JPn Neuroscience

The Journal of Physiology

J Physiol 591.10 (2013) pp 2647–2666

Differential responses of fast- and slow-conducting pyramidal tract neurons to changes in accuracy demands during locomotion

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Key points

- The motor cortex is highly involved in performing complex movements including skilled locomotion.
- Slow-conducting pyramidal tract neurons (PTNs) in the motor cortex are much more numerous than fast-conducting PTNs, but little is known about their function during movements.
- We find here that slow-conducting PTNs show vigorous and concerted changes to their activities during accurate targeted stepping *versus* simple locomotion over a flat surface, while changes to the activities of fast-conducting PTNs vary.
- This suggests that slow-conducting PTNs are involved to a greater extent in control of accuracy during locomotion.
- The results may be relevant to developing therapies for stroke and traumatic brain injury.

Abstract Most movements need to be accurate. The neuronal mechanisms controlling accuracy during movements are poorly understood. In this study we compare the activity of fast- and slow-conducting pyramidal tract neurons (PTNs) of the motor cortex in cats as they walk over both a flat surface, a task that does not require accurate stepping and can be accomplished without the motor cortex, as well as along a horizontal ladder, a task that requires accuracy and the activity of the motor cortex to be successful. Fast- and slow-conducting PTNs are known to have distinct biophysical properties as well as different afferent and efferent connections. We found that while the activity of all PTNs changes substantially upon transition from simple locomotion to accurate stepping on the ladder, slow-conducting PTNs respond in a much more concerted manner than fast-conducting ones. As a group, slow-conducting PTNs increase discharge rate, especially during the late stance and early swing phases, decrease discharge variability, have a tendency to shift their preferred phase of the discharge into the swing phase, and almost always produce a single peak of activity per stride during ladder locomotion. In contrast, the fast-conducting PTNs do not display such concerted changes to their activity. In addition, upon transfer from simple locomotion to accurate stepping on the ladder slow-conducting PTNs more profoundly increase the magnitude of their stride-related frequency modulation compared with fast-conducting PTNs. We suggest that slow-conducting PTNs are involved in control of accuracy of locomotor movements to a greater degree than fast-conducting PTNs.

(Received 22 March 2012; accepted after revision 1 February 2013; first published online 4 February 2013)
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Abbreviations CV, coefficient of variability; *dM*, depth of modulation; *M*, coefficient of modulation; PEF, period of elevated firing; PTN, pyramidal tract neuron; Sw, swing phase of the stride; St, stance phase of the stride.

DOI: 10.1113/jphysiol.2012.232538

Introduction

Most movements require accuracy to be successful. This is true for everything: a finger tap on a keyboard, a reach for a coffee mug, a step over a puddle. Accuracy is perhaps one of the most important characteristics of the majority of movements that we make, and thus the mechanics of it have received considerable experimental attention (e.g. Woodworth, 1899; Fitts, 1954; Goodale *et al.* 1986; Soechting & Flanders, 1989; Prablanc & Martin, 1992; Gordon *et al.* 1994; Messier & Kalaska, 1999; Novak *et al.* 2002; Dounskaia *et al.* 2005; Beloozerova *et al.* 2010). In contrast, the neuronal mechanisms that impart accuracy to movements remain poorly understood. While it is well known that lesions to a variety of brain centres significantly hamper accuracy (e.g. Liddell & Phillips, 1944; Martin & Ghez, 1993; Bastian *et al.* 2000; Beer *et al.* 2000; Mihaltchev *et al.* 2005), there had been only a handful of studies that directly examined individual neuronal responses to changes in accuracy demand during movements (e.g. Beloozerova & Sirota, 1993*a*; Gomez *et al.* 2000; Beloozerova *et al.* 2010).

Locomotion is one of the most essential and common motor behaviours. Locomotion often requires precise stepping, as humans and animals have to navigate through complex natural environments filled with obstacles and variable support surfaces. It has been shown that lesions to the motor cortex or even its short-lasting inactivation deprive subjects of the ability to step accurately (Trendelenburg, 1911; Liddell & Phillips, 1944; Chambers & Liu, 1957; Beloozerova & Sirota, 1988, 1993*a*; Drew *et al.* 1996; Metz & Whishaw, 2002; Friel *et al.* 2007). It has also been shown that when stepping has to be accurate during negotiation of obstacles or walking on crosspieces of a horizontal ladder, the activity of neurons in the motor cortex differs dramatically from that during simple locomotion over flat terrain (Beloozerova & Sirota, 1993*a*; Drew, 1993; Marple-Horvat *et al.* 1993; Widajewicz *et al.* 1994; Sirota *et al.* 2005). Moreover, we recently found that, as accuracy demand during stepping progressively increases, 30% of neurons in the motor cortex progressively refine their discharge timing, producing activity more precisely in specific phases of the stride (Beloozerova *et al.* 2010). Thus, it appears that during accurate stepping the discharges of neurons in the motor cortex contain cortical commands for accurate foot placement.

The motor cortex is connected to the spinal cord via pyramidal tract neurons (PTNs), large pyramid shaped cells located in the layer V of the cortex. In the spinal cord PTNs synapse mostly on interneurons (Hoff & Hoff, 1934; Lloyd, 1941; Dyachkova *et al.* 1971; Antal, 1984; Lacroix *et al.* 2004; Rosenzweig *et al.* 2009). Based on their axonal conduction velocity, PTNs can be subdivided into two distinct groups: 'fast' PTNs, conducting with velocities of 21 to >80 m s^{-1}, and 'slow' PTNs, conducting with velocities below 21 m s^{-1} (Lassek & Rasmussen, 1940; Brookhart & Morris, 1948; Bishop *et al.* 1953; Takahashi, 1965). Fast-conducting PTNs have larger somas but account for only 10–20% of the PTN population, while slow-conducting neurons represent the smaller-bodied majority of PTNs (Calvin & Sypert, 1976; Humphrey & Corrie, 1978). In addition to axonal conduction velocities, a number of other biophysical properties such as the duration of the spike, membrane resistance, amplitude of after-hyperpolarization, and others distinguish fast- and slow-conducting PTNs (Takahashi, 1965; Baranyi *et al.* 1993). Fast- and slow-conducting PTNs are also distinct in the manner by which they contact neurons within the cortex and subcortically (e.g. Towe *et al.* 1968; Takahashi, 1965; Ghosh & Porter, 1988; Lemon & Porter, 1993; Canedo, 1997). For example, in the spinal cord, fast-conducting PTNs preferentially influence distal muscle-related networks, while slow-conducting PTNs influence both proximal and distal muscle-related networks (Brookhart, 1952; Wiesendanger, 1981; Canedo, 1997). The activity of fast- and slow-conducting PTNs was compared in primates during movements of the forelimb (Evarts, 1965; Fromm & Evarts, 1977, 1981; Fromm *et al.* 1984). It was found that slow-conducting PTNs are more readily activated by small movements, whereas many of fast-conducting PTNs only engage during large movements. Based on this observation and considering the nature of axonal projections of slow-conducting PTNs, Fromm & Evarts (1977) suggested that slow-conducting PTNs may have a special role in control of accuracy of movements. No experiments so far, however, have actually been designed to provide direct data on whether fast- and slow-conducting PTNs transmit differing cortical commands regarding accuracy during movements. It remains unclear whether the efficient activation of slow-conducting PTNs during small movements is truly due to the accuracy requirements of small tasks, or merely due to a low activation threshold for these PTNs.

In our study, we presented subjects with two motor tasks that required movements of similar amplitude but different accuracy demand. Cats walked on a flat surface where there were no restrictions on foot placement, and on crosspieces of a horizontal ladder, where they had to step precisely on the crosspieces. The distance between the crosspieces was set to be the modal length of steps on the flat surface. We recorded from fast- and slow-conducting PTNs in the forelimb representation of the motor cortex and found that while the individual cells of both varieties vigorously respond to accuracy demands during locomotion, the activity of slow-conducting PTNs changes in more respects and often more intensively than that of fast-conducting PTNs. We suggest that during locomotion slow-conducting PTNs may have a greater role in adaptation of locomotor movements to the accuracy

demands of the environment. Based on known differences in biophysical properties and synaptic connections of fast- and slow-conducting PTNs we speculate on what influence these different PTNs may exert over the neuronal networks of the spinal cord.

Preliminary results have been published in abstract form (Stout & Beloozerova, 2010).

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 (Sirota *et al.* 2005; Beloozerova *et al.* 2010; Stout & Beloozerova, 2012), however, the selection of neurons for this study is unique. Methods of data collection and spike trains analysis have been described (Beloozerova & Sirota, 1993*a*; Prilutsky *et al.* 2005; Beloozerova *et al.* 2010) and will be briefly reported below. All experiments were conducted in accordance with NIH 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) accurate stepping on the crosspieces of a horizontal ladder (Fig. 1*A*). A box 2.5 m long and 0.6 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. The crosspieces of the horizontal ladder were flat and 5 cm wide. The width of the crosspieces was chosen to exceed the cat's mean foot length (3 cm), so that cats had full foot 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 flat floor at a self-selected pace (Beloozerova & Sirota 1993*a*; Beloozerova *et al.* 2010). Crosspieces were elevated 6 cm above the floor of the chamber.

It has been demonstrated in several studies that, while locomotion over a flat surface can be successfully performed after the motor cortex has been ablated or inactivated, locomotion that requires accurate foot placement, including on a horizontal ladder, depends on the activity of the motor cortex (Trendelenburg, 1911; Liddell & Phillips, 1944; Chambers & Liu, 1957; Beloozerova & Sirota, 1993*a*; Metz & Whishaw, 2002; Friel *et al.* 2007). In our early publications we showed that neurons in the motor cortex, including PTNs, substantially change their activity upon transition from locomotion over a flat surface to walking along a horizontal ladder (Beloozerova & Sirota, 1993*a*; Sirota *et al.* 2005). In our recent study we examined 229 full-body biomechanical parameters of cats walking on the flat surface and along horizontal ladder in a similar experimental setup (Beloozerova *et al.* 2010). We found that on the ladder, cats step on the support surface with much less spatial variability, use a slightly more bent-forward posture, and the wrist flexion moment is lower throughout stance; however, the horizontal velocity trajectories of paws are symmetric and smooth, and do not differ from those seen during walking on the flat surface. Most other biomechanical parameters do not differ between the tasks. Based on these data, in this study we have used a comparison between 'non-accurate' locomotion on the flat surface and 'accurate' stepping on the horizontal ladder as a tool to reveal a portion of PTN activity that is related to accuracy constraints during stepping.

Cats were accustomed to wearing a cotton jacket, a light backpack with connectors, and an electro-mechanical sensor on the paw for recording of swing and stance phases of stride. The floor in the chamber and the crosspieces

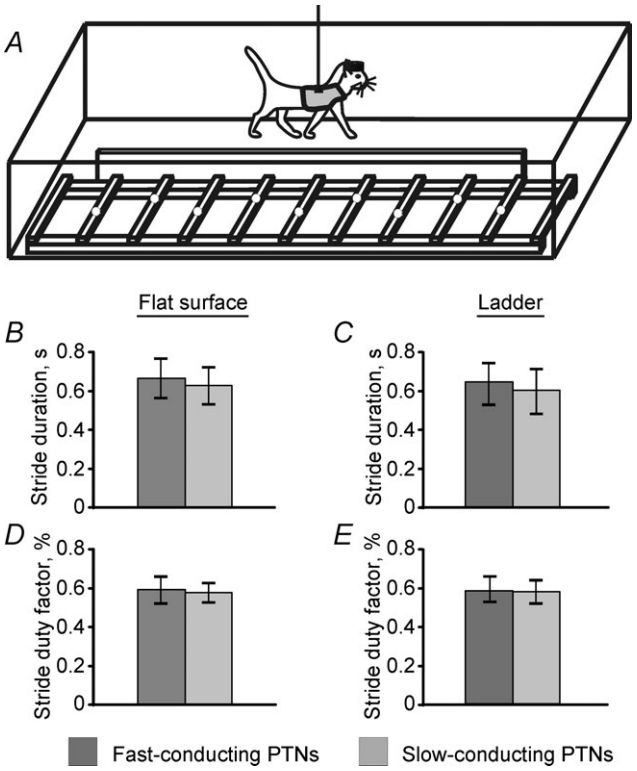

Figure 1. Locomotion tasks
A, the experimental box was divided into two corridors. In one of the corridors, the floor was flat, while the other corridor contained a horizontal ladder. White circles on the crosspieces of the ladder schematically show placements of cat forelimb paws. *B*, *C*, average durations of the step cycle for both fast- and slow-conducting PTN recordings during simple (*B*) and ladder (*C*) locomotion. *D*, *E*, average stride duty factor (the ratio of stance duration to cycle duration) for both fast- and slow-conducting PTN recordings during simple (*D*) and ladder (*E*) locomotion. In *B*–*E* error bars are SD.

Table 1. PTNs recorded in different subjects. In brackets are numbers of fast- and slow-conducting PTNs that were simultaneously recorded with the same electrode

Cat no.	Gender	Mass (kg)	Fast-conducting PTN tracks	Fast-conducting PTNs	Slow-conducting PTN tracks	Slow-conducting PTNs	F & S common tracks	PTNs in common tracks (F/S)	Total PTNs
1	Male	3.9	7	14	4	10	3	6/9	24
3	Female	3.0	7	12	5	7	3	4/4	19
4	Male	3.8	9	13	13	22	4	4/10 (2/2)	35
7	Female	2.7	6	12	6	11	3	8/5 (2/2)	23
8	Male	4.5	10	12	2	4	1	2/1	16
9	Male	3.9	13	13	7	9	4	4/4 (1/1)	22
11	Female	3.7	8	11	3	4	3	3/4 (1/1)	15
12	Male	4.0	7	8	3	3	2	3/2	11
Total 8			67	95	43	70	23	34/39	165

of the ladder were covered with an electro-conductive rubberized material. During locomotion the duration of the swing and stance phases of the right 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. 4*A* and *F*) (Beloozerova & Sirota, 1993*a*,*b*; Beloozerova *et al.* 2010). The passage of a cat through the beginning or the end of each corridor was monitored using infrared photodiodes. While walking in the chamber, cats occasionally changed direction from clockwise to counterclockwise. After each round, food was dispensed into a feeding dish in one of the corners (Skinner, 1938; Pryor, 1975). 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 walked in the experimental chamber on the flat floor and horizontal ladder for 1–2 h per day for 5–6 days a week for at least one month before recordings were made. Thereafter, experiments proceed 6 days a week for 5–10 weeks. On a particular day, experiments lasted for as long as the cat was interested in walking for food reward.

Surgical procedures

After cats were trained, surgery was performed. Anaesthesia was induced using ketamine (8 mg kg^{-1}), which was followed by 2–5% isofluorane mixed with oxygen (flow rate 0.8 l min^{-1}) administered by inhalation for the length of the surgical procedure. The skin and fascia were removed from the dorsal surface of the skull. At ten 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, cats were held rigidly by this base. The base was also used to fixate connectors, a miniature micro-drive, a pre-amplifier, contacts for stimulating electrodes, and a protective cap. A portion of the skull and dura above the left motor cortex (approximately 0.6 cm^2) were removed. The approximate area of the motor cortex was identified by surface features and photographed (Fig. 3*A*–*H*). The aperture was then covered by a 1 mm thick acrylic plate. The plate was pre-perforated with holes of 0.36 mm in diameter spaced 0.5 mm, and holes were filled with bone wax. The plate was fastened to the surrounding bone by orthodontic resin (Densply Caulk). 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 a depth of H0. They were later used for physiologically guided insertion of stimulating electrodes into the pyramidal tract (Prilutsky *et al.* 2005). These electrodes were used for identification of PTNs in the awake animal. Immediately after the surgery and then 12 h thereafter an analgesic buprenorphine was administered intramuscularly.

Cell recording and identification

Experiments were initiated after 7–10 days of recovery when cats resumed normal preoperative behaviour. The animal was positioned on a comforting pad and encouraged to take a 'sphinx' position. After the cat rested in this posture for several minutes, the base attached to the skull during surgery was fastened to an external frame so that the resting position of the head was approximated. Over 3–5 days, a number of sessions of increasing duration (5–30 min) were used to accustom the cat to the head restrainer. Cats quickly learned to sit quietly with their head restrained. They did not seem to be disturbed by the restraint, as they frequently fell asleep.

Extracellular recordings were obtained using conventional tungsten varnish-insulated microelectrodes

(120 μm o.d., Frederick Haer & Co; Bowdoin, ME, USA) or platinum–tungsten quartz insulated microelectrodes (40 μm o.d.) pulled to a fine tip and mechanically sharpened using a diamond grinding wheel (Reitboeck, 1983). The impedance of both types of electrodes was 1–3 MΩ at 1000 Hz. A custom made light-weight (2.5 g) manual single-axis micromanipulator chronically mounted to animal's skull was used to advance the microelectrode. Signals from the microelectrode were pre-amplified with a miniature custom made pre-amplifier positioned on the cat's head, and then further amplified with CyberAmp 380 (Axon Instruments). After amplification, signals were filtered (0.3–10 kHz band pass), digitized with a sampling frequency of 30 kHz, displayed on a screen, fed to an audio monitor, and recorded to the hard disk of a computer by means of a data acquisition hard- and software package (Power-1401/Spike-2 System, Cambridge Electronic Design, Cambridge, UK). Example recordings from pyramidal tract neurons during locomotion are shown in Fig. 4*A* and *F*.

A detailed description of the area of recording has been given previously (Beloozerova *et al.* 2005). In brief, the area immediately adjacent to and inside the lateral half of the cruciate sulcus in the cat is considered to be the motor cortex. This is based on data obtained by means of inactivation, stimulation, and recording techniques (Nieoullon & Rispal-Padel, 1976; Vicario *et al.* 1983; Armstrong & Drew, 1985; Beloozerova & Sirota, 1993*a*; Drew, 1993; Martin & Ghez, 1993), as well as on histological considerations (Hassler & Muhs-Clement, 1964; Ghosh, 1997*a*; Myasnikov *et al.* 1997). A parasagittal section through the frontal cortex with a reference electrolytic lesion next to giant pyramidal cells in cortical layer V, which are characteristic of motor cortex area 4γ, is shown in Fig. 3*I* and *J*. Selection of neurons for this study was as follows. All successfully recorded slow-conducting PTNs were taken. The main criterion for selection of fast-conducting PTNs was their location. First, preference was given to cells recorded from the same microelectrode tracks as slow-conducting PTNs, and they compose 1/3 of fast-conducting PTNs. Additional PTNs were selected from tracks that, when combined from all cats, would cover approximately same area of the cortex as tracks with slow-conducting PTNs (Fig. 3*A*–*H*).

Determination of axonal conduction velocity

All encountered neurons were tested for antidromic activation using pulses of graded intensity (0.2 ms duration, up to 0.5 mA) delivered through a bipolar stimulating electrode in the medullary pyramidal tract. The stimulating electrode consisted of two platinum–iridium wires 200 μm in outer diameter, insulated with Teflon to within 0.4 mm of the tip. Animals gave no sign of discomfort or of noticing the stimulation, suggesting that current did not spread to afferent pathways. The criterion for identification of antidromic responses was the test for collision of spikes (Bishop *et al.* 1962; Fuller & Schlag, 1976), illustrated in Fig. 2*A* and *B*. The distance between electrodes in the medullary pyramidal tract and at recording sites in the pre-cruciate cortex was estimated at 51.5 mm, which includes the curvature of the pathway, as well as the spread of current and the refractory period at the site of stimulation. Neurons were classified as fast- or slow-conducting based on the criteria of Takahashi (1965): neurons with a conduction velocity of 21 m s^{-1} or higher were considered to be fast conducting, while those with conduction velocities below this were considered to be slow conducting. A bimodal distribution of PTN conduction velocities had been documented in a number

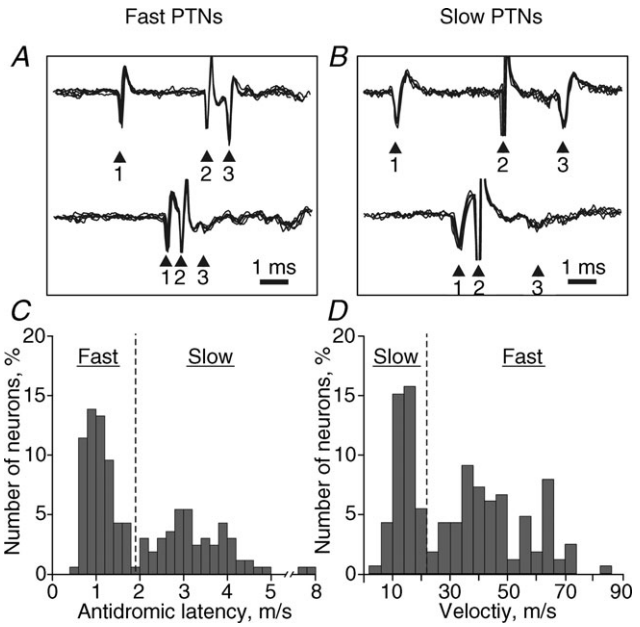

Figure 2. Identification of PTNs
A, *B*, a collision test determines whether a neuron's response is antidromic for fast- (*A*) and slow-conducting (*B*) PTNs. *A*, top trace, the PTN spontaneously discharges (arrowhead 1), and the pyramidal tract is stimulated 3 ms later (arrowhead 2). The PTN responds with latency of 1 ms (arrowhead 3). *A*, bottom trace, the PTN spontaneously discharges (arrowhead 1) and the pyramidal tract is stimulated 0.7 ms later (arrowhead 2). 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. *B*, results are analogous for the slow-conducting PTN, with a latency of 2.5 ms. *C*, distribution of latencies of PTN responses to stimulation of the pyramidal tract. The dashed line denotes the division between fast- and slow-conducting PTNs. *D*, axonal conduction velocities of PTNs. The dashed line denotes the division (21 m s^{-1}) between fast- and slow-conducting PTNs.

of previous studies (e.g. Towe *et al.* 1963; Takahashi 1965; Calvin & Sypert 1976; Humphrey & Corrie, 1978; Armstrong & Drew 1984*a*; Vigneswaran *et al.* 2011; see Fig. 2*C*, *D*). Neurons were checked for antidromic activation before, during, and after testing during locomotion. In addition, waveform analysis was employed to identify and isolate the spikes of a single neuron using the Power-1401/Spike-2 system waveform-matching algorithm. Only the neurons with a stable response latency and spike shape that consistently satisfied the collision test were used for analysis.

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. It was previously shown that the strides in the middle of the corridor are normally made at a nearly constant speed with no acceleration or deceleration, and that their average length during flat surface and ladder locomotion is identical (Beloozerova *et al.* 2010). For the comparison of discharges of individual neurons between two locomotion tasks we selected strides from different tasks of as similar duration as possible. The onset of swing phase was taken as the beginning of 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 (Fig. 4*C*, *E*, *H*, *J*). The discharge frequency in a bin was derived according to the method of Udo *et al.* (1982), which averages the instantaneous frequency of inter-spike intervals that fall within the bin and also accounts for those intervals that overlap with bin's beginning and end. The phase histogram was smoothed by recalculating the value of each bin as follows:

$$F_n' = 0.25F_{n-1} + 0.5F_n + 0.25F_{n+1},$$

where F_n is the original value of a bin. The first bin was considered to follow the last one; the last bin was considered to precede the first one. The coefficient of stride-related frequency modulation, M, was calculated using the histogram. It was defined as $M = (1 - F_{min}/F_{max})100\%$, where F_{min} and F_{max} are the minimal and the maximal frequencies of discharge in the histogram. In addition, the 'depth' of modulation, dM, characterizing fluctuation in probability of the discharge, was calculated as $dM = (N_{max} - N_{min})/N100\%$, where N_{max} and N_{min} are the number of spikes in the maximal and minimal histogram bins, and N is the total number of spikes in the histogram. The two measures for the modulation, M and dM, enabled characterization of fluctuation of PTN discharge rate both in terms of variation in frequency (M) and probability (dM) of discharge. Neurons with $dM > 4\%$ and $M > 50\%$ were judged to be stride related. This was based on an analysis of

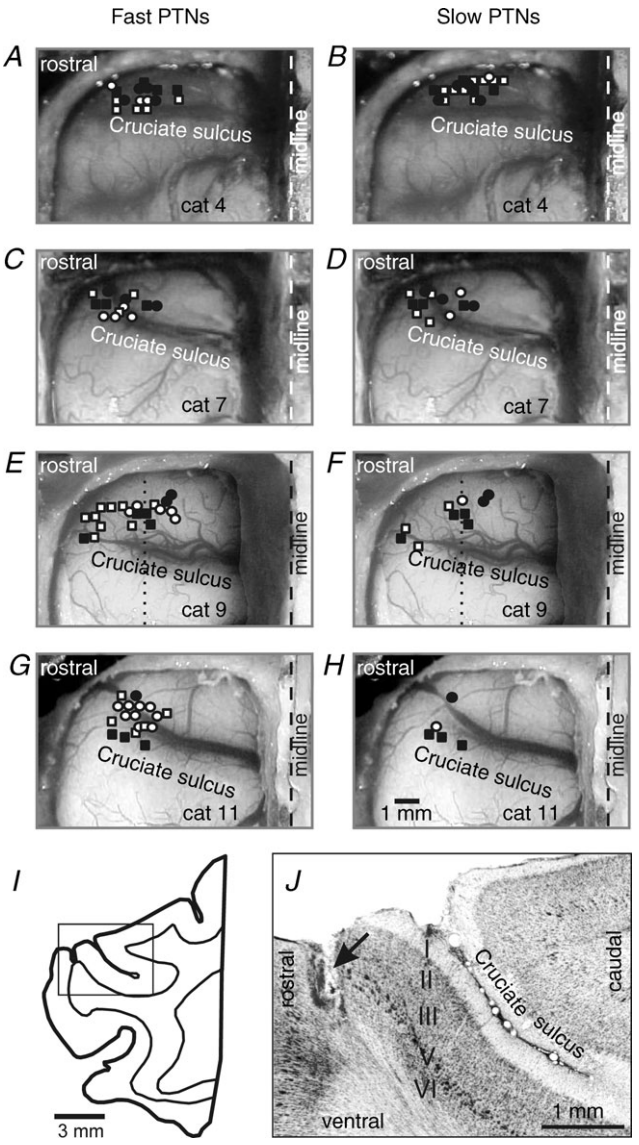

Figure 3. Location of PTNs
A–H, areas of recording in the forelimb representation of the left motor cortex. Microelectrode entry points into the cortex were combined from pairs of cats whose cortices were most similar and are shown as shapes on photographs of one of the cat's cortex for fast- (*A*, *C*, *E*, *G*) and slow-conducting (*B*, *D*, *F*, *H*) PTNs. Tracks were both fast- and slow-conducting PTNs were recorded are shown with filled shapes. *A*, *B*, a photograph of cat 4 cortex; microelectrode entry points into this cat cortex are indicated by squares and approximate positions of tracks in an additional cat, cat 1, are shown by circles. *C–H*, analogous presentation of data for cats 7 and 3 (*C*, *D*), cats 9 and 12 (*E*, *F*), and cats 8 and 11 (*G*, *H*). In *E* and *F*, the position of the parasagittal section shown in *I* and *J* is indicated by a dotted line. *I*, drawing of a parasagittal section through the frontal cortex. Thin line shows border between the grey and white matter. The square approximately indicates the area shown in the photomicrograph in *J*. *J*, photomicrograph of a parasagittal section through the motor cortex, stained with Cresyl Violet. Layers of the cortex are numbered. Giant cells in layer V that are characteristic for area 4γ are visible throughout the pre-cruciate cortex. The arrow points to a reference electrolytic lesion.

fluctuation in the activity of neurons in the resting animal. For this analysis, the activities of 100 neurons recorded while the cat was sitting in the head-restraining device were processed as if the cat was walking (Marlinski *et al.* 2012). The timing of steps made by the same cat during the preceding walking test was used to construct the histogram. This analysis showed that at rest, the values of *dM* exceeded 4% in only five cells. Therefore, when the *dM* of activity of a neuron was greater than 4% during locomotion, we could conclude with 95% confidence that it was due to stride-related modulation. In stride-related neurons, 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' (PEF; illustrated in Fig. 4*C*, *E*, *H*, *J*). The 'preferred phase' of discharge of each neuron with a single PEF was assessed using circular statistics (Batshelet, 1981; Drew & Doucet, 1991; Fisher, 1993; see also Beloozerova *et al.* 2003; Sirota *et al.* 2005), while neurons exhibiting two or more PEFs were excluded from this analysis. The coefficient of variability of discharge rate, CV, was defined as $CV = \sigma^2/m$, where σ is standard deviation and m is mean firing rate. The activity during standing was assessed by averaging discharges during all 1 s periods occurring a second after the right forelimb (contralateral to the recorded cortex) was placed on ground when cat stopped for food reward at the end of each walking round.

For comparisons of the discharge rate, depth of modulation, preferred phase, and duration of PEF of individual neurons between the two tasks differences equal or greater than ±20%, ±20%, ±10%, and ±20%, respectively, were considered significant. These criteria were established based on the results of a bootstrapping analysis (Efron & Tibshirani, 1993), which compared differences in discharges between various reshufflings of strides of the same locomotion task and found that natural PTN activity fluctuations remain within these limits with 95% confidence (Stout & Beloozerova, 2012). Thus, when these limits were exceeded, we assumed that it was the difference between locomotion tasks that caused it. Parameters of activity of groups of neurons were compared using Student's unpaired *t* test. When data were categorical, a nonparametric χ^2 test was used. For all the tests, the significance level was set at $P = 0.05$. Unless noted otherwise, for all mean values, the standard error of the mean (SEM) is given.

Histological procedures

At the termination of experiments, cats were deeply anaesthetized 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% paraformaldehyde 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. Zoning of the cortex was performed according to criteria established by Hassler & Muhs-Clement (1964). The positions of recording tracks in the cortex were estimated in relation to the reference lesions (Fig. 3*I*, *J*). The position of stimulation electrodes in the medullar pyramids was verified.

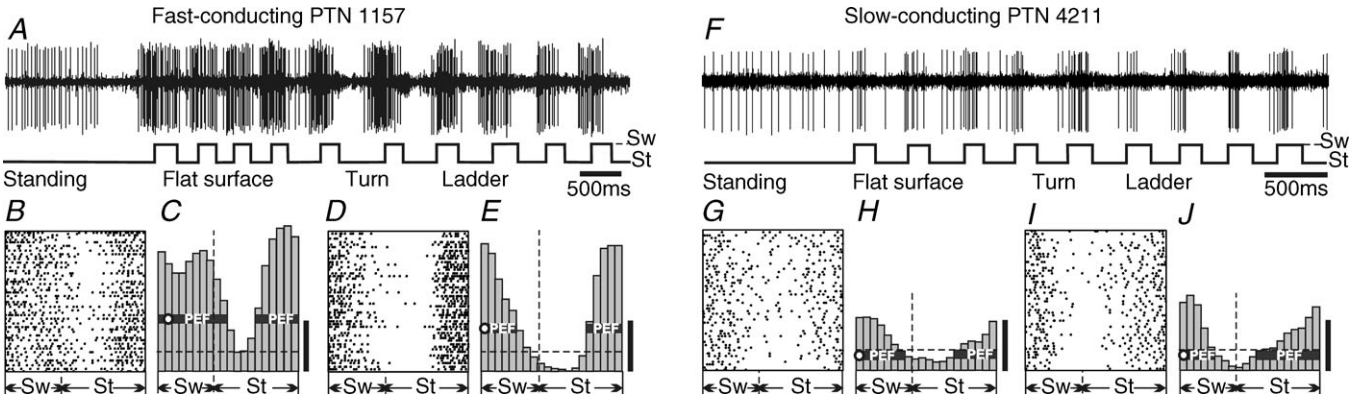

Figure 4. Example activity of fast- and slow-conducting PTNs
A, *F*, activity of individual fast- (*A*) and slow-conducting (*F*) PTNs during standing, simple, and ladder locomotion. The bottom trace shows the swing (Sw) and stance (St) phases of the step cycle of the right forelimb that is contralateral to the recording site in the cortex. *B*, *C*, *G*, *H*, activities of the same neurons during simple locomotion are presented as rasters of 50 step cycles (*B*, *G*) and as histograms (*C*, *H*). The duration of step cycles is normalized to 100%. In the histogram, the horizontal 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 as defined in the Methods section. *D*, *E*, *I*, *J*, activities of the same neurons during ladder locomotion are presented as rasters (*D*, *I*) and as histograms (*E*, *J*). In *C*, *H* and *E*, *J* the vertical scale bar equals 20 imp s^{-1}.

Table 2. Selected parameters of locomotion-related activity of fast- and slow-conducting PTN populations

	Parameters of PTN activity	Fast-conducting, all $n = 95$	Fast-conducting, in same tracks with slow PTNs $n = 33$	Slow-conducting, all $n = 70$	Slow-conducting, in same tracks with fast PTNs $n = 39$
	Proportion of cells with receptive fields (%)	**87**	86	**68**	71
Standing	Proportion of active cells (%)	**100**	100	**94**	98
	Average activity (spikes s^{-1})	**16.0 ± 1.0**	17.6 ± 1.9	**9.4 ± 0.8**	8.9 ± 0.9
	Discharge variability, CV	**2.2 ± 0.36**	2.4 ± 0.6	**1.08 ± 0.11**	1.0 ± 0.1
Simple locomotion	Average activity (spikes s^{-1})	**16.6 ± 1.1**	17.1 ± 1.6	**11.4 ± 0.9**	9.9 ± 0.8
	Discharge variability, CV	**1.85 ± 0.12**	1.8 ± 0.2	**1.79 ± 0.13**†	1.7 ± 0.1†
	Proportion modulated (%)	**98**	97	**96**	97
	Proportion with 0 sp s^{-1} in any bin (%)	**84**	5.4	**17.1**	22
	Mean peak rate (spikes s^{-1})	**35.2 ± 2.1**	37.4 ± 3.7	**23.0 ± 1.8**	21.5 ± 2.1
	Depth of modulation, *dM* (%)	**10.6 ± 0.5**	11.1 ± 0.9	**9.6 ± 0.5**	9.8 ± 0.6
	Coefficient of modulation, *M* (%)	**87.3 ± 1.4**	89.2 ± 2.2	**86.1 ± 1.8**	86.7 ± 2.2
	Duration of PEF (% of cycle)	**56.5 ± 2.0**	56.5 ± 2.5	**60.5 ± 14**	60.5 ± 2.0
	Proportion with single PEF (%)	**76**	84	**82**	83
Ladder locomotion	Average activity (spikes s^{-1})	**18.1 ± 1.2**	20.3 ± 2.0	**13.5 ± 1.2**∗	11.4 ± 1.2∗
	Discharge variability, *CV*	**1.72 ± 0.08**	1.6 ± 0.1	**1.49 ± 0.09**∗	1.6 ± 0.1∗
	Proportion modulated (%)	**100**	100	**96**	97
	Proportion with 0 sp s^{-1} in any bin (%)	**14.7**	16	**18**	21
	Mean peak rate (spikes s^{-1})	**41 ± 2.7**	47.2 ± 4.5	**29.1 ± 2.6**	25.8 ± 2.7
	Depth of modulation, *dM* (%)	**11.0 ± 0.4**	12.9 ± 0.6	**11.2 ± 0.5**∗	11.1 ± 0.6∗
	Coefficient of modulation, *M* (%)	**91.0 ± 1.1**∗	92.6 ± 1.6∗	**91.7 ± 1.2**∗	91.4 ± 1.5∗
	Duration of PEF (% of cycle)	**56.5 ± 2.5**	56.5 ± 2.5	**60.5 ± 2.0**	60.5 ± 2.5
	Proportion with single PEF (%)	**77**	81	**90**	93

Underlined values are statistically significantly different between fast- and slow-conducting PTNs according to Student's unpaired *t* test for averages (mean ± SEM) or according to the χ^2 test for proportions. Comparisons are made separately between entire fast- and slow-conducting populations and between fast- and slow-conducting groups of neurons recorded in the same microelectrode tracks. † values that are statistically significantly different between standing and simple locomotion, ∗ values that are statistically significantly different between simple and ladder locomotion.

Results

Characteristics of locomotion tasks

During the recording of each individual PTN cats walked between 10 and 100 (typically 20–40) times down each of the chamber's corridors. From these runs, 25–150 strides (70 ± 30) taken in the middle of each corridor (during walking on the flat surface or along the horizontal ladder) were selected for analysis. Walking speeds varied during each of the locomotion tasks between 0.6 and 1.2 m s^{-1}. Previous studies showed that only a minority of neurons in the motor cortex respond to changes in the velocity of walking (Armstrong & Drew, 1984*a*; Beloozerova & Sirota, 1993*b*). Nevertheless, for the comparison of discharges of individual neurons between two locomotion tasks in this study we selected strides from different tasks of as similar duration as possible. For 80% of neurons we were able to select 25 or more strides, for which the average duration of the strides in the two tasks differed by less than 10%. And for both fast- and slow-conducting PTN populations, the average duration of the chosen strides was similar during simple and ladder locomotion (Fig. 1*B*, *C*), as was the ratio of the stance duration to the cycle duration, the stride duty factor (Fig. 1*D*, *E*).

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 recently been reported elsewhere (Beloozerova *et al.* 2010). Stepping on the ladder is associated with dramatically greater precision in foot placement compared to walking on the flat surface, while the overwhelming majority of other forelimb-related biomechanical parameters, with the exception of slightly more bent-forward posture and lower wrist flexion moment during stance, are similar between the tasks. Therefore, in the current study, selection of steps of similar durations and duty factors for the two locomotion tasks enabled us to ascribe most between-task differences in neuronal activities to the main distinction between the tasks: the low variability of step lengths and high accuracy during the ladder task, *versus* high variability of step lengths and low accuracy during the flat walking task.

Characteristics of neurons

The activity of 165 PTNs was included in the analysis (Table 1). Of these PTNs, 95 were fast-conducting (21–80 m s^{-1}), and 70 were slow-conducting (5–20 m s^{-1}; Fig. 2*C*, *D*). Cells were collected from a total of 87 microelectrode tracks, with an average 10 ± 4 (mean $\pm$ SD) tracks used per cat (Fig. 3*A*–*H*). In Fig. 3*A*–*H*, shapes overlaying the cortex schematically show microelectrode entry points into the cortex for tracks, in which PTNs of different groups were recorded during locomotion. Filled shapes indicate the 23 tracks where both fast- ($n = 33$) and slow-conducting ($n = 39$) PTNs were recorded, typically 1–2 of each type per track (Table 1). This included five pairs of fast- and slow-conducting PTNs recorded simultaneously by the same electrode. Histological inspection confirmed that all neurons were collected from the motor cortex area 4γ. A drawing of a parasagittal section through the frontal cortex, whose approximate position is indicated by a dotted line in Fig. 3*E* and *F*, is given in Fig. 3*I*. Figure 3*J* shows a photomicrograph of a portion of the cortex that is outlined by a square in *I*. Numerous giant pyramidal neurons characteristic of area 4γ can be seen in layer V throughout the pre-cruciate cortex.

Responses of 83 fast- and 53 slow-conducting PTNs to somatosensory stimulation were tested. A somatosensory receptive field was found in 87% (72/83) of fast-conducting PTNs, but in only 68% (36/53) of slow-conducting PTNs, a significantly lower proportion (χ^2 test, $P = 0.037$). In both PTN groups, all receptive fields were located on the contralateral (right) side of the body and all but two were excitatory. Both PTNs responding with inhibition were slow conducting. Among slow-conducting PTNs, approximately equal numbers of neurons had receptive fields on the shoulder, elbow, and wrist/paw. In the fast-conducting group, however, there were more neurons that responded to movements of the shoulder than to either elbow or wrist/paw (χ^2 test, $P < 0.03$). This bias is due to the fact that slow-conducting PTNs were often found in the medial regions of the motor cortex, and many fast-conducting PTNs in the same tracks were also recorded (Fig. 3); these regions are more likely to contain neurons with proximal receptive fields. In both fast- and slow-conducting populations, several neurons were activated by a movement in both shoulder and elbow. Neurons activated by a joint movement often had a preferred direction. Fast-conducting PTNs with receptive fields on the shoulder were more often excited by the shoulder extension or abduction than by flexion or adduction (χ^2 test, $P = 0.018$). At the same time, elbow- and wrist/paw-related fast-conducting PTNs, as well as any slow-conducting neurons, were as likely to respond to flexion as to extension.

Example activities of individual fast- and slow-conducting PTNs during standing, simple and ladder locomotion are shown in Fig. 4. Both PTNs were steadily active during standing. When locomotion began, they both were highly active during the second half of stance and during swing. Rasters in Fig. 4*B*, *D*, *G* and *I* show that the activity of both neurons were very consistent across 50 strides of simple (*B*, *G*) and ladder (*D*, *I*) locomotion. Activities were summed in Fig. 4*C*, *E*, *H* and *J*, showing histograms of PTN firing rates across the step cycle during simple (*C*, *H*) and ladder (*E*, *J*) locomotion. PEFs are indicated by black horizontal bars, and preferred phases of the activity are depicted with circles. During ladder locomotion, the discharge of the fast-conducting neuron during the second half of swing was lower than during simple locomotion, while the discharge of the slow conducting neuron not only was lower during the transition from swing to stance, but also was higher during the first half of swing. Thus, the magnitude of frequency modulation for both PTNs was larger during ladder locomotion, but to a greater extent for slow-conducting PTNs.

Selected parameters of locomotion-related activity of fast- and slow-conducting PTN populations are given in Table 2.

Activity during standing and simple locomotion

During standing, all fast-conducting and 66 of 70 slow-conducting PTNs were active. Fast-conducting neurons discharged at 16 spikes s^{-1} and their discharge variability, CV, was 2.2; slow-conducting PTNs were less active but more consistent in their discharge (t test, $P < 0.05$; Table 2).

With the start of simple locomotion, the discharge rate of most neurons changed in both the fast- (82%, 78/95) and slow-conducting (79%, 55/70) populations. Among fast-conducting PTNs, 45% of cells increased it by 15–400% and 37% decreased by 10–80%. Changes in slow-conducting PTNs were similar, and overall, the discharge rates of both populations remained similar to those during standing (t test, $P > 0.05$; Table 2). The CV in the slow-conducting population, however, became much higher during walking while in the fast-conducting group it did not change (t test, $P < 0.05$; Table 2).

During simple locomotion, the discharges of 93 of 95 fast-conducting PTNs and 67 of 70 slow-conducting PTNs were modulated with respect to the stride: they were higher in one phase of the stride and lower in another phase. The great majority of both fast- and slow-conducting neurons exhibited a single PEF (Table 2), while the rest had two PEFs.

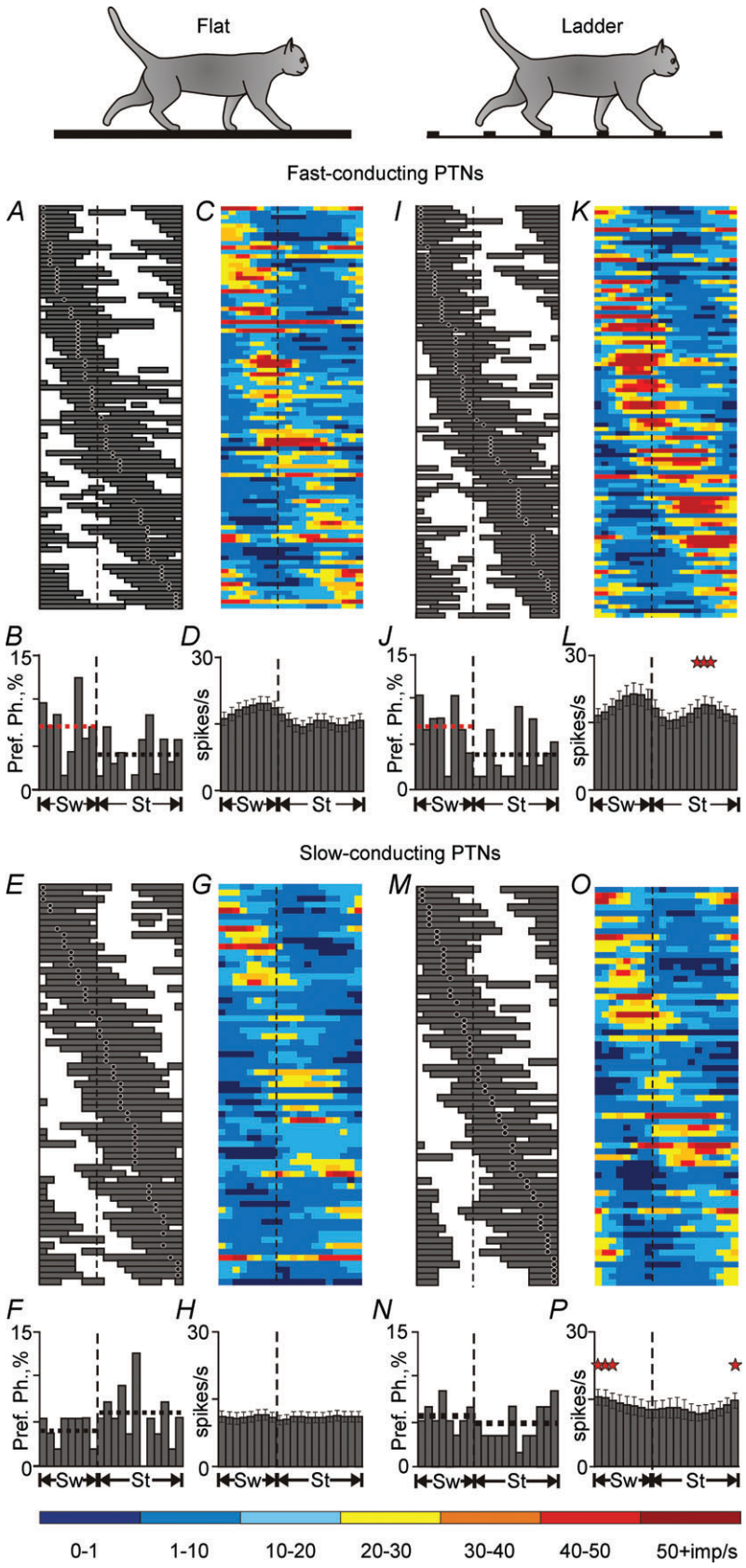

Figure 5. Phase distribution of PEFs, preferred phases, and discharge rates of fast- and slow-conducting PTNs during locomotion
A, *E*, *I*, *M*, distribution of PEFs of individual fast- (*A*, *I*) and slow-conducting (*E*, *M*) PTNs in the step cycle of simple (*A*, *E*) and ladder (*I*, *M*) locomotion. Each trace represents the PEF of one PTN (see definition in Methods). Neurons are rank ordered so that those whose preferred phase is earlier in the cycle are plotted on the top of the graph. Circles indicate preferred phase of neurons with one PEF. *C*, *G*, *K*, *O*, corresponding phase distribution of discharge frequencies. The average discharge frequency in each 1/20th portion of the cycle is colour-coded according to the scale shown at the bottom of the figure. *B*, *F*, *J*, *N*, distribution of preferred phases of fast- (*B*, *J*) and slow-conducting (*F*, *N*) PTNs across the step cycle during simple (*B*, *F*) and ladder (*J*, *N*) locomotion. Horizontal red and black dashed lines show the mean percentages of neurons with preferred phases during swing and stance, respectively. Red indicates that the percentage was statistically significantly higher than expected by chance (χ^2 test, $P < 0.05$). *D*, *H*, *L*, *P*, phase histogram of the average firing rate of PTNs across the step cycle during simple (*D*, *H*) and ladder (*L*, *P*) locomotion. Red stars in *L* and *P* indicate portions of the cycle when the activity during ladder locomotion was statistically significantly higher then during simple locomotion (Student's *t* test, $P < 0.05$). Sw, swing phase; St, stance phase.

Rasters of the PEFs of all fast-conducting PTNs, as well as the preferred phases of those with one PEF are shown in Fig. 5*A* and *B*. The PEFs were distributed throughout the step cycle. Their duration varied between 20 and 85% of the cycle (Table 2). Preferred phases of 55% (41/71) of neurons with a single PEF occurred during swing, which was significantly more than the 40% that would be expected by chance (χ^2 test, $P < 0.05$; Fig. 5*B*). About 10% of cells were completely silent for a part of the step cycle; the majority, however, were active throughout the cycle, while their discharge rate was modulated (Fig. 5*C*). The average coefficient of modulation, *M*, was 87%, and *dM* was 10.6%. The mean peak discharge rate averaged over one histogram bin (1/20th of the cycle) was 35 spikes s^{-1}. There was a subtle peak in population activity during the swing phase (Fig. 5*D*).

Rasters of the PEFs of all slow-conducting PTNs and the preferred phases of those with one PEF are shown in Fig. 5*E* and *F*. Similarly to the fast-conducting group, the PEFs of slow-conducting PTNs were distributed throughout the step cycle and varied in duration from 30 to 85% of the cycle (Table 2). However, the activity of the slow-conducting PTN population was steady throughout the stride (Fig. 5*F*, *H*). The magnitude of modulation in individual neurons varied. About 17% of cells were completely silent for a part of the step cycle; the majority, however, were active throughout the cycle, while their discharge rate was modulated (Fig. 5*G*). The average coefficients of modulation were similar to those in the fast-conducting group (t test, $P > 0.05$; Table 2). However, the peak discharge rate averaged over one histogram bin was more than 10 spikes s^{-1} less

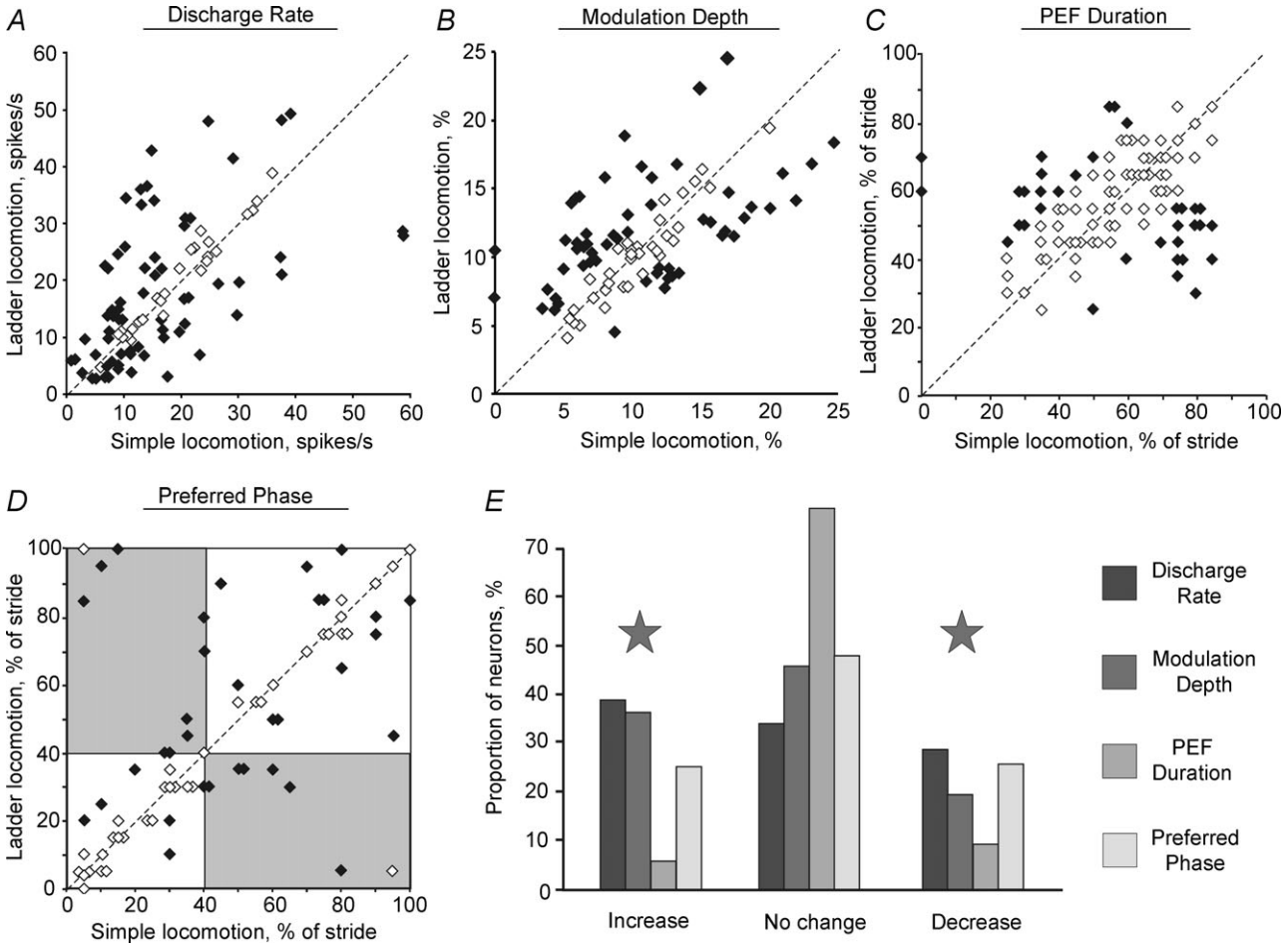

Figure 6. Comparison of activity characteristics of individual fast-conducting PTNs between simple and ladder locomotion

A–D, the abscissa and ordinate of each point show the values of a characteristic of a neuron during simple and ladder locomotion, respectively. Neurons whose characteristics were statistically significantly different during the two tasks (according to criteria established using a bootstrapping analysis, see Methods) are shown as filled diamonds; others are shown as open diamonds. In *D*, areas that correspond to the swing phase during one task but stance phase during the other task are shaded. *E*: percentage of neurons significantly changing a parameter upon transition from simple to ladder locomotion. Stars indicate that significantly more neurons increased than decreased the depth of modulation (χ^2 test, $P < 0.05$).

than in the fast-conducting population (t test, $P < 0.05$; Table 2).

Subpopulations of fast- and slow-conducting PTNs recorded in the same track, simultaneously or sequentially, were similar to the larger populations in all parameters tested (Table 2).

Activity during ladder locomotion

Locomotion over the ladder required accuracy during stepping on crosspieces. However, by design of the task, the length and duration of strides were kept similar to those during simple locomotion. During walking along the ladder the activity of all fast-conducting PTNs and nearly all slow-conducting PTNs were modulated in the rhythm of strides. Similar to simple locomotion, 73 of 95 fast- and 60 of 67 slow-conducting neurons had one PEF, while the rest had two PEFs. However, during ladder locomotion slow-conducting PTNs had a significantly smaller proportion of two-PEF cells than fast-conducting PTNs (χ^2 test, $P < 0.05$; Table 2).

Upon transition from simple to ladder locomotion 90 of 95 fast-conducting and 66 of 70 slow-conducting PTNs experienced significant changes to their activity characteristics (Figs 6*E*, 7*E*). To facilitate comparison between the characteristics of individual neurons during two tasks, we used scatter diagrams. In Figs 6*A* and 7*A* the mean discharge rate of individual neurons during ladder locomotion is plotted against that during simple walking for fast- and slow-conducting PTNs, respectively. The great majority of both fast- and slow-conducting PTNs changed discharge rate upon transition from simple to ladder locomotion: in 39% and 40%, respectively, the rate increased twofold on average, while in 33% and 27%, respectively, it decreased, on average by one-half. As a result, the average activity of the slow-conducting PTN population rose to 13.5 spikes s^{-1}, and was now greater than during both standing and simple locomotion

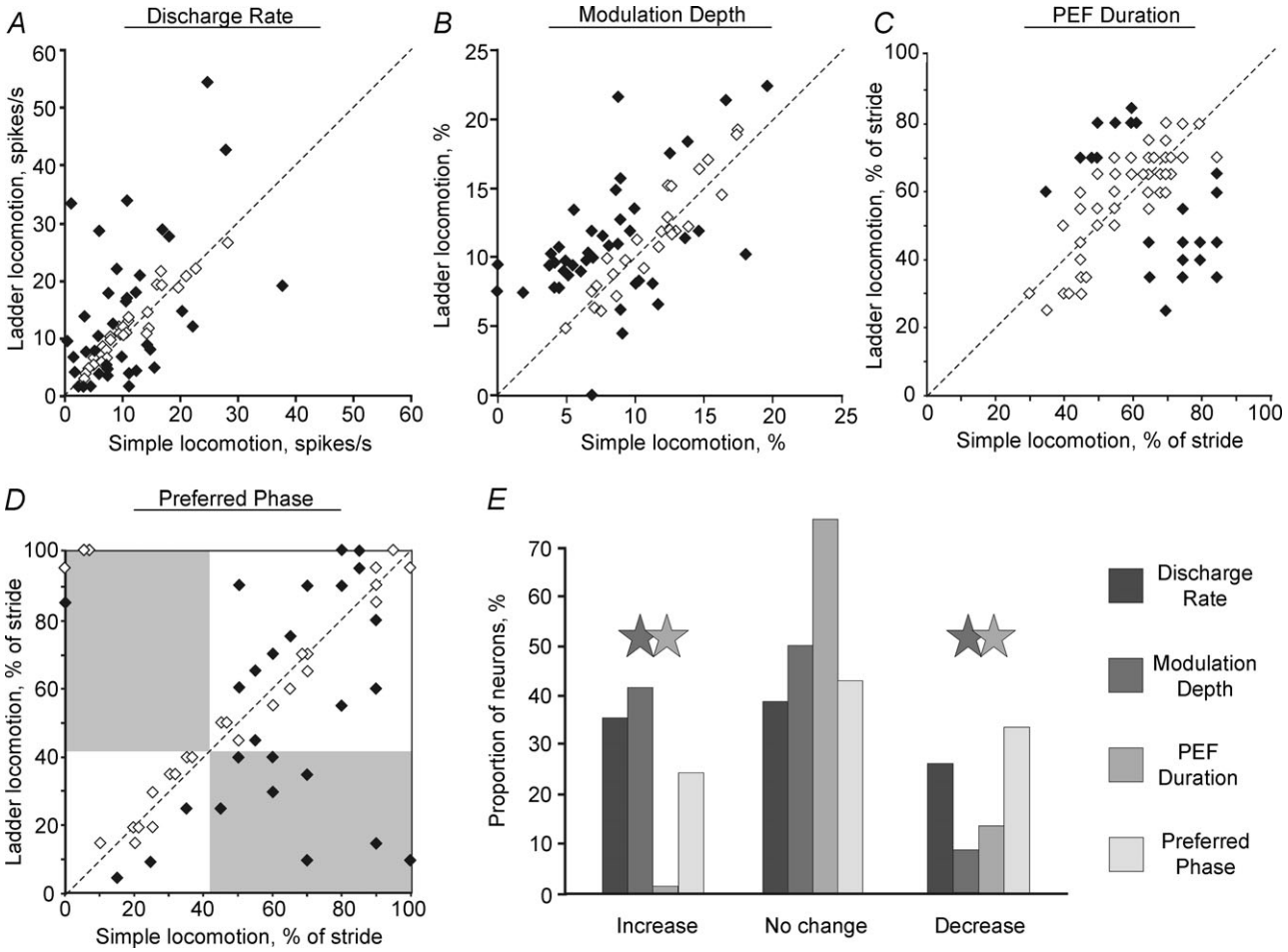

Figure 7. Comparison of activity characteristics of individual slow-conducting PTN between simple and ladder locomotion (organized in the same fashion as in Fig. 6)
In *E*, darker stars indicate that significantly more neurons increased than decreased the depth of modulation, and lighter stars indicate that significantly more neurons decreased than increased the duration of PEF (χ^2 test, $P < 0.05$).

(t test, $P < 0.05$; Table 2), and highest during the beginning of stance and end of swing (Fig. 5*P*). In addition, the discharge variability of slow-conducting PTNs during ladder locomotion diminished compared to simple walking (t test, $P < 0.05$; Table 2). For the fast-conducting population, neither the mean discharge rate nor the discharge variability changed (t test, $P > 0.05$; Table 2).

Upon transition from simple to ladder locomotion, the magnitude of stride-related modulation in the majority of PTNs, both fast- and slow-conducting, changed, and in both populations, it increased rather than decreased in significantly more neurons: 36% *vs.* 23% in the fast-conducting and 48% *vs.* 15%, in the slow-conducting population (χ^2 test, $P < 0.05$; Figs 6*B* and 7*B*). The disparity, however, was greater in the slow-conducting group. This resulted in an increase in the average depth of modulation, *dM*, in the slow-conducting population, while the *dM* of the fast-conducting PTN population did not increase (Table 2). In contrast, changes to the depth of modulation in fast-conducting PTNs tended towards a set point: neurons with a lower depth of modulation during simple locomotion were more likely to raise it on the ladder, while neurons with higher depth of modulation were more likely to lower it (Fig. 6*B*); this effect was not observed in the slow-conducting population (Fig. 7*B*). This led to a narrower distribution of modulation depths during ladder walking compared to simple locomotion (Fig. 6*B*). The frequency-based coefficient of modulation, *M*, for the fast-conducting population was, however, higher during ladder walking than during simple locomotion, as it was for the slow-conducting PTNs (t test, $P < 0.05$; Table 2).

Increases to the depth of modulation in both fast- and slow-conducting PTNs most often occurred either by a purely 'subtractive' mechanism, when the activity of the neurons outside of the PEF further decreased (in 17 of 35 fast-conducting PTNs with increasing modulation and in 13 of 32 such slow-conducting PTNs; Figs 4*A*–*E* and 8*A*) or by a purely 'additive' mechanism, when the activity within the PEF further increased (in 9 of 35 and 8 of 32 fast- and slow-conducting PTNs, respectively, Fig. 8*B*). Decreases in depth of modulation also most often occurred by either a purely subtractive mechanism when the activity within the PEF decreased (in 9 of 22 fast- and 2 of 10 slow-conducting PTNs with decreasing modulation; Fig. 8*C*) or a purely additive mechanism when the activity outside of the PEF became more intense (in 10 of 22 and 3 of 10 fast- and slow-conducting PTNs, respectively; Fig. 8*D*).

One-third of PTNs in both populations changed the duration of their PEF upon transition from simple to ladder locomotion: increasing or decreasing it generally by 20–50% of the cycle (Figs 6*C* and 7*C*). The duration of the PEF tended to a set point in both populations: neurons with a longer PEF often decreased the PEF duration, while neurons with a shorter PEF tended to increase it. As a result, the range of PEF durations during walking on the ladder was smaller than during simple locomotion.

Upon transition from simple to ladder locomotion, many neurons changed their preferred phase. That change could occur either because of a phase shift of the same discharge pattern, or because of re-formation of the pattern, such that the neuron had a one-PEF pattern during one locomotion task and a two-PEF pattern during another task. Nearly half of PTNs from both populations that had one PEF during both locomotion tasks (35/71 and 26/54, respectively) changed their preferred phase between tasks (Figs 6*D* and 7*D*). The preferred phases of the majority of them remained in the same phase of the stride (swing or stance), however, and in most neurons the change was small, constituting only 10% of the stride. Fast-conducting neurons did not have any predilection as to where to shift their preferred phase upon transfer from simple to ladder locomotion, while slow-conducting

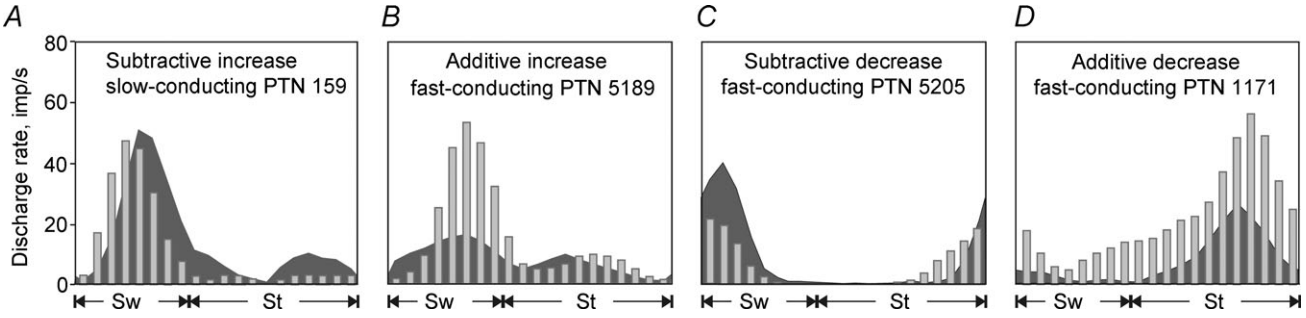

Figure 8. Typical changes in the depth of modulation upon transition from simple to ladder locomotion
Area histograms show the activity of a typical PTN during simple locomotion. The histograms show activity of the same PTN during ladder locomotion. *A*, increase in the depth of modulation by a subtractive mechanism: the activity of the neuron outside of the PEF further decreases. *B*, increase in the depth of modulation by an additive mechanism: the activity within the PEF further increased. *C*, decrease in the depth of modulation by a subtractive mechanism: the activity within the PEF decreased. *D*, decrease in the depth of modulation by an additive mechanism: the activity outside of the PEF increases.

Table 3. Fast- and slow-conducting PTNs with different numbers of PEFs during simple and ladder locomotion

N of PFEs on flat surface / *N* of PFEs on ladder	Fast-conducting PTNs			Slow-conducting PTNs		
	0	1	2	0	1	2
0		0	0		1	0
1	1		11	1		9
2	1	10		0	5	

PTNs had a tendency to shift the preferred phase from the stance to the swing phase (Fig. 7*D*, compare the lower highlighted area on the right with the upper one on the left).

Twenty-three fast-conducting and sixteen slow-conducting PTNs changed the number of PEFs on transition from simple to ladder locomotion (Table 3). In neurons with two PEFs during simple locomotion and one PEF during the ladder task, the pattern change typically occurred because of an increase in the activity during one of the inter-PEF intervals, joining the previously distinct PEFs. In neurons that discharged one PEF during simple locomotion and two PEFs during walking on the ladder the change occurred either because a new PEF emerged within a period of relative silence during simple locomotion or because the pre-existing subtle sub-peaks intensified into two full PEFs.

Fast- and slow-conducting PTNs recorded from the same track, simultaneously or sequentially, exhibited the same activity characteristics as the larger populations (Table 2). Upon transition from simple to ladder locomotion, fast and slow PTNs recorded in the same track were more likely to exhibit the same changes to discharge rate than would be expected based on the characteristics of the overall population (t test for proportions, $P < 0.05$), but were more likely to show *different* changes to modulation depth (t test for proportions, $P < 0.05$). For PTNs recorded simultaneously, the same changes were observed for three out of five pairs with regard to discharge rate and for two out of five pairs with regard to modulation strength.

In summary, while fast- and slow-conducting PTNs had much in common, there were several notable differences in activity. Slow-conducting PTNs were: (i) considerably less active during all tasks, but upon transfer from simple to ladder locomotion they (ii) decreased discharge variability, (iii) more profoundly increased magnitude of stride-related frequency modulation, (iv) almost always discharged only one PEF per cycle, (v) had a tendency to shift their preferred phase of activity to the swing phase, and (vi) as a population increased mean discharge rate.

Discussion

A bimodal distribution of PTN conduction velocities, revealing 'fast-' and 'slow-conducting' neurons, has been documented in many previous studies (e.g. Towe *et al.*, 1963; Takahashi, 1965; Calvin & Sypert, 1976; Humphrey & Corrie, 1978, Armstrong & Drew, 1984; Vigneswaran *et al.*, 2011). There is a good agreement that the divide between fast- and slow-conducting neurons is set at 20–25 m s^{-1}. Our current database represents fast- and slow-conducting PTN populations by similar groups of cells collected from the same or neighboring microelectrode tracks through the motor cortex (Fig. 3). The characteristics of discharges during locomotion that we found within these PTN groups are consistent with earlier reports (Armstrong & Drew 1984; Beloozerova & Sirota 1985, 1993*a*,*b*; Drew, 1993; Beloozerova *et al.* 2010; Stout & Beloozerova, 2012). Namely, the activity of nearly all PTNs was step cycle-modulated, with the great majority of neurons exhibiting one PEF per cycle, and PEFs of different neurons distributed widely across the cycle. Upon transition from walking on the flat surface to accurate stepping on the horizontal ladder, the majority of PTNs changed their activity, depth of modulation, and/or duration of the PEF.

The main finding of this study is that, upon transfer from simple locomotion to accurate stepping over a ladder, fast- and slow-conducting PTN responded differently to the accuracy demand of the ladder with slow-conducting PTNs altering their activity more vigorously, concertedly, and in more ways than fast-conducting PTNs. This suggests that slow-conducting PTNs may play a greater role than fast-conducting PTNs in managing accuracy demands during locomotion.

The activity of fast- and slow-conducting PTNs during simple locomotion has been previously compared by Armstrong & Drew (1984*a*). These authors also found that fast-conducting PTNs have higher mean and peak discharge rates than slow-conducting PTNs. Armstrong & Drew (1984*a*) reported, however, that during locomotion there was a tendency for fast-conducting PTNs to discharge discrete step-related bursts of activity separated by near silence, while slow-conducting PTNs more often fired continuously throughout the cycle, exhibiting a lesser magnitude of frequency modulation. However, our data obtained from a significantly larger population of slow-conducting PTNs ($n = 70$ *vs.* $n = 16$) show that the activity of slow-conducting PTNs is not any less modulated in relation to stride than that of fast-conducting PTNs. This result is based on two assessments of modulation magnitude, dM and M, and also on the proportion of neurons that were completely silent for any 1/20th portion of the cycle. Our failure to find any tendency for slow-conducting PTNs to discharge more 'tonically' or fast-conducting PTNs to be active more 'phasically'

during locomotion also contrasts with previously reported data on activities of these neuronal populations during isolated limb movements in primates. Specifically, in primates it was found that slow-conducting PTNs are typically active tonically at rest and respond with a sustained discharge to passive ramp-form displacements of the forearm whereas fast-conducting PTNs are usually nearly silent at rest and exhibit transient responses (Evarts, 1965; Fromm & Evarts, 1977, 1981; Tanji *et al.* 1978; Fromm *et al.* 1984). The difference between this and our locomotion data is likely to be explained by the fact that during walking the cats in our study only made comparatively large amplitude movements that effectively activated both fast- and slow-conducting PTNs. When the activities of these PTN subpopulations were compared during this mutually engaging condition, they differed only in discharge rates, and not in strength of the stride-related frequency modulation. Apart from the discharge rate, the only other difference between the activity of fast- and slow-conducting PTNs during simple locomotion is the slightly different distribution of their preferred phases, which in the fast-conducting group show a mild concentration during the swing phase, while slow-conducting PTNs as a group discharge roughly evenly throughout the stride cycle (Fig. 5*A*, *B* and *E*, *F*).

The motor cortex does not appear, however, to exert decisive control over simple locomotion, as lesions or even short reversible inactivations of it have no effect on performance of this task (Trendelenburg, 1911; Liddell & Phillips, 1944; Chambers & Liu, 1957; Beloozerova & Sirota, 1988, 1993*a*; Drew *et al.*, 1996). We have previously suggested that the stride-related frequency modulation of neuronal activity in the motor cortex during simple locomotion has an informational character, allowing motor cortical neurons, when a need arises, to integrate with and influence the spinal locomotor mechanism to correct movements in a manner that does not disturb the overall stepping rhythm (Beloozerova & Sirota, 1993*a*).

The ladder imposes accuracy constraints on the locomotion task, as cats have to step accurately on crosspieces. It has previously been demonstrated that locomotion with accurate feet placement requires the activity of the motor cortex to be successful (Trendelenburg, 1911; Liddell & Phillips, 1944; Chambers & Liu, 1957; Beloozerova & Sirota, 1988, 1993*a*; Drew *et al.* 1996; Metz & Whishaw, 2002; Friel *et al.* 2007). On the ladder, the overwhelming majority of PTNs, both fast- and slow-conducting, changed their activity compared to simple locomotion (Figs 6 and 7). The activity of slow-conducting PTNs, however, changed in more aspects and, in regard to the magnitude of modulation, more intensively than that of fast-conducting PTNs.

First, while the average activity of the fast-conducting PTN population remained unchanged upon transition from simple to ladder locomotion, despite significant changes in the discharge rates of most individual neurons, mean discharge rates of the slow-conducing PTN population rose (Table 2). The activity increase was most prominent during the late stance and early swing phase of the stride, and was partly due to a shift of preferred phases of some neurons into the swing phase (Fig. 7*D*). The increased discharge rates almost certainly made the influence of the slow-conducting PTN group on its synaptic targets more effective. Furthermore, this strengthened signal was also more consistent, as the slow-conducting PTNs significantly decreased the variability of their discharges between steps during locomotion on the ladder. This effect was not seen in the fast-conducting group (Table 2). We have previously suggested that the more vigorous activity of motor cortical neurons shortly before paw-off and during the early swing may contribute to control of stride length and thus more accurate paw placement during complex locomotion (Beloozerova *et al.*, 2010). The conclusion that the motor cortex may play a role in control of position of paw landing during walking was also reached by Amos *et al.* (1990) and Friel and colleagues (2007) based on results of movement perturbations and motor cortex inactivation experiments.

Second, while both fast and slow PTN populations increased the averaged peak discharge rates and the frequency-based coefficients of modulation *M* upon transition from simple to ladder locomotion, the average value of the frequency-corrected modulation coefficient *dM*, which reflects magnitude of modulation in probability of discharge, increased only in the slow-conducting group (Table 2). The increased activity modulation made the influence of all PTNs more salient and thus probably more effective, but to a greater degree within the slow-conducting group.

Finally, while fast-conducting PTNs retained an approximately 3:1 split between one-PEF and two-PEF discharge patterns during locomotion on the ladder, many of the two-PEF slow-conducting PTNs lost their second PEF – to the extent that 90% exhibited only one PEF during the ladder task. Such a transformation in the discharge pattern typically occurred via an increase in the activity of a neuron during one of its inter-PEF intervals, which joined the previously distinct PEFs, thus making the PEF longer, that is, increasing the neuron's duration of influence.

The observed differences in the activities of fast- and slow-conducting PTNs cannot be explained by the difference in their receptive field properties. Slow-conducting neurons tend to lack somatosensory receptive fields and one may suggest that their population activity profiles during simple and ladder locomotion are due to the large proportion of non-responsive PTNs (Figs 4*H* and 7*G* in Stout & Beloozerova, 2012). However, we found that slow-conducting PTNs are the ones to most strongly increase the depth of locomotion-related

modulation upon transition to the accuracy-demanding ladder task. This is in contrast to the typical behaviour of non-responsive PTNs, which more often than any other PTNs decrease the depth of modulation during ladder locomotion (Fig. 6*D* in Stout & Beloozerova, 2012). Similarly, the activity of fast-conducting PTNs, which were most likely to have receptive fields on the shoulder, cannot be explained by this bias. Their population activity profiles are dissimilar to shoulder-related PTNs, and do not show the pronounced response to accuracy demand of the ladder task exhibited by shoulder-related PTNs (Stout & Beloozerova, 2012).

The above group of observations on differences in responses of fast- and slow-conducting PTNs to accuracy requirement during locomotion suggests a greater role for slow-conducting PTNs in addressing the accuracy demands of complex environments compared to fast-conducting PTNs. The lower discharge rates of slow-conducting PTNs, by $\sim$5 spikes s^{-1} on average (18.1 ± 1.2 *vs.* 13.5 ± 1.2 spikes s^{-1}), are likely to be more than compensated for by the significantly greater number of slow-conducting PTNs in the cortex (Calvin & Sypert, 1976; Humphrey & Corrie, 1978; Wiesendanger, 1981).

Fast- and slow-conducting PTNs differ in their connections to the spinal cord, such that fast-conducting PTNs preferentially influence distal muscle-related networks, while slow-conducting PTNs influence both proximal and distal muscle-related networks (Brookhart, 1952; Wiesendanger, 1981; Canedo, 1997). Therefore, more intensive involvement of slow-conducting PTNs in control of accuracy of movements during locomotion means that the accuracy of stepping is predominantly achieved not by adjustments of movements in distal limb segments, but by a more careful planning of the whole limb transfer, in which proximal limb-related networks significantly participate. It has been shown that during limb movements, individual joints make unique contributions to the overall movement, as proximal joints greatly affect movements of distal joints, while distal joints have only small influence on movements of proximal joints (e.g. Grillner & Rossignol, 1978; Galloway & Koshland, 2002; Dounskaia, 2005).

The contribution of fast-conducting PTNs may be indispensable for the most rapid adjustments of locomotion movements that are needed when walking across fast-changing surfaces such as for example a ladder with a displaceable crosspiece (Amos *et al.* 1990; Marple-Horvat *et al.* 1993; Beloozerova *et al.*, 2007) and, possibly, during very high-speed locomotion by fast trot or gallop.

The specific mechanism by which PTNs assist accuracy of stepping remains to be determined. While one may suggest that observed differences in PTN discharges during locomotion on a flat surface and on the ladder are a non-specific reflection of increased cortical involvement, it has been shown that during increasingly accuracy-demanding walking tasks, the corresponding changes in PTN activities become increasingly vigorous (Beloozerova & Sirota, 1993*a*; Drew *et al.*, 2008; Beloozerova *et al.*, 2010). Therefore, it seems likely that PTNs are directly involved in accurate movements.

This study was inspired, in part, by an earlier observation by Fromm & Evarts (1977, 1981) that slow-conducting PTNs are more readily activated by small movements than are fast-conducting PTNs and the hypothesis of these authors that slow-conducting PTNs may have a special role in control of accuracy of limb movements. In their experiments, however, Fromm & Evarts (1977, 1981) compared firing properties of fast- and slow-conducting PTNs during small, ostensibly precise movements and large-amplitude, ballistic movements that lacked a requirement for accuracy. Thus, from their data it remained unclear whether the effective activation of slow-conducting PTNs during small movements was truly due to the accuracy requirement of small-amplitude tasks, or merely due to the low activation threshold of these PTNs. Our study separated these characteristics. The two locomotion tasks tested differed solely in the accuracy demands on stepping, and were nearly identical in terms of other kinematic parameters. We have recently shown that when cats walk in an experimental setup similar to that used in this study, there are only a few differences in the kinematics between simple and ladder locomotion: a somewhat more bent-forward posture, a lower wrist flexion moment during stance, and slightly enhanced activity of selected distal muscles during walking on the ladder with thin crosspieces (Beloozerova *et al.*, 2010). Thus, the different responses of PTNs between simple and ladder locomotion in our study can be nearly entirely ascribed to the differences in the accuracy requirements of the tasks, rather than other kinematic differences. Therefore, our study, in relation to locomotion, provides data from a targeted experiment to support the previous observation of Fromm & Evarts (1977) that slowly conducting PTNs have the most selective relations to accurately controlled movements.

We want to note that most studies of the discharges of individual neurons in the motor cortex over the years have been strongly biased toward fast-conducting PTNs, on account of their comparatively large size, and thus relative ease of recording. With the recent wide adoption of commercially available chronically implantable microarrays for cortical neuronal recording, this biasing has become an even larger issue. However, the vast majority of PTNs are of the slow-conducting variety (Calvin & Sypert, 1976; Humphrey & Corrie, 1978), and these neurons have anatomical and physiological properties that are quite distinct from those of fast-conducting PTNs. Fast- and slow-conducting PTNs have different dendritic field ranges (Deschênes *et al.* 1978; Sakai,

1982), different distributions throughout the motor cortex (Takahashi, 1965; Towe *et al.* 1968) and may receive input of different types (Deschênes *et al.* 1982). In addition, neurons of the two types influence one another in different ways: fast-conducting PTNs commonly make inhibitory disynaptic connections to slow-conducting PTNs, while slow-conducting PTNs often make excitatory monosynaptic connections to fast-conducting PTNs (Takahashi, 1965; Tsukahara *et al.* 1968; Ghosh & Porter, 1988; Canedo, 1997). While neurons of either type are equally likely to synapse upon the spinal cord, and both produce facilitation of their target muscles (Fetz & Cheney, 1980), the facilitation produced by fast-conducting PTNs is larger (Lemon & Porter, 1993). These differences in biophysical and connective properties strongly suggest that fast- and slow-conducting PTNs may have quite distinct functional roles in the control of movements. The results of our study suggest that they may have different roles during accuracy-constrained stepping.

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Author contributions

Both authors contributed to the conception and design of the study, analysis and interpretation of data, drafting the article, revising it critically for important intellectual content, and to final approval of the version to be published. The experiments were conducted at the Barrow Neurological Institute, Phoenix, Arizona, USA.

Acknowledgements

The authors are thankful to Dr Zinaida Tamarova for help with data acquisition, to Mr Peter Wettenstein for excellent engineering assistance, and to Drs Mikhail Sirota and Vladimir Marlinski for valuable suggestions and discussions. The research reported in this paper was supported by NIH grants R01 NS-39340 and R01 NS-058659 to I.N.B.