from this and other laboratories (Armstrong and Drew 1984b; Be-

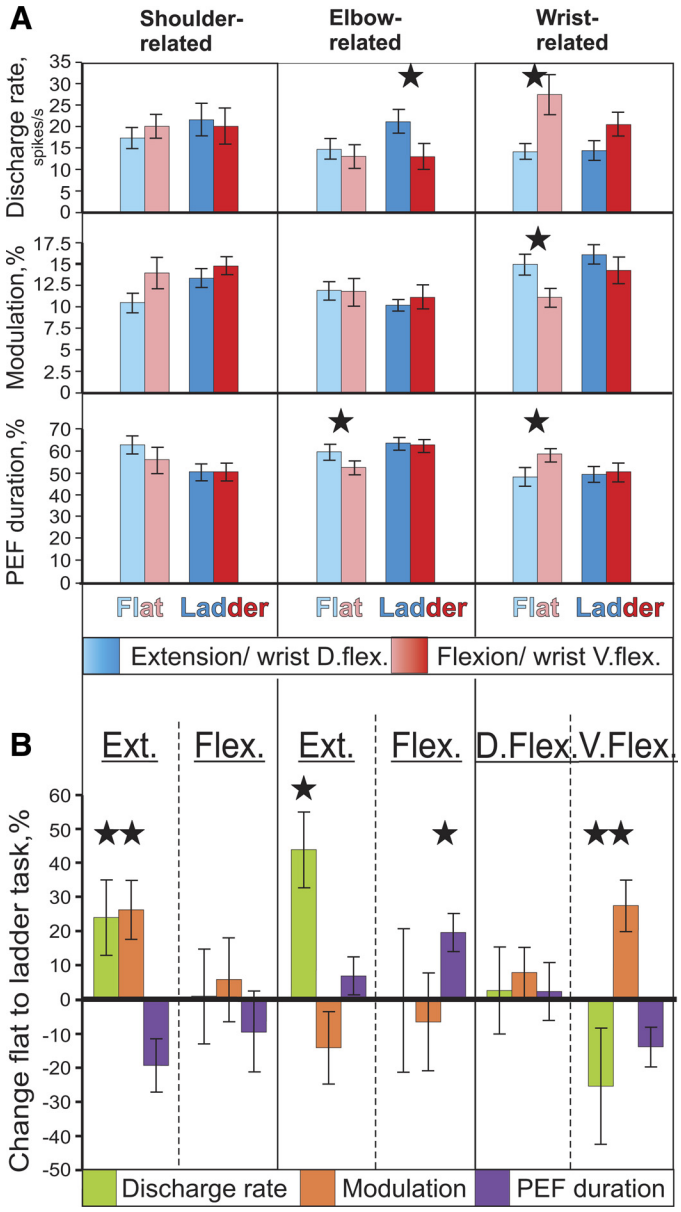

Fig. 8. Comparison of activities of flexion- and extension-receptive PTNs. *A*: discharge rate, modulation depth, and PEF duration are compared for flexion-extension pairs of each PTN group during simple (Flat) and complex (Ladder) locomotion. Extension-receptive cells, including wrist dorsal flexion-related, are colored a lighter color. *B*: % change in activity parameters for each group. *A* and *B*: significant changes are denoted with a star (*t*-test, $P < 0.05$); error bars are SE.

loozerova et al. 2003c, 2005; Drew 1993; Karayannidou et al. 2008). While it is true that somatosensory receptive fields during active movements may be somewhat different from those observed at rest (Chapman et al. 1988; Ghez and Pisa 1972), the above group of observations suggest that some factors other than stimulation of somatosensory receptive field drive PTN discharges during locomotion. In fact, in decerebrated cats neurons of both reticulospinal and rubrospinal tracts display locomotion-related modulation of their activity even during fictive locomotion when the subject is motionless and thus no rhythmic afferentation is present (Arshavsky et al. 1988; Perret 1976), suggesting that the spinal cord locomotor central pattern generator (CPG) plays a significant role in modulating their discharges. It is quite likely that during simple locomotion the activity of PTNs of the motor cortex also, rather than being driven by stimulation of somatosensory receptive fields, is significantly influenced by signals from the spinal locomotion CPG. If so, then the influence appears to be somewhat different for PTNs associated with different joints of the forelimb (Fig. 4), as we found that PTNs with receptive fields involving different joints—PTNs with receptive fields in different locations on the limb—tend to discharge differently during simple locomotion. Shoulder-related PTNs are most active during the late stance and early swing, elbow-related

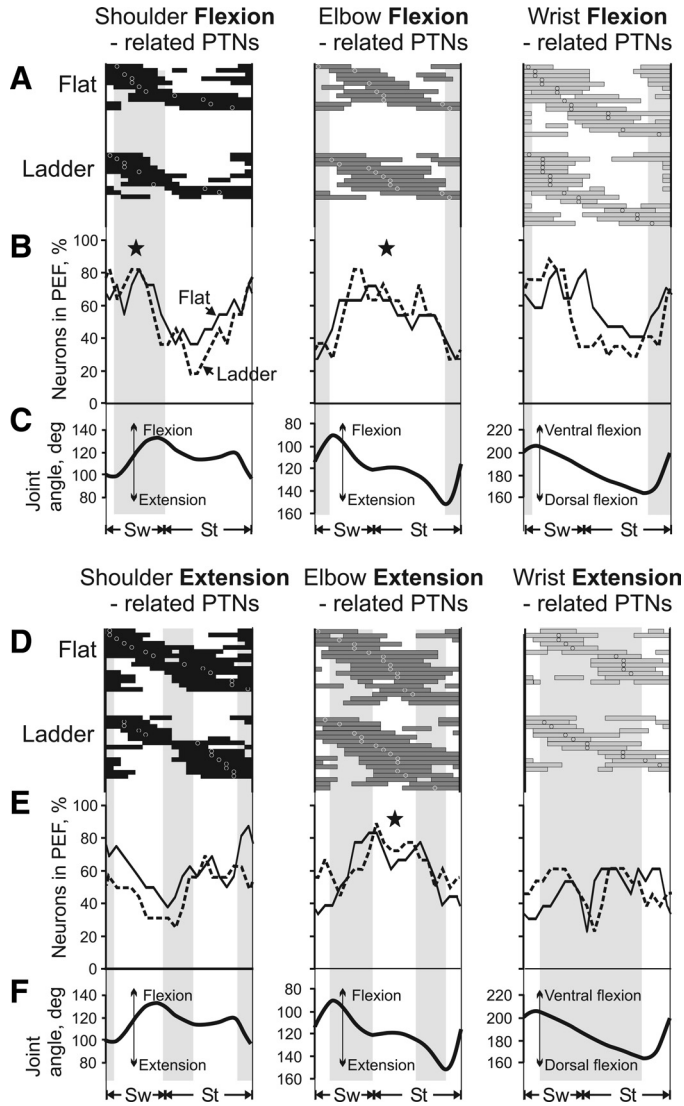

Fig. 9. Proportions of PTNs firing in phase with activation of their receptive field during locomotion. *A* and *D*: distribution of PEFs of individual flexion-related (*A*) and extension-related (*D*) PTNs in the step cycle. Each trace represents the PEF of 1 PTN. PEFs during simple (Flat) and ladder locomotion (Ladder) are individually rank ordered. *B* and *E*: proportion of flexion-related (*B*) and extension-related (*E*) PTNs active during the step cycle during simple (solid line) and ladder (dashed line) locomotion. *C* and *F*: movements in forelimb joints during the step cycle (Prilutsky et al. 2005). In *A–C*, periods of the step cycle when the joint flexes are highlighted in gray. In *D–F*, periods when the joint extends are highlighted in grey. Sw, swing phase; St, stance phase. Stars indicate significant difference between the average number of PTNs that were in their PEF when the associated joint movement was occurring (in-phase firing) and when it was not (out-of-phase firing) (*t*-test, $P < 0.05$).

PTNs are most active during the late swing and early stance, and the activity of wrist-related PTNs is roughly even throughout the step cycle.

The motor cortex does not appear, however, to exert decisive control over simple locomotion, because a lesion or even short reversible inactivation of it has no effect on performance (Drew et al. 1996; Beloozerova and Sirota 1988, 1993a; Chambers and Liu 1957; Liddell and Phillips 1944; Trendelenburg 1911). We have previously suggested that the stride-related modulation of activity that the motor cortex exhibits during simple locomotion has an informational character, allowing the motor cortex to influence the spinal locomotor mechanism during correction of movements without disturbing the overall stepping rhythm (Beloozerova and Sirota 1993a).

Locomotion on the ladder adds accuracy constraints to the locomotion task, as cats are required to step precisely on the crosspieces. It was previously demonstrated that this task requires the activity of the motor cortex to be successful (Beloozerova and Sirota 1988, 1993a; Chambers and Liu 1957; Drew et al. 1996; Liddell and Phillips 1944; Trendelenburg 1911). On the ladder, most PTNs changed their activity compared with simple locomotion (Figs. 5–7). Again, this change does not appear to be caused by a difference in somatosensory afferentation between the two tasks. Indeed, we have shown that mechanical parameters of simple and ladder locomotion differ only very slightly, making it likely that only small dissimilarities exist in the afferent signals that arrive to the motor cortex during these two tasks (Beloozerova et al. 2010). For the forelimbs, we found that on the ladder compared with simple locomotion cats only rotate their neck down, increase flexion in the metacarpophalangeal joint, and reduce the wrist flexion moment during stance. Other mechanical variables, out of >200 tested, are similar during the two tasks. On the basis of this evidence, we feel that the small differences in joint kinematics are insufficient to cause the very pronounced differences observed in neuronal discharges. On the other hand, we found that cats move their eyes and look at the walking pathway in a very different manner during simple and ladder locomotion (Beloozerova et al. 2010; Rivers et al. 2009, 2010, 2011) and that ladder locomotion is not possible in complete darkness (Beloozerova and Sirota 2003). Considering rather similar motor patterns in the two locomotion tasks but dramatically different gaze behaviors and the need for vision, we have previously suggested that during locomotion on the ladder, which requires visual guidance of stepping, motor cortex PTNs transmit processed visual information by modulating their simple locomotion-related discharges (Beloozerova et al. 2010). These integrated visuomotor signals appear to control accurate placing of feet on crosspieces of the ladder. The purpose of the present study was to investigate whether and how these PTN signals vary depending on the part of the forelimb they control.

In this study we took an advantage of the fact that in small loci in the forelimb representation of the motor cortex, a relationship exists between afferent input and motor output. This relationship makes it possible to infer the forelimb joint that an individual PTN influences from the somatosensory receptive field that it has. Indeed, although axons of individual PTN from the forelimb representation of the motor cortex give off several branches along cervical and thoracic segments of the spinal cord most often synapsing upon interneurons of laminae IV–VII (Chamber and Liu 1957; Shinoda et al. 1986), and there is a rich spinal interneuron network that mediates signals from PTNs to motoneurons, earlier reports have shown that microstimulation in the forelimb region of the motor cortex typically produces contraction in single muscles or in small groups of muscles in the area that composes the receptive field at the stimulation site (Armstrong and Drew 1985a; Asanuma et al. 1968; Murphy et al. 1975; Rosen and Asanuma 1972; Sakata and Miyamoto 1968) and affects monosynaptic reflexes of only one or two muscles (Asanuma and Sakata 1967). Even when series of pulses of 20 μA were used in locomoting subjects, microstimulation of a quarter of sites within forelimb motor cortex still affected only one or two muscles (Fig. 3 in Armstrong and Drew 1985b). Experiments that used spike-triggered averaging of EMGs in primates showed that although many PTNs excite several motoneuron pools including those related to muscles on two different segments of the limb or occasionally even across the entire forelimb, about half of PTNs influence motoneuron pools that only innervate muscles on one segment of the limb (Buys et al. 1986; McKiernan et al. 1998).

For this study we selected only PTNs with a receptive field constrained to a single forelimb segment, and we found that these PTNs—PTNs with receptive fields in different localized locations—tend to discharge differently during complex locomotion. On the basis of the information above, and also taking into account the limitations of those experiments, which have been carefully reviewed by Schieber (2001), we believe that our main result can be restated as follows: PTNs assumedly influencing different joints of the forelimb have different activity characteristics during visually guided locomotion. These PTNs exert influence in distinct manners, and therefore have different roles in control of locomotion.

On the ladder, most PTNs changed their activity compared with simple locomotion; however, each group changed it in a specific and unique way (Figs. 5–7). Shoulder-related PTNs often increased their discharge rate and depth of modulation while reducing discharge duration. They typically did not change their preferred phase but as a group became more active at the end of stance. Such activity modifications are consistent with the hypothesis that during precise stepping shoulder-related PTNs have a significant role in planning of limb transfer, which is hypothesized to occur at the end of stance phase (Hollands and Marple-Horvat 1996; Laurent and Thomson 1988), as well as in the initial phases of limb transfer when adjustment of the foot trajectory is still possible (Marigold et al. 2006; Reynolds and Day 2005). Also, during the second half of stance, accurate paw placement of the opposing limb is taking place, and precise posture maintenance from the supporting limb is important to maintain balance. This could be another reason that shoulder-related PTNs, specifically those related to shoulder extension, increase their activity and modulation during stance (Figs. 5, 8).

Wrist-related PTN activity was fairly evenly distributed throughout the cycle during simple locomotion, but during complex locomotion wrist-related PTNs became strongly modulated as a group, exhibiting a prominent activity peak during swing (Fig. 7*G*). In contrast to shoulder-related PTNs, individual wrist-related PTNs often decreased discharge rate while also increasing depth of modulation and reducing their discharge duration. Such activity modifications are consistent

with the hypothesis that wrist-related PTNs, specifically those related to the wrist plantar (ventral) flexion, are involved in distal limb transfer during challenging tasks. This view is further supported by the fact that wrist ventral flexion-related PTNs increased their depth of modulation more than wrist dorsal flexion-related PTNs (Fig. 8), and indeed in a previous study we found that during locomotion on the ladder the wrist is more flexed in the plantar (ventral) direction compared with simple locomotion (Beloozerova et al. 2010).

Although both shoulder- and wrist-related PTNs often increased modulation during complex locomotion compared with simple walking, they generally did so by using different mechanisms (Fig. 6). Shoulder-related PTNs commonly achieved an increase in modulation by increasing their peak discharge rate. This would result in a more intensive signal to the spinal network, often along with a more specific timing of the discharge. Wrist-related PTNs achieved increases in modulation almost exclusively by decreasing the firing outside of PEF, increasing the salience of the signal without making it more intense. This modification could specifically improve the temporal precision of the controls for limb transfer during a precision stepping task.

In contrast to shoulder- and wrist-related PTNs, elbow-related PTNs did not often change activity, modulation depth, or discharge duration upon transition from simple to complex locomotion but often changed their preferred phase. Their group activity became evenly distributed throughout the cycle during complex locomotion (Fig. 7*G*). Their generally elevated activity during ladder locomotion might improve overall limb control during locomotion tasks that require accurate foot placement. Nonreceptive PTNs showed no changes to discharge rate, modulation depth, discharge duration, or preferred phase between tasks. The functions of these cells as well as their spinal targets remain to be determined. The diversity of responses between different PTN groups suggests that each group exerts influence within a different domain of the movement control.

An effective way for PTNs to differentially influence different joints of the forelimb during locomotion would be to individually influence the respective locomotion pattern formation networks of the CPG (Markin et al. 2011; McCrea and Rybak 2008), modulating the amplitude and potentially the timing of their output. Indeed, Asante and Martin (2010) recently found in the mouse that spinal projections from shoulder-, elbow-, and wrist-related areas in the motor cortex primarily contact those spinal premotor circuits that connect to shoulder-, elbow-, and wrist-related motoneuron pools, respectively. On the basis of results of experiments with microstimulation in the motor cortex, analogous mechanisms for control of limb joints have been previously suggested by Drew (1991) for the forelimb and by Bretzner and Drew (2005) for the hindlimb of the cat. However, these authors now stress the likelihood that the motor cortex controls locomotion movements based on muscle synergies that appear to form during stepping (Drew et al. 2008; Krouchev et al. 2006). While the concept of synergies is indeed very helpful for understanding the organization and neuronal control of movements (e.g., reviewed in Bizzi et al. 2008 and Latash 2008), it does not exclude a possibility that, within the entire limb or even the entire body locomotor synergy, individual elements of the synergetic network may receive individual commands when conditions of the task warrant it. The inability of the cat to continue on the ladder for even a single step after lights are turned off (Beloozerova and Sirota 2003) and the persistent visual sampling of every single crosspiece of the ladder on every run (Rivers et al. 2009, 2010, 2011) strongly suggest that, despite significant training, our cats did not establish a "ladder locomotion" synergy but controlled foot landing on each crosspiece step by step. Our data suggest that within the basic locomotion synergy, spinal mechanisms related to different joints of the forelimb receive different commands from the motor cortex during ladder locomotion.

Although the neuronal mechanisms underlying the differences in motor cortex controls for different forelimb joints have never been directly studied, there exists evidence suggesting that the mechanisms for their controls during tasks other than locomotion might be different. For example, it has been found that nearly all neurons in the shoulder/elbow area of the motor cortex modulate their activity during reaching in accordance with the posture of the arm (Scott and Kalaska 1997), while the activity of only a fraction of neurons in the hand area is wrist posture related (Kakei et al. 2003).

While in our study the inference about the area of the forelimb that is controlled by individual PTNs may be imprecise, the data nevertheless suggest that there is likely to be a significant distinction in the commands that are sent from the motor cortex to different joints of the forelimb during complex locomotion.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: E.E.S. and I.N.B. conception and design of research; E.E.S. and I.N.B. performed experiments; E.E.S. and I.N.B. analyzed data; E.E.S. and I.N.B. interpreted results of experiments; E.E.S. and I.N.B. prepared figures; E.E.S. and I.N.B. drafted manuscript; E.E.S. and I.N.B. edited and revised manuscript; E.E.S. and I.N.B. approved final version of manuscript.

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