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Known and unexpected constraints evoke different kinematic, muscle, and motor cortical neuron responses during locomotion

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Abstract

During navigation through complex natural environments, people and animals must adapt their movements when the environment changes. The neural mechanisms of such adaptations are poorly understood, especially in respect to constraints that are unexpected and must be adapted to quickly. In this study, we recorded forelimb-related kinematics, muscle activity, and the activity of motor cortical neurons in cats walking along a raised horizontal ladder, a complex locomotion task requiring accurate limb placement. One of the crosspieces was motorized, and displaced before the cat stepped on the ladder or at different points along the cat's progression over the ladder, either toward or away from the cat.

We found that when the crosspiece was displaced before the cat stepped onto the ladder, kinematic modifications were complex and involved alterations of dynamics of all forelimb joints. When the crosspiece displaced unexpectedly while the cat was on the ladder, kinematic modifications were minimalistic and primarily involved distal joints. The activity of M. triceps and M. extensor digitorum communis differed based on the direction of displacement. Out of 151 neurons tested, 69% responded to at least one condition; however, neurons were significantly more likely to respond when crosspiece displacement was unexpected. Most often they responded during the swing phase. These results suggest that different neural mechanisms and motor control strategies are used to overcome constraints for locomotor movements depending on whether they are known or unexpectedly emerge.

Keywords

Motor Control; Cat; Perturbation; Kinematics

INTRODUCTION

The motor cortex is highly involved in the control of single limb movements, locomotion and posture. During locomotion, nearly all layer V neurons of motor cortex discharge in rhythm with the step cycle (Beloozerova and Sirota, 1993 a,b; Drew, 1993; Fitzsimmons et

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al., 2009; Stout and Beloozerova, 2012, 2013), and the characteristics of this activity are often specialized to the task being performed. In many behaviors, visual information about the environment must be used in order to navigate obstacles and accurately guide foot placement. The contributions of the motor cortex are essential in managing the complexities posed by irregular surfaces, including those found in the natural environment: when the motor cortex is inactivated or ablated, subjects lose the ability to successfully traverse complex terrain (Trendelenburg, 1911; Liddell and Phillips, 1944; Chambers and Liu, 1957; Beloozerova and Sirota 1993a; Friel at al., 2007).

During planned gait adaptations on complex terrain, preparatory kinematic adjustments are often made (Mohagheghi et al., 2004), and frequently, the smallest kinematic adjustments that are sufficient to overcome environmental complexities are preferred (Patla et al., 2004). Such anticipatory adjustments are thought to be driven through integration of feedforward and feedback signals (Drew et al. 1996). However, many natural behaviors require adaptations to changes in the environment, which are dynamic and unexpected. Preparatory movements are often impossible in these situations, and the selection of movement strategies may be constrained based on the amount of time available to accommodate the perturbation (Patla, 1999). It has been suggested that planned and unplanned adaptations may be mediated through different neural pathways, as the latency of unexpected obstacle avoidance behaviors is shorter than the latency of planned movement modifications (Pettersson et al., 1997; Weerdesteyn et al., 2004). Indeed, it was recently shown that during reaching to unexpectedly shifting targets, differential neuronal processing occurs based on when the shift occurs (Ames et al. 2014).

Therefore, it appears likely that the neuronal motor control strategies employed to overcome task-related constraints may be dependent on the amount of time between constraint perception and motor adaptation, as well as whether the constraint is known or unexpected. Little is known, however, about the motor control processes used to compensate for emergent or unexpected changes in the movement environment during locomotion. To investigate this function, kinematics, muscle activity, and the activity of motor cortical neurons was recorded as cats walked along a raised horizontal ladder, a complex locomotor task that required accurate limb placement. One of the crosspieces was motorized, and could be displaced either prior to the cat stepping on the ladder or at different points along the cat's progression along the ladder, either towards or away from the cat. To successfully continue along the ladder, cats needed to make a longer or shorter step. Forelimb kinematics, EMGs, and motor cortex activity during shorter or longer steps with these various displacement timings were compared.

A brief account of a part of this study was published in abstract form (Stout et al., 2012).

METHODS

Recordings were obtained from two adult male cats (weight 11 and 8.5 lb). Methods of data collection and spike trains analysis have been previously reported (Prilutsky et al., 2005; Beloozerova et al., 2010; Stout and Beloozerova, 2012, 2013) and will be described briefly

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

Positive reinforcement (food) was used to adapt cats to the experimental situation and to engage them in locomotion (Skinner, 1938; Pryor, 1975). A walkway, 2.5 m long and 0.3 m wide on each edge, served as an experimental chamber (Fig. 1A). Cats passed sequentially and repeatedly through the two corridors of the chamber in a counter-clockwise direction. In one of the corridors, the floor was flat, while the other corridor contained a horizontal ladder with 10 crosspieces. Crosspieces were spaced 25 cm apart, which is half of the mean stride length observed in the chamber during locomotion on flat floor at a self-selected pace (Beloozerova and Sirota, 1993; Beloozerova et al., 2010). The tops of crosspieces were flat and 5 cm wide. The width of the crosspieces was chosen to slightly exceed the cat's mean foot length (3 cm), so that cats had full foot support on a crosspiece. Crosspieces were elevated 6 cm above the floor of the chamber. One crosspiece (the seventh from the left side of the ladder, Fig. 1A) was connected to an electric motor. When displaced, it was shifted 5 cm in either direction, such that there was no overlap between the crosspiece's position before or after the displacement. Displacement was completed within 145 ms of initiation. On the side of the crosspiece facing the cat, there was a yellow LED lamp. It was lit as soon as the triggering of the crosspiece displacement occurred, regardless of the direction of the initiated move. This illumination attracted the cat's attention to the crosspiece when it was displacing. Auditory cues from the activation of the motor also alerted the cat to a rung displacement. Regardless of the crosspiece's displacement or the cat's performance, after each round of walking, the cat received food in a feeding dish located in one of the chamber's corners.

This apparatus allowed us to compare several locomotion tasks by displacing the crosspiece at various time points along the cat's progression. Only passages where the cat stepped on the displaceable crosspiece with right feet were studied. Seven conditions were used (Fig. 1 B): control, when the crosspiece remained in its original location; and three groups of conditions where the crosspiece was displaced either toward or away from the cat at different times along the cat's progression through the chamber, and the cat had to make a larger or smaller step to successfully traverse the ladder. In "known displacement" (*Kn*) conditions, the crosspiece was displaced while the cat was at the feeder. In these conditions, the cat did not see movement of the crosspiece. The ladder was in its final configuration when the cat stepped onto it. The cat had two full strides: a stride from crosspiece #1 onto crosspiece #3, and a stride from crosspiece #3 onto crosspiece #5, before making a larger or smaller step to reach the displaced crosspiece #7. In unexpected "long-notice" conditions (*Ul*), the rung was displaced when the cat's right forelimb stepped on crosspiece #3. The cat had one full locomotion cycle to complete before needing to adjust. In unexpected "short notice" conditions (*Us*), the crosspiece was displaced when the cat's right forelimb stepped on crosspiece #5 and the very next transfer of the forelimb had to be adjusted. A sequence of 21 conditions was repeated pseudorandomly by a computer program, occasionally resetting at random times that were different for different experimental days and subjects. All

conditions were presented an approximately equal number of times, and the cat could develop no fore-knowledge of which condition would be presented.

Cats were accustomed to wearing a cotton jacket, a light backpack with connectors and preamplifiers for electromyographic (EMG) signals, and an electro-mechanical sensor on the right paw for recording duration of swing and stance phases of stride. They were also trained to wear LEDs on lateral aspects of the right forelimb. The floor in the chamber and the crosspieces 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 was monitored by measuring the electrical resistance between the right foot and the floor with the electromechanical sensor (Fig. 1 C, the bottom trace). The passage of the cat through the beginning and end of each corridor was monitored using infrared photodiodes.

Surgical procedures

After cats were trained, surgery was performed under isoflourane anesthesia using aseptic procedures. 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; they served as a fixation and a common ground. The screw heads and the wire were inserted into a plastic cast to form a circular base. Later, while searching for neurons before locomotion tests, awake cats were rigidly held by this base. The base was also used 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 area of the motor cortex was identified by the surface features and photographed (Fig. 2A). 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).

For muscle activity recordings, a pair of leads constructed from Teflon-insulated multistrand stainless steel wire (AS632, Cooner Wire, Chatsworth, CA) was implanted into m. triceps lateralis and m. extensor digitorum communis. The electrode placements were verified by stimulation through the implanted wires before closure of the incision. The wires were led subcutaneously and connected to sockets on the head base. Immediately after surgery, and then 12 hours thereafter, an analgesic buprenorphine was administered intramuscularly.

Cell recording

Experiments were initiated after several days of recovery when cats resumed their normal preoperative behavior. The animal was positioned in the restraining device, 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 several days, a number of sessions of increasing duration were used to accustom the cat to the head restrainer. Cats fast learned to sit quietly with their head restrained. They did not seem to be disturbed by the restraint because they frequently fell asleep.

Extracellular recordings were obtained using conventional tungsten varnish-insulated microelectrodes (120 μm OD, Frederick Haer & Co). The impedance of electrodes was 1-3 MΩ at 1000 Hz. A custom made light-weight (2.5g) manual single-axis micro-manipulator permanently affixed to the head base was used to advance the microelectrode (see Fig. 2E in Prilutsky et al., 2005) . Signals from the microelectrode were pre-amplified with a miniature custom made preamplifier positioned on the cat's head, and then amplified with the 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, led to an audio monitor, and recorded to the hard disk of a computer by means of data acquisition hard- and software package (Power-1401/Spike-2 System, Cambridge Electronic Design, Cambridge, UK). After a neuron with a good signal to noise ratio was isolated, the animal's head was released from the restraining device, and the cat was placed in the walking chamber. An example of recording from a neuron during locomotion is shown in Figure 1C.

A detailed description of the area of recording has been given previously (Beloozerova et al. 2005). In brief, in the cat, the area immediately adjacent to and inside the lateral half of the cruciate sulcus is considered to be the motor cortex (Fig. 2A). This is based on a considerable body of data obtained by means of inactivation, stimulation and recording techniques (Nieoullon and Rispal-Padel, 1976; Vicario et al., 1983; Armstrong and Drew, 1985; Beloozerova and Sirota, 1993a; Drew, 1993; Martin and Ghez, 1993), as well as on histological considerations (Myasnikov et al., 1997; Ghosh, 1997). The area and depth of recording was confirmed postmortem using reference lesions (Fig. 2C); at this depth, the cortex was populated with large pyramidal neurons characteristic of layer V of area 4 γ (Fig. 2D).

Motion capture and joint kinematics analysis

Mechanics of locomotion for the right forelimb were recorded using the computerized, active-marker three-dimensional real-time motion capture and analysis system Visualeyez (VZ-4000, Phoenix Technologies Inc., Canada). Six wide-angle LEDs were placed on the shaved lateral aspects of the right forelimb using double-side adhesive tape: the greater tubercle of the humerus (shoulder joint), approximate elbow joint center, ulna styloid process (wrist joint), base of the fifth metacarpals (metacarpophalangeal joints, MCP), tip of the middle toe, and the trunk anatomical landmark the right scapula. The definitions of forelimb joint angles and the segment orientation are shown in Figure 2 B. Three-dimensional positions of LEDs were recorded at 111.1 Hz throughout the duration of the experiment. Accuracy of measuring distances on a rigid test object was better than 2.3 mm. Joint dynamics were calculated using provided functions from the VZ Analyzer software package. Kinematics were analyzed using a minimum of 10 strides of the same condition, all recorded during the same testing session, and compared between the tasks.

Processing of EMGs

Muscle activity was pre-amplified using miniature preamplifiers on the cat's backpack. The activity was additionally amplified and filtered (30 – 1500 Hz band pass) using CyberAmp 380 amplifier (Axon Instruments), sampled at 3 kHz, and stored on a computer hard drive. For analysis, raw EMGs were full-wave rectified and averaged using a central moving

average with a time window of 20 ms (Fig. 2E). For each locomotor task (Fig. 1B), muscle activity was averaged over 10-40 strides recorded during the same testing session, and compared between the tasks.

Processing of neuronal activity

Neuronal data from right foot steps that landed on the displaceable crosspiece #7 were analyzed. The onset of stance phase on crosspiece #5 was taken as the beginning of the stride to crosspiece #7. The duration of each stride was divided into 20 equal bins. Neuronal activity during strides in each of the seven conditions were compared for overall similarity using a support vector machine (SVM) trained on spiking activity during individual runs (Cortes and Vapnik, 1995; Stark and Abeles, 2007; Jochumsen et al., 2013). Specifically, to test the similarity of a neuron discharge during a pair of conditions, data from each of the two conditions was segmented into two groups, one to train a SVM classifier (training group), and one to test the classifier (test group). To minimize uncontrolled variables such as walking speed, segmentation into training and test groups was stratified, with every other step being placed into the training (or test) group. Optimal splitting criteria between the two conditions were developed based on the neuronal activity in the training group (e.g., Figs. 3 A,B show individual traces on the top and average activity profiles at the bottom for two selected conditions). The splitting criteria were applied to the test group, and used to classify steps into one of the two conditions (Fig. 3C). Individual neuron responses were analyzed in a minimum of 20 strides, and compared between the tasks.

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 (Fig. 2C). 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. Positions of recording tracks in the motor cortex were estimated in relation to the reference lesions.

Statistical Analyses

To assess differences between kinematic and EMG waveform data among the locomotor tasks, the difference between the initial and minimal values of the waveform and the difference between the final and minimal values during the swing phase of the stride were calculated. These two metrics are termed “initial amplitude” and “final amplitude”, respectively, and are demonstrated for a sample waveform (elbow joint dynamics) in Figure 3D. In addition, comparisons were performed for either true-time (kinematic) or normalized-cycle (EMG) traces through the stride from crosspiece #5 to crosspiece #7. To assess differences in kinematic or EMG parameters during different conditions, an unpaired t-test was used. To determine characteristic responses to each condition, initial and final amplitude calculations for kinematic and EMG data were averaged between cats, with equal weight given to data from each subject, and a final composite was developed.

Activity of each recorded neuron was analyzed individually, and neuronal populations collected from each cat were compared in aggregate to ensure that neuronal properties were similar between subjects. To assess overall differences in neuronal activity between tasks, the prediction accuracy of SVM methods was tested. If SVM methods correctly identified which group a particular step belonged to more often than would be expected by chance (Fig. 3C), the neuron was considered to distinguish between the two conditions. Theoretical chance levels for classifying between conditions are 50%, and to test for classifier bias, a bootstrapping procedure with data from the same condition was performed. This procedure produced mean classification accuracy of 50.3%, not significantly different from the theoretical chance level. The SVM procedure was repeated for all combinations of conditions (n= 21). To assess bin-wise differences in neuronal activity between tasks, an unpaired t-test was used with a significance level of $p<0.05$. To assess the significance of correlation, the t-test was applied to the Fisher transformation of Pearson's R coefficient.

Statistical methods for comparison of discharges of individual neurons between locomotion tasks have been reported elsewhere (Stout and Beloozerova 2012, 2013), and will be briefly outlined here. A 20 bin phase histogram of spike activity of the neuron in the stride cycle was generated and averaged over all selected cycles. The first bin was considered to follow the last one; the last bin was considered to precede the first one. The "depth" of modulation, dM, characterizing fluctuation in probability of the discharge, was calculated as $dM = (Nmax - Nmin)/N * 100\%$, where Nmax and Nmin are the number of spikes in the maximal and the minimal histogram bin, and N is the total number of spikes in the histogram. Neurons with $dM>4\%$ were judged to be stride-related. In stride-related neurons, the period of elevated firing (PEF) was defined as the portion of the cycle in which the activity level exceeds the minimal activity by 25% of the difference between the maximal and minimal frequencies in the neuronal discharge histogram. The "preferred phase" of discharge of each neuron with a single PEF was assessed using circular statistics (Batshelet, 1981; Drew and 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.

For comparisons of the discharge rate, depth of modulation, and preferred phase of individual neurons between the tasks, differences equal or greater than ±20%, ±20%, and ±10%, respectively, were considered significant. These criteria were established based on the results of a bootstrapping analysis (Efron and Tibshirani, 1993), which compared differences in discharges between various reshufflings of strides of the same locomotion task and found that natural activity fluctuations of neurons in layer V of the motor cortex remain within these limits with 95% confidence (Stout and 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.

RESULTS

Recordings of the activity of 151 neurons from layer V of the motor cortex, 2 forelimb muscles, and forelimb kinematics were obtained from two cats.

Movement adaptation strategies between known and unexpected perturbations are distinct

In each condition, the kinematics of the stride to the displaced crosspiece were adjusted such that the limb could successfully land on it. The kinematic strategies used, however, depended on the timing of the displacement of the crosspiece. This was true for both longer and shorter strides. During the unexpected displacement conditions, to make the step either smaller or larger than normal, the cat produced accurate steps by altering the duration of the swing, making it shorter or longer, respectively (Fig. 4A). However, when the location of the crosspiece was known already by the time the cat stepped on the ladder (*Kn* condition), the cat produced accurate steps by increasing or decreasing limb velocity without altering the duration of the swing phase (Fig. 4B). Additionally, in the *Kn* condition, preparatory adjustments were observed: during the stride preceding the disturbed one, the cat stepped on the crosspiece #5 either slightly further along in the direction of motion (when a larger step on crosspiece #7 was upcoming) or less far along, when a smaller step on crosspiece #7 was required (t=2.06 for large and t=2.25 for small strides; P<0.05; Figs. 4C). However, the kinematics of the preparatory stride, as well as those of the stance phase of the step onto the displaced crosspiece, were similar among all conditions in every other respect (not shown).

The joint displacements during disturbed steps also differed by crosspiece displacement condition. Two major differences were found during the swing phase of the disturbed step between known and unexpected conditions. First, during the *Kn* condition, kinematic alterations occurred during the early parts of the swing phase (red stars in Figs. 4D-G, H and J), while during the *Ul* and *Us* conditions, kinematic adaptations only began immediately prior to footfall (Figs. 4G, I, J, K). Second, while most joints exhibited changes during perturbed steps in the known displacement condition (Figs. 4 D-O), alterations during the unexpected displacement conditions primarily involved more distal joints (Figs. 4 G,K,O).

Muscles respond to a change in the size of the stride

The activity of both recorded muscles (elbow extensor m. triceps, and wrist extensor EDC) during the entirety of the preparatory step from crosspiece #3 on crosspiece #5 (Fig. 1B) and the stance phase of the disturbed step on crosspiece #7 were similar among conditions (not illustrated). Both muscles exhibited changes to activity during swing phase of the disturbed step, decreasing it during a small step and increasing during a large step (Fig. 5A-D). These changes were observed regardless of the crosspiece displacement condition. The observed changes in terms of initial and final amplitude during the swing phase were generally consistent between unexpected long- and short-notice conditions - in 7/8 comparisons, either both were significantly different from control, or neither were (Fig. 5E-H). In 5/8 comparisons were the changes observed in the *Kn* condition in common with those in the unexpected displacement conditions.

Motor cortex neurons respond to adaptation of movement

Recordings of the activity of 151 neurons from layer V of the motor cortex were obtained. The activity of 114 neurons was recorded during all seven conditions (Fig. 1B); the activity of the remaining 37 neurons was recorded only during control and four unexpected displacement conditions.

Neuronal data was collected from 37 tracks through the motor cortex: from 13 tracks in cat 1 and 24 tracks in cat 2 (Fig. 2A). The activity of 59 neurons from cat 1 and 92 neurons from cat 2 was analyzed. During locomotion in the control condition on the ladder with evenly spaced crosspieces, the preferred phases of discharges for the population were distributed throughout the step cycle, although more neurons had preferred phases during swing (Fig. 6A). To test if neuronal responsivity to displacement of the crosspiece was similar between cats, overall SVM prediction accuracy was calculated between the control and test conditions, and compared. Mean SVM prediction accuracy was similar between the neuronal populations collected from each cat (57.2±3.1% vs. 58.7±2.5%, $p>0.05$). Sixty nine percent of all neurons (91/151) responded to the disturbance of the stride on the motorized crosspiece. Neurons exhibiting a response fell into two major categories. Unidirectional neurons, representing 40% of the total population, responded only to large or small steps, but not both, and bidirectional neurons, representing 30% of the population, responded to both large and small steps, most often increasing activity during large steps and decreasing activity during small steps. The remaining 30% did not exhibit a response. Examples of a unidirectional and a bidirectional neuron are shown in Figures 6B and 6C, respectively.

To make the step larger than normal, 29.6% of unidirectional neurons increased their average discharge rate by 17.3±2.6% on average while 13.9% decreased it by approximately the same amount. In addition, 23.7% of unidirectional neurons increased the depth of their stride-related activity modulation by 25.8% while another 29.0% decreased it by 32.7%. Only 5.3% of unidirectional neurons changed the preferred phase of their discharge during large steps and that change was 10% of the step cycle on average.

To make the step smaller than normal, 44.4% of unidirectional neurons decreased their average discharge rate by 18.5±2.8% on average while only 2.8% increased it. Changes to discharge were distributed roughly equally throughout the step cycle for unidirectional neurons responding to large and small steps (Fig. 6D). In addition, 19.4 % of unidirectional neurons increased the depth of their stride-related activity modulation by 24.3% while another 24.1% decreased it by approximately the same amount. In contrast to large steps, 23.2% of unidirectional neurons changed the preferred phase of their discharge during small steps by 15-17.5% of the step cycle on average. Changes to preferred phase were equally likely to shift the neuron’s activity earlier or later in the cycle.

Bidirectional neurons responded differently to steps that were smaller or larger than normal. Specifically, more bidirectional cells responded to large steps by increasing their average discharge rate than did so in response to small steps (39.7% vs. 28.3%; $t=2.44$, $P=0.019$). This increase was 22.4% on average. Similarly, more bidirectional cells responded to small steps by decreasing their average discharge rate than did so in response to large steps (31.2% vs. 17.7%; $t=3.49$, $P=0.0011$). This decrease was 26.1% on average. For both large and small steps, changes to discharge rate occurred primarily during the swing phase (Fig. 6E). Bidirectional neurons also distinguished between large and small steps in how they changed the depth of their stride-related modulation. More bidirectional cells responded to large steps by increasing their depth of modulation (19.4% vs. 11.4%; $t=2.87$, $P=0.0062$) by 23.8%, and more cells responded to small steps by decreasing their depth of modulation (50.5% vs.

38.1%; t=2.41, P=0.020) by 26.8%. In addition, bidirectional cells more often changed the preferred phase of their activity in response to small than large steps (29.5% vs. 17.6%; t=3.14, P=0.003). However, during both large steps and small steps, bidirectional neurons cells nearly always shifted they preferred phase to a position later in the cycle.

Neuronal response likelihood depends on whether disturbance is known or unexpected

Neurons were considerably more likely to respond during either of the unexpected displacement conditions than during the known displacement condition, especially during large steps (Fig. 7A). However, neurons commonly responded to multiple conditions, and 20% of the total population responded to steps on the displaced crosspiece during all three timing conditions (Fig. 7B). Many neurons responded to only two out of the three conditions. Of these, neurons responding to the *Ul* and *Us* conditions, but not the *Kn* condition, were the most common type, and represented 11% of the total population (Fig. 7B). Neuronal responses during the swing phase of the disturbed step were nearly twice as common as responses during the stance phase (Fig. 7C). While this characteristic was observed across all conditions, responses during the late stance and early swing phases were significantly more common for the unexpected displacement conditions (Fig. 7D).

Neuronal responses are direction-sensitive

To understand what factors influence whether or not motor cortical neurons respond during the different test conditions, the relationship between SVM classification accuracy during the various disturbed conditions was compared to determine if neurons that responded to a certain disturbance would respond to other disturbances that were similar, either in the direction of crosspiece displacement, or the timing at which the crosspiece displacement occurred. A representative scatterplot showing direction-sensitivity is shown in Figure 8A. Neurons exhibiting a response during a short or long step in one timing condition were more likely to exhibit a response during steps of the same size in another timing condition. This relationship was uniformly stronger for larger-than-normal steps (Fig. 8C). Large steps, whether they were made in the *Kn*, *Ul*, or *Us* condition, were generally similar in terms of changes to the discharge rate, depth of modulation, and preferred phase of discharge. A representative example of a unidirectional neuron responding similarly to all large steps, but not small steps, is shown in Figure 8E. There were a few notable differences, however. For larger-than-normal steps, as the time available for the stride modification increased from *Us* to *Ul* to *Kn* conditions, progressively more neurons responded by decreasing the average discharge rate (10.6% vs. 17.8% vs 20.0%, respectively) or by shifting their preferred phase to a position later in the step cycle (8.5% vs. 11.1% vs. 20.0%, respectively). For smaller-than-normal steps, the reduction in the average discharge rate was significantly greater for the small step made in the *Us* condition than for such a stride made in the *Ul* or *Kn* conditions (−32.7% vs. −22.4% and −19.3%, respectively).

A representative scatterplot showing latency-sensitivity of neuronal responses is shown in Figure 8B. In contrast to exhibiting generally consistent responses to steps of the same size, neurons exhibiting a response during a large or a small step were no more or less likely to exhibit a response when the crosspiece was displaced in a different direction at the same latency (Fig. 8D).

Responses to unexpected disturbances preferentially involve neurons that are already active

The relationship between SVM classification accuracy and a variety of neuronal activity characteristics, including discharge rate, modulation with respect to the stride cycle, and preferred phase of discharge, were compared to determine which characteristics might predict a neuron's responses to disturbance in the stepping. Of these characteristics, only discharge rate was found to exhibit a consistent relationship with neuronal responses. Figures 9 A-C show scatter plots of neuronal discharge rate during swing phase and mean SVM classification accuracy for the *Kn*, *Ul*, and *Us* conditions, respectively. As the time available for stride modification decreased, swing discharge rate became increasingly related to the likelihood of neurons to respond to a larger or smaller step (R^2=0.0326 vs. R^2=0.0449 vs. R^2=0.0739, respectively). However, this correlation was only significant for unexpected disturbances (Fig. 9D). Therefore, in the *Ul* and *Us* conditions, the neurons which responded tended to be those that were already active when the step was not disturbed, while in the known displacement condition, many neurons responded that were not active when the step was not disturbed.

Neuronal responses to unexpected short-notice disturbances are often unique

The majority of neurons distinguished between short and long steps when crosspiece displacement occurred at the same time (64% of neurons, 99/151). Such differences were more common in either of the unexpected displacement conditions (Fig. 10A). On the other hand, it was far less common for neurons to exhibit different responses to displacements occurring in the same direction, but with different timing: only 25% of neurons (38/151; t=16.13, P=4.3E-20) responded in this manner. However, such "unique" responses between conditions involving steps of the same size did occur, and an example is shown in Figure 10B. Unique responses were most common for the unexpected short-notice displacement condition (Fig. 10C). Most frequently, the difference in the neuronal responses occurred during the stance-to-swing phase transition, for both small and large steps (Figs. 10D,E).

DISCUSSION

It is apparent from our data that the strategies used to adapt to constraints in the walking environment differ depending on whether those constraints are known or unexpected, and that these strategies are consistent whether crosspiece displacement cause strides to be longer or shorter than normal. These distinctions persist despite the fact that both known and unexpected displacement conditions imposed identical constraints on foot placement, strongly suggesting that distinct motor planning and control processes are at work in the known versus unexpected displacement conditions. Analysis of motor cortical neuron responses supports this conclusion, showing different likelihoods of response depending on whether crosspiece displacement is known or unexpected. Neuronal responses during unexpected displacement conditions were significantly higher during the transition between stance and swing phases, and neuronal adaptations were more likely to involve neurons that were discharging at high rates during the control locomotion task. While the activity in recorded muscles was similar between known and unexpected displacement conditions, this is not unexpected. These muscles are primarily active during the final portions of the swing

phase, and during this portion of the movement, joint kinematics during both known and unexpected displacement conditions were altered in a similar manner.

While this study is the first to directly demonstrate that different neuronal and kinematic mechanisms are employed during unexpected and expected gait modifications, the results are consistent with those observed in previous studies. The kinematic and EMG profiles observed during locomotion are consistent with those of previous reports from our laboratory and other investigators (e.g., Drew 1988; Prilutsky et al., 2005; Krouchev et al. 2006; Gregor et al. 2006; Beloozerova et al. 2010). In addition, the motor adaptations employed to step onto the displaced crosspiece are similar to data reported by Drew (1988), in that increases of the length of the step (large steps) involved increased EMG activity in the triceps and EDC muscles, and, commonly, increases in the discharge rates of motor cortical neurons. We additionally found that when the length and trajectory of the step decreased (small steps), EMG activity and neuronal discharge rates often decreased as well. Although Drew (1988) observed that EMG adjustments principally involved flexor muscles, this author also saw consistent changes in extensors, albeit on a smaller scale. Motor adaptations during unexpected displacement conditions exhibited minimalistic changes to kinematics, as Patla and colleagues (2004) demonstrated for visually guided trajectory modifications during walking in humans.

In respect to neuronal activities, during the known displacement condition, we found similar proportions (~40%, Fig. 7) of motor cortical neurons responding to gait adjustments in landing on crosspieces located closer to or farther away from the cat, as Amos and colleagues (1990) found for landing on crosspieces displaced vertically higher or lower. However, while Marple-Horvat and colleagues (1993) commonly observed fast motor cortical responses to unexpected crosspiece displacements at approximately 40 ms following displacement onset, we observed no such response. This is likely due to the fact that their paradigm involved displacement of the crosspiece only after the forelimb was placed upon it, likely activating proprioceptive feedback circuits, while ours involved displacement in advance of paw placement.

Known and unexpected motor adaptations reflect feedforward and feedback-driven motor control processes

The different motor adaptations observed in the known and unexpected displacement conditions suggest the use of feedforward and feedback-driven motor control processes, respectively. Motor adaptations during the *Ul* and *Us* conditions were restricted to the final 25% of the swing phase, and no preparatory adjustments were observed (Fig. 4). These adjustments followed a classical profile observed in many feedback-driven human reaching tasks, in which corrective motor adaptations are made during the final portions of a visually-guided, accuracy-dependent movement (e.g., Woodsworth, 1899; Milner 1992; Meyer et al. 1988). In contrast, motor adaptations during the *Kn* condition exhibited preparatory adjustments in advance of the step onto the displaced crosspiece, as well as differences throughout the movement (Fig. 4C-O), which is consistent with a forward-modeled motor plan (e.g., Wolpert and Kawato, 1998; Kawato, 1999).

Implications for the role of the motor cortex in adaptations to locomotion

The observed preference for already-active neurons to respond in the unexpected displacement conditions (Fig. 9) may reflect complexities in integrating motor adaptations to movements that are currently in progress. It might be expected that the comparatively extensive alterations observed in the kinematics of limbs displaced in the *Kn* condition would require more substantive changes to motor cortex activity than in the unexpected displacement conditions. This was not the case. Rather, neuronal responses in the known condition were significantly less frequent than in unexpected displacement conditions (Fig. 7A). This sheds light on how corrective motor commands are generated in these two situations. It was shown that the posterior parietal cortex is involved in planning gait adaptations during complex locomotion tasks (Andujar et al. 2010, Marigold et al., 2011), and lesions to this structure compromise gait modifications (Lajoie and Drew, 2007). Because many neurons in the posterior parietal cortex discharge well in advance of gait modifications, this structure may selectively activate efficient synergies of neurons (Drew et al. 2008), or activate alternate descending tracts involved in corrective motor commands, such as the rubrospinal (Pettersson et al. 1997) or reticulospinal tracts (Pettersson and Perfiliev, 2002). This latter activation would require less extensive motor cortical adaptations to successfully place the paw on the displaced rung. During the unexpected displacement conditions, however, due to time constraints, already-activated synergies could be modified to accommodate the crosspiece displacement, regardless of whether these synergies are the most efficient for the task or not. This would entail modification of already active neurons in the unexpected displacement conditions, which we have observed, and activation of otherwise inactive neuronal populations, as was observed in the *Kn* condition (Fig. 8A-D).

The differences in neuronal adaptations found in this experiment also suggest that a dynamical model of the motor cortex, which has been posed for reaching tasks (e.g. Churchland et al. 2010, 2012), could potentially be generalized to locomotion as well. Under this framework, there is an optimal neuronal preparatory state for the generation of a given movement task (Churchland and Shenoy, 2007), and the movement proceeds mechanistically from that state (Churchland et al. 2010). Indeed, the idea of a dynamical system guiding behavior is not new; researchers as early as Nikolai Bernstein recognized that the effect of centrally-generated impulses on the body is not constant over time, but rather is strongly dependent on the state of the motor periphery (Bernstein, 1967). During locomotion, the coordination of proprioceptive reflexes among different joints of the leg changes over the course of the movement, and on this basis it was suggested that centrally generated mechanisms act to optimally organize and prepare the periphery for the production of movement behaviors.

Our results support this assertion. Regardless of whether the condition is *Kn*, *Ul* or *Us*, all large steps are of the same size, and all small steps are of the same size. Thus, the spatial constraints on how long the step can be are identical in all 3 test conditions, and all that differs between the conditions is how much time the cat has to prepare. If generation of movements in the motor cortex is occurring as a dynamical system, neuronal discharges should exhibit two specific characteristics. First, because the spatial constraints are identical,

neural activity should be nearly identical. Thus, neuronal activity during long steps should be relatively similar to one another, and neuronal activity during short steps should be similar to one another. We observed that neuronal responses to displacements in the same direction were often similar for all steps of the same size (Fig. 8A,C), suggesting that similar adjustments in neuronal activity are used to make all such steps, regardless of when the crosspiece displaced. Second, when the preparatory state is incorrect due to an unexpected change in the movement environment, neuronal activity should rapidly adjust to converge with the optimal preparatory state, as observed in Ames et al. (2014). In figure 10D and 10E, the similarity in neuronal activity for small and large steps, respectively, between the *Kn*, *Ul*, and *Us* conditions is shown. What we see is that prior to the swing phase onto the moving rung, neuronal activity is most different (stars). Once the swing phase begins, neuronal activity in each of the test conditions converges and becomes very similar to one another.

Motor adaptations do not correspond to energetic cost minimization

It is difficult to reconcile the results of this study with predictions of the expected outcomes from optimal feedback control theory (OFCT) using an energy-minimizing cost function (e.g. Todorov, 2004; Diedrichsen et al., 2009). In the known displacement condition, the cat does not directly observe the crosspiece shift positions – the shift occurs before the cat enters that portion of the corridor. Thus, when the cat first observes the ladder, the crosspiece is already in its final (shifted) position and the cat can plan its movements ahead of time. For this reason, one might expect that the trajectory modifications to step onto the displaced crosspiece in the *Kn* condition would be “optimal” and involve the minimal energetic cost relative to the control step, and that the motor control strategy used in the *Ul* and *Us* conditions might be “sub-optimal” and involve higher energetic cost, as the cat has little time for preparation and must adapt its walking trajectory on the fly. While we were not able to directly measure the energetic costs involved in each condition, the observed kinematic responses do not correspond to this prediction. The observed kinematic responses were far more extensive in the known condition, involving both proximal and distal joints, while the responses in the unexpected conditions primarily involved the more distal joints (Fig. 4). This does not comply with the principle of muscle effort minimization or the related principle of minimal muscle torque change (Nakano et al. 1999).

It appears more likely that the global motor control strategy during locomotion, perhaps including selection of synergies, is determined well in advance of the step in question, and may not correspond to energetic cost minimization. This global strategy may then be tuned to arrive at a *locally* optimal control strategy based on any unexpected or emergent constraints imposed on the behavior. Local optimality may be defined by the minimal kinematic adjustment required to successfully accommodate the disturbance (Patla et al., 2004), or may reflect the simplest adjustment to compute, given the hierarchical relationship between joints (Dounskaia, 2005). However, it appears that there is a fundamental distinction between the neuronal, muscular, and kinematic motor control strategies employed when a constraint is known and planned for, and when one unexpectedly emerges and must be immediately adapted to.

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CITATIONS

Ames KC, Ryu SI, Shenoy KV. Neural dynamics of reaching following incorrect or absent motor preparation. Neuron. 2014; 81(2):438–451. [PubMed: 24462104]

Amos A, Armstrong DM, Marple-Horvat DE. Changes in the discharge patterns of motor cortical neurones associated with volitional changes in stepping in the cat. Neurosci Lett. 1990; 109(1):107–112. [PubMed: 2314625]

Andujar JÉ, Lajoie K, Drew T. A contribution of area 5 of the posterior parietal cortex to the planning of visually guided locomotion: limb-specific and limb-independent effects. J Neurophysiol. 2010; 103(2):986–1006. [PubMed: 20018828]

Armstrong DM, Drew T. Electromyographic responses evoked in muscles of the forelimb by intracortical stimulation in the cat. J Physiol. 1985; 367:309–326. [PubMed: 4057101]

Beloozerova IN, Sirota MG. Activity of neurons of the motosensory cortex during natural locomotion in the cat. Neirofiziologia. 1985; 17(3):406–408.

Beloozerova, IN.; Sirota, MG. The role of motor cortex in control of locomotion. In: Gurfinkel, VS.; Ioffe, ME.; Massion, J.; Roll, JP., editors. Stance and Motion. Facts and Concepts. Plenum Press; New York: 1988. p. 163-176.

Beloozerova IN, Sirota MG. The role of the motor cortex in the control of accuracy of locomotor movements in the cat. J Physiol (L). 1993a; 461:1–25.

Beloozerova IN, Sirota MG. The role of the motor cortex in the control of vigour of locomotor movements in the cat. J Physiol (L). 1993b; 461:27–46.

Beloozerova IN, Sirota MG. Integration of motor and visual information in the parietal area 5 during locomotion. J Neurophysiol. 2003; 90(2):961–971. [PubMed: 12904498]

Beloozerova IN, Sirota MG, Orlovsky GN, Deliagina TG. Activity of pyramidal tract neurons in the cat during postural corrections. J Neurophysiol. 2005; 93:1831–1844. [PubMed: 15525811]

Beloozerova IN, Farrell BJ, Sirota MG, Prilutsky BI. Differences in movement mechanics, electromyographic, and motor cortex activity between accurate and non-accurate stepping. J Neurophysiol. 2010; 103:2285–2300. [PubMed: 20164404]

Bernstein, N. The Co-ordination and Regulation of Movements. Pergamon Press; Oxford, London: 1967. p. 196

Chambers WWV, Liu CN. Corticospinal tract of the cat. An attempt to correlate the pattern of degeneration with deficit in reflex act following neocortical lesions. J Com Neurol. 1957; 108:23–26.

Churchland MM, Shenoy KV. Delay of movement caused by disruption of cortical preparatory activity. J Neurophysiol. 2007; 97:348–359. [PubMed: 17005608]

Churchland MM, Cunningham JP, Kaufman MT, Ryu SI, Shenoy KV. Cortical preparatory activity: representation of movement or first cog in a dynamical machine? Neuron. 2010; 68:387–400. [PubMed: 21040842]

Churchland MM, Cunningham JP, Kaufman MT, Foster JD, Nuyujukian P, Ryu SI, Shenoy KV. Neural population dynamics during reaching. Nature. 2012; 487:51–56. [PubMed: 22722855]

Cortes C, Vapnik V. Support-vector networks. Machine Learning. 1995; 20:273–297.

Diedrichsen J, Shadmehr R, Ivry RB. The coordination of movement: optimal feedback control and beyond. Trends Cog. Sci. 2009; 14:31–39.

Dounskaia N. The internal model and the leading joint hypothesis: implications for control of multi-joint movements. Exp Brain Res. 2005; 166:1–16. [PubMed: 16132966]

Drew T. Motor cortical cell discharge during voluntary gait modification. Brain Res. 1988; 457(1): 181–187. [PubMed: 3167563]

Drew T. Motor cortical activity during voluntary gait modifications in the cat. I. Cells related to the forelimbs. J Neurophysiol. 1993; 70:179–993. [PubMed: 8360715]

Drew T, Jiang W, Kably B, Lavoie S. Role of the motor cortex in the control of visually triggered gait modifications. Can J Physiol Pharmacol. 1996; 74:426–442. [PubMed: 8828889]

Drew T, Kalaska J, Krouchev N. Muscle synergies during locomotion in the cat: a model for motor cortex control. J Physiol. 2008; 586:1239–1245. [PubMed: 18202098]

Emerick AJ, Kartje GL. Behavioral recovery and anatomical plasticity in adult rats after cortical lesion and treatment with monoclonal antibody IN-1. Behav Brain Res. 2004; 152:315–325. [PubMed: 15196799]

Efron, B.; Tibshirani, RJ. Monographs on statistics and applied probability. Vol. 57. Chapman & Hall; New York: 1993. An introduction to the bootstrap.

Fitzsimmons NA, Lebedev MA, Peikon ID, Nicolelis MA. Extracting kinematic parameters for monkey bipedal walking from cortical neuronal ensemble activity. Frontiers in integrative neuroscience. 2009; 3

Friel KM, Drew T, Martin JH. Differential activity-dependent development of corticospinal control of movement and final limb position during visually guided locomotion. J Neurophysiol. 2007; 97(5):3396–4406. [PubMed: 17376849]

Ghosh S. Identification of motor areas of the cat cerebral cortex based on studies of cortical stimulation and corticospinal connections. J Comp Neurol. 1997; 380:191–214. [PubMed: 9100132]

Gregor RJ, Smith DW, Prilutsky BI. Mechanics of slope walking in the cat: quantification of muscle load, length change, and ankle extensor EMG patterns. J Neurophysiol. 2006; 95(3):1397–1409. [PubMed: 16207777]

Jochumsen M, Niazi IK, Mrachacz-Kersting N, Farina D, Dremstrup K. Detection and classification of movement-related cortical potentials associated with task force and speed. J Neu Eng. 2013; 10:056015.

Krouchev N, Kalaska JF, Drew T. Sequential activation of muscle synergies during locomotion in the intact cat as revealed by cluster analysis and direct decomposition. J Neurophysiol. 2006; 96(4): 1991–2010. [PubMed: 16823029]

Lajoie K, Drew T. Lesions of area 5 of the posterior parietal cortex in the cat produce errors in the accuracy of paw placement during visually guided locomotion. J Neurophysiol. 2007; 97(3):2339–2354. [PubMed: 17215501]

Liddell EGT, Phillips CG. Pyramidal section in the cat. Brain. 1944; 67:1–9.

Marigold DS, Andujar JE, Lajoie K, Drew T. Chapter 6--motor planning of locomotor adaptations on the basis of vision: the role of the posterior parietal cortex. Prog Brain Res. 2011; 188:83–100. [PubMed: 21333804]

Marple-Horvat DE, Amos AJ, Armstrong DM, Criado JM. Changes in the discharge patterns of cat motor cortex neurones during unexpected perturbations of on-going locomotion. J Physiol. 1993; 462(1):87–113. [PubMed: 8331599]

Martin JH, Ghez C. Differential impairments in reaching and grasping produced by local inactivation within the forelimb representation of the motor cortex in the cat. Exp Brain Res. 1993; 94:429–443. [PubMed: 8359257]

Martin JH, Donarummo L, Hacking A. Impairments in prehension produced by early postnatal sensory motor cortex activity blockade. J Neurophysiol. 2000; 83:895–906. [PubMed: 10669503]

Meyer DE, Abrams RA, Kornblum S, Wright CE, Keith Smith JE. Optimality in human motor performance: ideal control of rapid aimed movements. Psychological review. 1988; 95(3):340. [PubMed: 3406245]

Milner TE. A model for the generation of movements requiring endpoint precision. Neuroscience. 1992; 49(2):487–496. [PubMed: 1436478]

Mohagheghi AA, Moraes R, Patla AE. The effects of distant and on-line visual information on the control of approach phase and step over an obstacle during locomotion. Exp Brain Res. 2004; 155:459–468. [PubMed: 14770275]

Myasnikov AA, Dykes RW, Leclerc SS. Correlating cytoarchitecture and function in cat primary somatosensory cortex: the challenge of individual differences. Brain Res. 1997; 750:95–108. [PubMed: 9098534]

Nieoullon A, Rispal-Padel L. Somatotopic localization in cat motor cortex. Brain Res. 1976; 105:405–422. [PubMed: 1260454]

Patla AE, Prentice SD, Rietdyk S, Allard F, Martin C. What guides the selection of alternate foot placement during locomotion in humans. Exp Brain Res. 1999; 128:441–450. [PubMed: 10541738]

Patla AE, Moraes R, Lewis MA. Strategies and determinants for selection of alternate foot placement during human locomotion: influence of spatial and temporal constraints. Exp Brain Res. 2004; 159:1–13. [PubMed: 15448958]

Pettersson LG, Lundberg A, Alstermark B, Isa T, Tantisira B. Effect of spinal cord lesions on forelimb target-reaching and on visually guided switching of target-reaching in the cat. Neurosci Res. 1997; 29(3):241–256. [PubMed: 9436650]

Pettersson LG, Perfiliev S. Descending pathways controlling visually guided updating of reaching in cats. Eur J Neurosci. 2002; 16(7):1349–1360. [PubMed: 12405995]

Prilutsky BI, Sirota MG, Gregor RJ, Beloozerova IN. Quantification of motor cortex activity and full-body biomechanics during unconstrained locomotion. J Neurophysiol. 2005; 94:2959–2969. [PubMed: 15888524]

Pryor, K. Lads before the wind. Harper and Row; New York: 1975.

Reitboeck HJ. Fiber microelectrodes for electrophysiological recordings. J Neurosci Methods. 1983; 8:249–262. [PubMed: 6312201]

Sirota MG, Swadlow HA, Beloozerova IN. Three channels of corticothalamic communication during locomotion. J. Neurosci. 2005; 25:5915–5925.

Skinner, BF. The Behavior of Organisms. Appleton-Century; Oxford, England: 1938.

Stark E, Abeles M. Predicting movement from multiunit activity. J. Neurosci. 2007; 27:8387–8394.

Stout EE, Beloozerova IN. Pyramidal tract neurons receptive to different forelimb joints act differently during locomotion. J Neurophysiol. 2012; 107:1890–903. [PubMed: 22236716]

Stout EE, Beloozerova IN. Differential responses of fast- and slow-conducting pyramidal tract neurons to changes in accuracy demands during locomotion. J Physiol. 2013; 591:2647–2666. [PubMed: 23381901]

Stout, EE.; Beloozerova, IN.; Sirota, MG. Changing steps on short notice: Insights from kinematics. Society for Neuroscience; New Orleans, LA: 2012.

Todorov E. Optimality principles in sensorimotor control. Nat Neurosci. 2004; 7:907–915. [PubMed: 15332089]

Trendelenburg W. Untersuchungen uber reizlose vorubergehende Aussaltung am Zentralnervensystem. III. Die extermitaten Region der Grosshirninde. Pflugers Archiv. 1911; 137:515–544.

Vicario DS, Martin JH, Ghez C. Specialized subregions in the cat motor cortex: a single unit analysis in the behaving animal. Exp Brain Res. 1983; 51:351–367.

Weerdesteyn V, Nienhuis B, Hampsink B, Duysens. Gait adjustments in response to an obstacle are faster than voluntary reactions. J Hum Mov Sci. 2004; 23:351–363.

Widajewicz W, Kably B, Drew T. Motor cortical activity during voluntary gait modifications in the cat. II. Cells related to the hindlimbs. J Neurophysiol. 1994; 72:2070–2089. [PubMed: 7884445]

Wolpert DM, Kawato M. Multiple paired forward and inverse models for motor control. Neural Networks. 1998; 11(7):1317–1329. [PubMed: 12662752]

Woodsworth RS. The accuracy of voluntary movements. Psychol Res Monogr [Suppl]. 1899; 3:1–114.

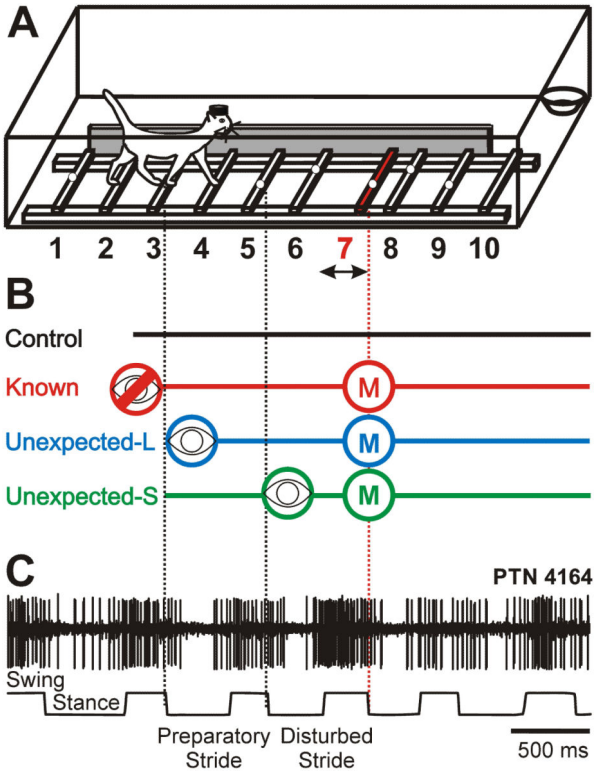

Figure 1.

Experimental Design. **A**: Cats walked through a rectangular, two-side chamber. One side contained a raised horizontal ladder, with one motorized crosspiece (#7, red) that was displaced at different times as the cat walked in the chamber. **B**: A total of seven conditions were analyzed: a control condition with the crosspiece remaining in its central position, when all crosspieces were equally spaced 25 cm apart, and six test conditions when the crosspiece moved away or towards the cat either before the cat stepped on the ladder (two "known" displacement conditions, *Kn*), or one stride away from it (two "unknown" long-notice displacement conditions, *Ul*), or during the current stride while the cat was about to initiate limb transfer to crosspiece #7 (two "unknown" short-notice displacement conditions, *Us*). Circles represent where the cat was along the ladder when the crosspiece displaced ("eye symbol" – visual stimulus) and when the step onto the disturbed rung was made ("M" – motor adaptation). **C**: An example of activity of a neuron (pyramidal tract neuron, PTN 4164) during locomotion along the ladder in the *Ul* long step condition.

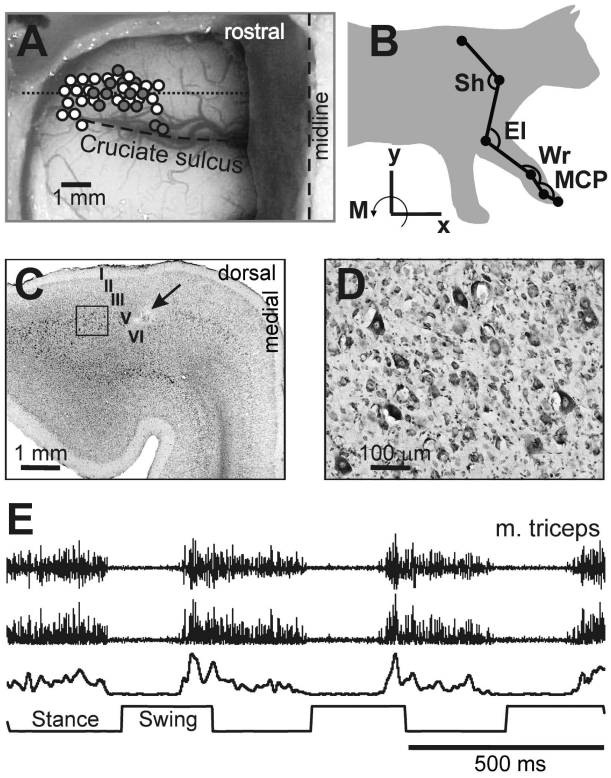

Figure 2.

Area of recording in the motor cortex, definition of forelimb joint angles, and example of muscle activity recording and initial processing. **A:** Area of recording in the forelimb representation of the left motor cortex. Microelectrode entry points into the cortex were combined from cat 1 (dark circles) and cat 2 (white circles) and superimposed on a photograph of cat 2 cortex. The position of the frontal section, whose photomicrographs are shown in C and D, is indicated by a dotted line. **B:** Markers placement for kinematics recording (see text for details) and definition of forelimb joint angles. **C**: Photomicrograph of a 50-μm-thick frontal section through the motor cortex, stained with cresyl violet. Layers of the cortex are numbered. Arrow points to the reference electrolytic lesions. Clusters of giant cells in layer V that are characteristic for area 4γ are visible around the lesions and are shown at a larger magnification in D (the square approximately indicates the area shown). **E:** Photomicrograph of layer V cells of the motor cortex area 4γ, cresyl violet stain. **F:** An example of EMG recording and initial waveform processing. Raw EMG signal (top trace) was rectified (middle trace) and smoothed using central moving average with a time window of 20 ms (bottom trace) prior to analysis.

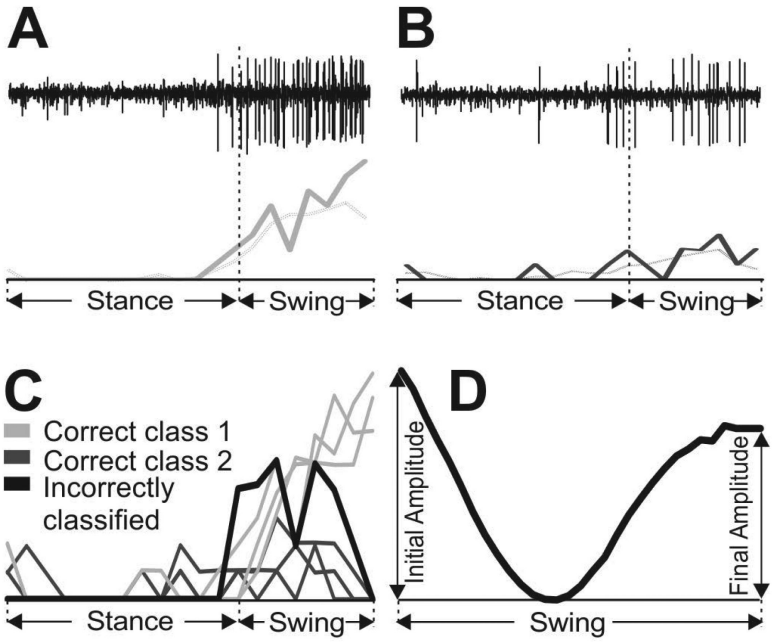

Figure 3.

Support Vector Machine (SVM) classification of neuronal activity and kinematics and EMG waveform metrics. **A, B:** Raw spiking activity of a neuron during a step cycle (top traces) recorded in two crosspiece displacement conditions, “a” and “b”. The raw activity was converted into a frequency histogram of the neuron firing rate (bottom traces; thick line represents that individual step, thin line represents the average for all steps in the condition). Groups of strides made in each condition were split into training and test sets. Strides in the training set were used to develop SVM splitting criteria between the two conditions (see text for details). **C:** Neuronal activity during steps in the test set was classified according to these splitting criteria. If neuronal activity was correctly classified more often than would be expected by chance, the neuron was considered to discharge differently between the two conditions, thus exhibiting a “response”. The classification accuracy in this example was 86%, so the neuron distinguished between the two conditions ($p<0.05$; t-test for proportions). **D**: Profiles of joint angles and EMG activity were compared between conditions using the amplitude of the difference between the initial and minimum value (Initial Amplitude) and the difference between the minimum value and the final value (Final Amplitude) during the swing phase of the step cycle. A typical averaged trace of elbow joint movement during the swing phase is shown.

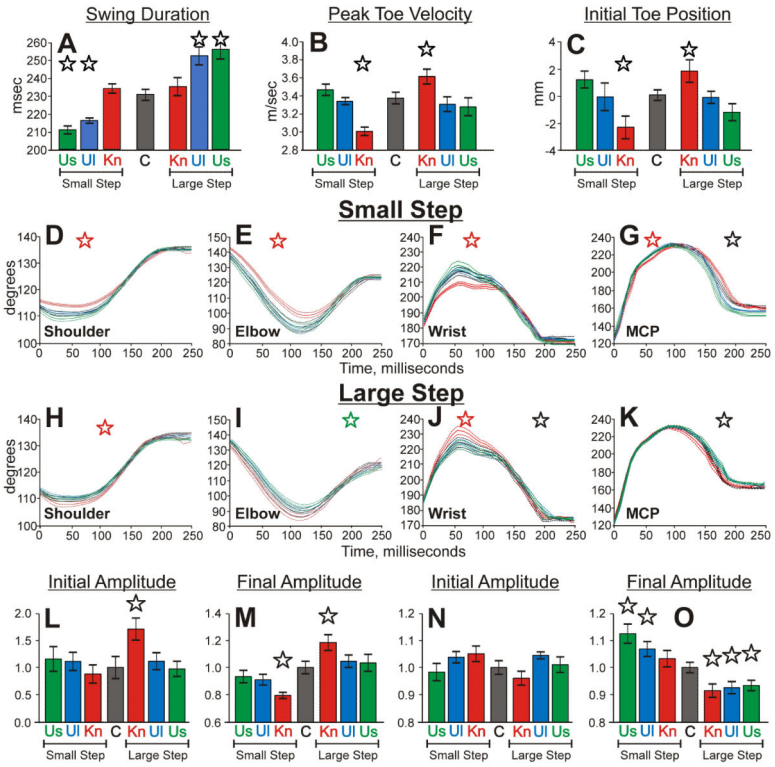

Figure 4.

Kinematic strategies for making smaller and larger than normal steps during known and unexpected crosspiece displacements. Data represent averages between cats and trials except where otherwise noted. **A**: Duration of the swing phase. **B:** Peak velocity of the toe during the swing phase in the direction of cat motion. **C:** Initial position of the toe on the crosspiece in the direction of motion, relative to control. **D-G:** Shoulder (D), elbow (E), wrist (F), and MCP (G) joint angles throughout the swing phase for control and smaller than normal steps. **H-K**: Shoulder (H), elbow (I), wrist (J), and MCP (K) joint angles throughout the swing phase for control and larger than normal steps. In **D-K**, representative examples obtained from one cat on one testing session are shown. **L, M**: The initial and final amplitude for the shoulder joint angle in different conditions. **N, O:** The initial and final amplitude for the MCP joint angle in different conditions. Black represents the control condition (50 cm step), red represents a known displacement requiring a small or large step (45 or 55 cm step, respectively), blue represents an unexpected long-notice disturbance requiring such a step, and green represents an unexpected short-notice disturbance. Stars represent significant differences against the control condition; colored stars represent significant differences between a single condition and control.

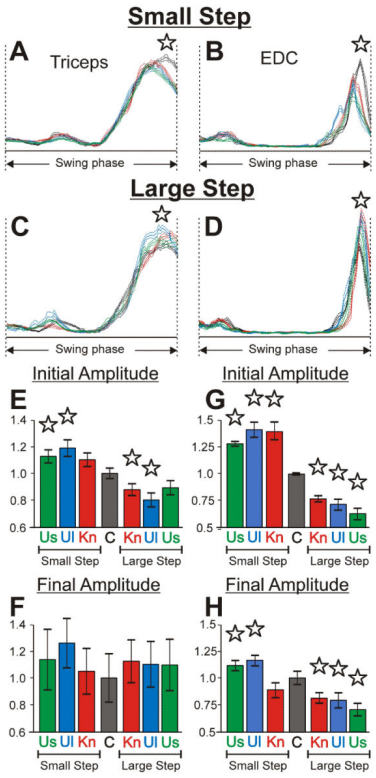

Figure 5.

Muscle activity during smaller and larger than normal steps in known and unexpected crosspiece displacement conditions. **A, B:** Traces of EMG activity in the right triceps medialis (**A**) and right extensor digitorum communis **(B)** muscles during swing phase of small steps. **C, D:** Traces of EMG activity for triceps medialis and EDC during swing phase of large steps. In **A-D**: representative examples obtained from one cat on one testing day are shown. **E-H**: The initial and final amplitudes for right triceps (**E-F**) and EDC (**G-H**) EMG activity during steps. Data are averaged between cats. Other designations as in Figure 4.

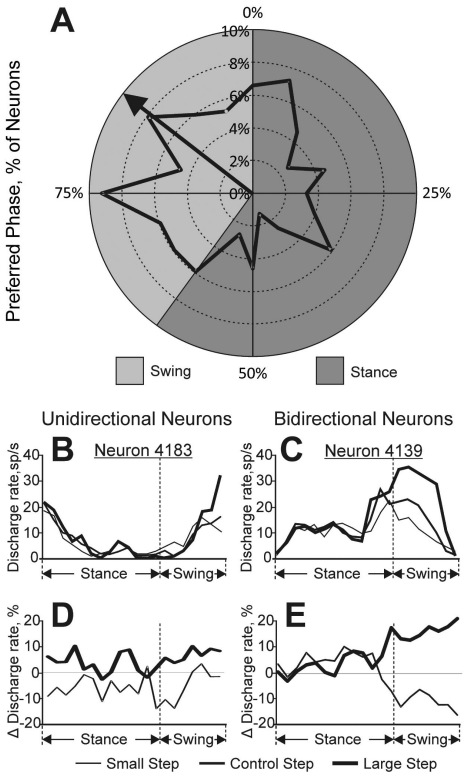

Figure 6.

Neuronal activity and response characteristics. **A:** Population preferred phase of discharge throughout the step cycle and mean population vector (black arrow). Light grey indicates the swing phase of the step cycle, and dark grey indicates the stance phase. **B, C:** Examples of SVM-identified neuronal responses to stepping on a disturbed crosspiece for a unidirectional neuron (Neuron #4183, **B**) and a bidirectional neuron (Neuron #4139, **C**). Thick traces show mean activity during large steps (higher activity), thin traces show that during small steps (lower activity), and medium-thick traces show mean activity during control steps. **D, E:** Average percentage change in discharge rate throughout the step cycle for unidirectional (**D**) and bidirectional (**E**) neurons. Thin lines represent small steps, and thick lines represent large steps.

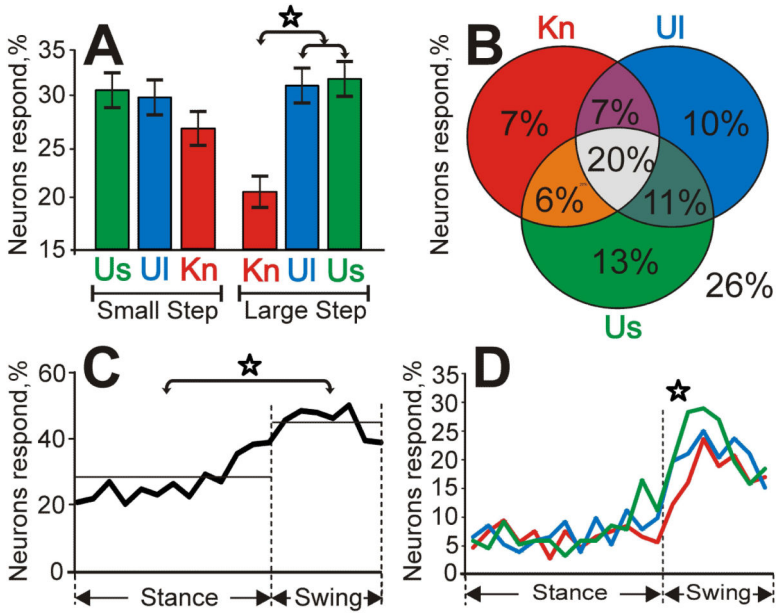

Figure 7.

A: Percentage of neurons showing a response during the disturbed step in the known, unexpected long-notice, or unexpected short-notice crosspiece displacement condition for small, normal, and large steps. **B:** Percentage of neurons showing a response to single or multiple displacement conditions during the disturbed step. For example, the orange area shows the percentage of neurons responding to the known and short-notice unexpected conditions, but not the long-notice unexpected condition. **C:** Percentage of neurons exhibiting significantly different activity (t-test, $p<0.05$) during different phases of the disturbed step. Horizontal bar represents the mean percentage responding during the stance and swing phases. **D:** Percentage of recorded neurons exhibiting significantly different activity (t-test, $p<0.05$), during the disturbed step in each bin between the control condition and crosspiece displacement conditions. Results for large steps are shown, and results for small steps were similar.

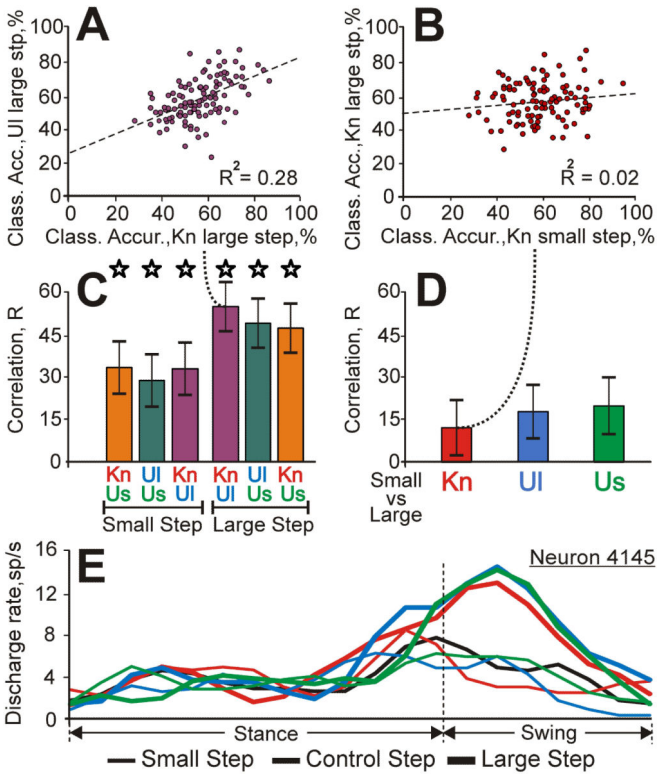

Figure 8.

Relationships in neuronal responsivity between conditions. **A,B:** Representative scatter plots comparing SVM classification accuracy for large steps in the known and unexpected long-notice conditions (**A**), and for small and large steps in the known condition **(B)**. Dotted lines are the regression best-fit trend lines, with correlation R^2 of the best fit shown in the bottom right. **C,D:** Comparison of Pearson correlation (R) between SVM classification accuracy for two crosspiece displacement conditions. **E**: Example of a neuron responding to all large steps and not small steps. Black line shows control steps, thin color lines show small steps, and thick color lines show large steps. Color designations as in Figure 7.

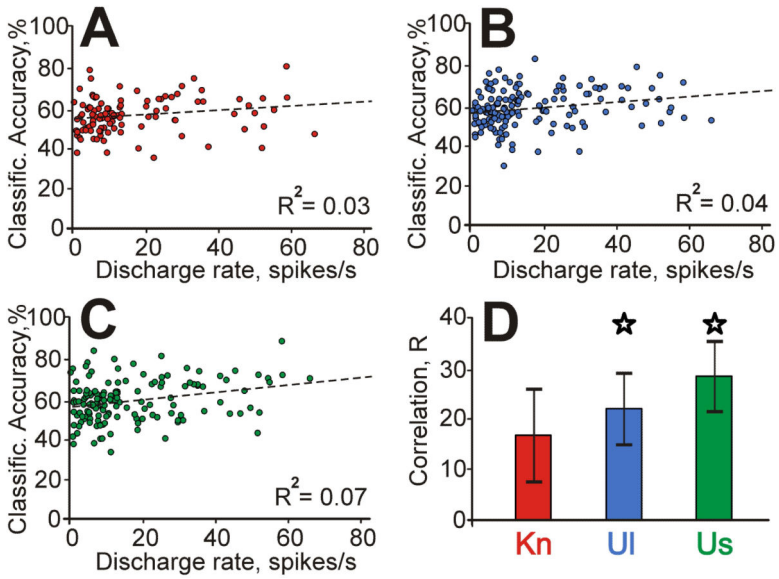

Figure 9.

Characteristics affecting neuronal responsivity **A-C:** Scatter plots comparing mean neuronal discharge rate during the swing phase of the disturbed step with SVM classification accuracy for the known-displacement condition **(A)**, unexpected long-notice condition **(B)**, and unexpected short-notice condition **(C).** Dotted lines are the regression best-fit trend lines, with correlation R^2 of the best fit shown in the bottom right. **D:** Comparison of Pearson correlation (R) between neuronal discharge rate during the swing phase of the control condition and SVM classification accuracy for crosspiece displacement conditions. Color designations as in Figure 6.

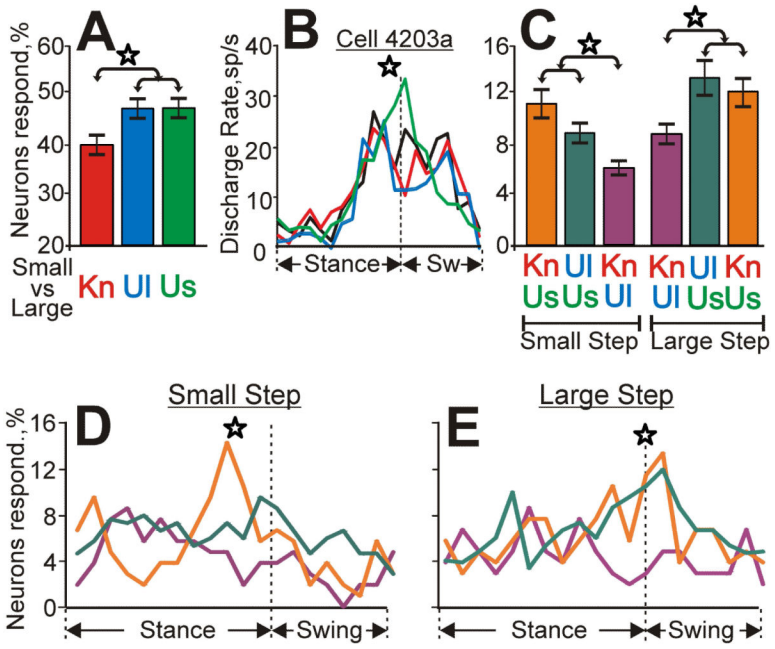

Figure 10.

Directional sensitivity in neuronal responses. **A:** Percentage of neurons exhibiting a different response between crosspiece displacements occurring at the same point of the cat's progression along the ladder, but in different directions. **B:** Example neuron exhibiting a response only during the crosspiece's unexpected short-notice displacement during longer-than-normal steps. **C:** Percentage of neurons exhibiting a different response between crosspiece displacements in the same direction, but occurring at different times. **D, E:** Percentage of neurons exhibiting significantly different activity (*t-test*, $P<0.05$) during different phases of the disturbed step for small (**D**) and large steps (**E**).