J Neurophysiol 130: 838–860, 2023.
First published August 23, 2023; doi:10.1152/jn.00114.2023

american physiological society

JNP JOURNAL OF NEUROPHYSIOLOGY

RESEARCH ARTICLE

Control of Movement

Activity of cat premotor cortex neurons during visually guided stepping

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Abstract

Visual control of steps is critical in everyday life. Several motor centers are implicated in visual control of steps on a complex surface, however, participation of a large cortical motor area, the premotor cortex, in visual guidance of steps during overground locomotion has not been examined. Here, we analyzed the activity of neurons in feline premotor cortex areas 6aa and 6ac as cats walked on the flat surface where visual guidance of steps is not needed and stepped on crosspieces of a horizontally placed ladder or over bar-riers where visual control of steps is required. The comparison of neuronal firing between vision-dependent and vision-independent stepping revealed components of the activity related to visual guidance of steps. We found that the firing activity of 59% of neurons was modulated with the rhythm of strides on the flat surface, and the activity of 83–86% of the population changed upon transition to locomotion on the ladder or with barriers. The firing rate and the depth of the stride-related activity modulation of 33–44% of neu-rons changed, and the stride phases where neurons preferred to fire changed for 58–73% of neurons. These results indicate that a substantial proportion of areas 6aa and 6ac neurons is involved in visual guidance of steps. Compared with the primary motor cortex, the proportion of cells, the firing activity of which changed upon transition from vision-independent to vision-dependent stepping, was lower and the preferred phases of the firing activity changed more often between the tasks.

NEW & NOTEWORTHY Visual control of steps is critical for daily living, however, how it is achieved is not well understood. Here, we analyzed how neurons in the premotor cortex respond to the demand for visual control of steps on a complex surface. We conclude that premotor cortex neurons participate in the cortical network supporting visual control of steps by modifying the phase, intensity, and salience of their firing activity.

accuracy; locomotion; PTN; vision; walking

INTRODUCTION

Locomotion on a complex terrain requires vision. Visual control of steps is critical for most daily activities, however, how it is achieved is not fully understood. Locomotion progresses due to coordinated rhythmic muscle activity, the basic pattern of which is generated by neural circuits in the spinal cord (1). Somatosensory signals from the limbs and body and descending signals from the brain modulate this pattern to adapt locomotor movements to conditions of the terrain (reviewed, e.g., in Refs. 2–5). Visual perception of the walking path is key for adaptation of the movements to a complex surface. How visual information about the walking surface is processed and adjusts the basic locomotor pattern is not well understood. This hampers construction of mechanistic models of nervous control of natural locomotion, which complicates development of rehabilitation approaches for people suffering from an inability to walk on complex surfaces.

In the cerebral cortex, several areas are implicated in control of visually guided stepping (reviewed, e.g., in Refs. 4 and 6). However, the contribution of a large motor area, the premotor cortex, to the visual control of steps on a complex surface has been only examined in a handful of studies. Here, we analyzed the activity of neurons in the premotor cortex during locomotion on the simple and complex surfaces with a goal to better understand how this cortical area may contribute to visual control of steps.

It was shown that lesions or inactivation in premotor cortex of humans and nonhuman primates cause limb-kinetic apraxia, i.e., inability to make accurate limb movements, as

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Submitted 15 March 2023 / Revised 13 July 2023 / Accepted 11 August 2023

well as inability to shape the hand correctly for grasping objects and avoid obstacles during reaching for objects (7–9). In Macaca monkeys, it was found that the discharge of two thirds of neurons in the dorsal premotor cortex correlates with the size of the target to which the monkey reaches, and thus with the required accuracy of the reach (10). For locomotion, it was shown in both monkeys and cats that the firing activity of neurons in premotor cortex is modulated with the rhythm of strides during treadmill locomotion (11, 12). In both humans and cats, neuronal activity changes when the subject encounters an obstacle on the treadmill belt (12, 13). In humans, the spectral power of the electrocortical signal increases in the 3–13-Hz frequency band shortly after an obstacle appears on the treadmill belt (13), and in cats, the activity of half of the neurons changes during strides leading up to an obstacle approaching on the treadmill belt and during stepping over the obstacle (12). However, treadmill locomotion complicates and likely alters normal visuomotor coordination by presenting a situation that almost never occurs naturally, namely, the locomotion that does not result in a progressive movement through the environment. How the activity of neurons in the premotor cortex is related to visually guided steps during normal overground locomotion has not been examined. Here, we have investigated it.

We used cats as experimental subjects because the activity of the cerebral cortex during locomotion is best studied in this specie. This includes the extensively researched feline primary motor cortex (14–28), secondary motor cortex (29), primary somatosensory cortex (30), and posterior parietal cortex (31–36). This substantial database provided background for the evaluation of how the premotor cortex may contribute to visual guidance of steps. We recorded single neuron activity in premotor area 6aa, the area of premotor cortex with the highest visual responsiveness (37), and adjacent region of area 6ac testing how this activity changes as cats walk on a flat surface, a horizontally placed ladder, or step over a series of barriers. Locomotion on the ladder and with barriers requires visual control of steps, whereas locomotion on the flat surface does not (e.g., Ref. 31). Comparison of neuronal activity during vision-demanding and vision-nondemanding locomotion tasks allowed us to isolate components of the activity that are related to control of visually guided stepping.

We found that during locomotion on the flat surface, the firing activity of 59% of neurons in premotor areas 6aa and 6ac is modulated with the rhythm of strides and the discharge of 83–86% of the population changes upon transition to visually guided stepping on the ladder or over barriers. We describe these changes and argue that the degree of neuronal participation in each particular activity change in these cortical areas is less than in the primary motor, primary somatosensory, or posterior parietal cortices, and that the manner of the participation is different. We conclude that premotor areas 6aa and 6ac are a part of the cortical network supporting visually guided stepping on the complex surface, and that they have their unique contribution to this vital function.

A brief account of a part of this study was published in abstract form (38).

METHODS

Experimental Strategy

Two tasks were initially used: locomotion on a flat surface where no vision was required, and locomotion on tops of 5-cm-wide crosspieces of a horizontally placed ladder that required visual control of steps (Fig. 1A). A number of studies showed that locomotion on the flat surface does not require vision and can be accomplished without the forebrain, while stepping on a complex surface, such as the ladder, relies on vision and participation of the cortex (17, 39–47). Thus, the neuronal activity in the cortex during locomotion on the flat surface is primarily related to the locomotor movement per se, whereas the activity during vision-guided stepping on the ladder represents a combination of the activity related to the locomotor movement and processing of visual information about the available site(s) for stepping and generation of a command for the required adjustment of the stride. When the biomechanics of the locomotor movement on the ladder are similar to those on the flat surface, the visual processing- and command generation-related components of neuronal activity can be isolated by subtracting the activity during locomotion on the flat surface from that on the ladder (e.g., Refs. 17, 22, 30, 31, 36, 42). We have previously shown that

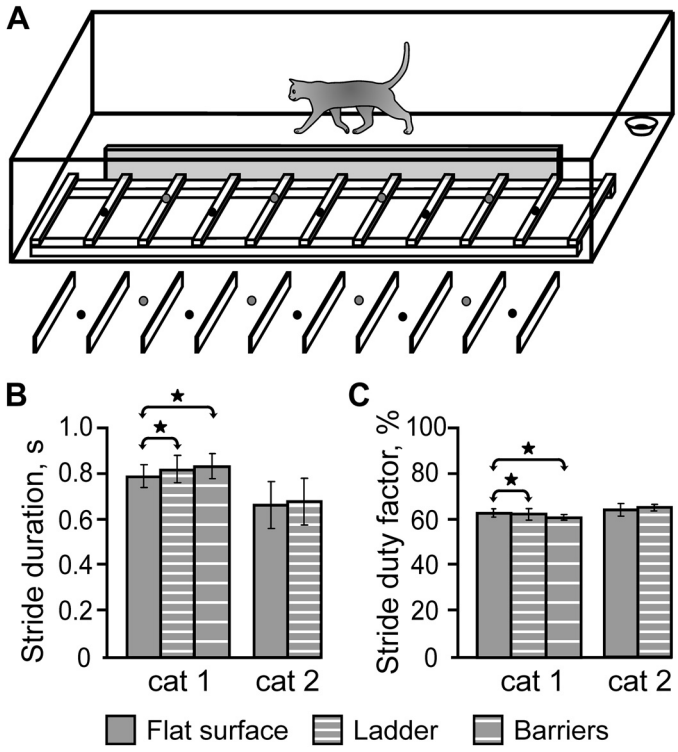

Figure 1. Locomotion tasks. A: the experimental box was divided into corridors. In one corridor, the floor was flat, whereas the other corridor(s) contained a horizontal ladder or a series of barriers. Cats walked with a self-selected speed. Filled circles on the crosspieces of the ladder and in-between barriers schematically show placements of the right (black) and left (gray) paws. See text for description of the ladder and barriers. B: duration of strides during locomotion on the flat surface, along the ladder, and, for cat 1, with barriers. C: stride duty factor (proportion of the stance phase in the step cycle) during locomotion on the flat surface, along the ladder, and with barriers. In B and C, error bars show SDs, stars indicate statistically significant difference between locomotion tasks ($P < 0.05$, t test; n, number of strides).

when the crosspieces of a horizontal ladder are placed at the distance of the cat's normal step length and have flat tops that are wide enough to fully support the paw, the biomechanics of ladder locomotion are very close to those on the flat surface (22). Thus, comparing neuronal activity during vision-dependent locomotion on such a convenient ladder with that during vision-independent locomotion on a flat surface allows us to reveal the components of neuronal activity that are related to the processing of visual information and generation of a command for accurate stepping on the ladder.

The activity of neurons of one of the cats was also tested as the cat stepped over a series of barriers. The 7.5-cm-tall barriers were placed at the same intervals as the crosspieces of the ladder, each obstructing the entire width of the walkway (Fig. 1A). The barriers were 0.5-cm thick, so the cat could not step on top of them but had to step over. During stepping over barriers, limbs had to be lifted higher than during locomotion on the ladder. This test allowed us to examine how muscle activity necessary to lift limbs high while stepping on a complex surface is reflected in discharges of premotor cortex neurons.

Animals

Extracellular recordings from single neurons in the premotor cortex areas 6aa and 6ac were obtained during chronic experiments in two adult domestic cats (Felis catus). The experimental protocol was in compliance with the National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals and was approved by the Barrow Neurological Institute Animal Care and Use Committee. A female (cat 1, 4.0 kg) and male (cat 2, 3.8 kg) cats were purchased from a certified commercial class B dealer. Data on the activity of motor, somatosensory and posterior parietal cortex, and the thalamus during vision-independent and vision-guided locomotion obtained from cat 1 have been included in previous publications (22, 25, 26, 30, 36, 48, 49). In addition, data on the movement of the head and gaze during vision-independent and vision-guided locomotion obtained from cat 1 were reported in Refs. 50–53.

Locomotion Tasks

Positive reinforcement by food was used to adapt cats to the experimental situation and to engage them in locomotion (54, 55). Cats were trained to walk in an experimental chamber that was an enclosure with two or three connected corridors 2.5 0.3 m each. In one corridor, the floor was flat, whereas the other corridor(s) contained a horizontal ladder or a series of barriers. The centers of the ladder crosspieces were spaced 25 cm apart, equal to one-half of the cat's average stride length during locomotion in the chamber with flat floor (17, 22). The crosspieces had flat tops 5-cm wide, which was only slightly greater than the 3-cm diameter support area of the cat paw (e.g., Ref. 56). During locomotion on the ladder, each limb overstepped two gaps and every other crosspiece of the ladder (Fig. 1A).

Ten 7.5-cm-tall barriers 0.5 cm wide were spaced 25 cm apart, at the same distance as crosspieces of the ladder. Each barrier obstructed the whole width of the walkway. During unobstructed walking in our experimental chamber, cats lift limbs by 2.5–4.0 cm (22, 25, 57). Thus, 7.5-cm-tall obstacles forced the cat to lift limbs two to three times higher than normal. Only cat 1 was tested with barriers.

While going around the chamber, cats passed through the corridors sequentially and repeatedly, occasionally changing direction from clockwise to counterclockwise. After each round, food was dispensed into a feeding dish in one of the corners. Cats were trained, upon arrival, to stand in front of the feeding dish quietly for 3–5 s. One second in the middle of this period was considered as "standing."

Cats were accustomed to wearing a cotton jacket, a light backpack with electrical connectors, and a sock with a small metal plate on the sole of the right forelimb paw for recording paw contact with the floor and ladder's crosspieces. The floor in the chamber and the crosspieces of the ladder were covered with an electrically conductive rubberized material. During locomotion, the duration of the swing and stance phases of the right forelimb, which was contralateral to the left cortex where the activity of neurons was recorded (see below), was monitored by measuring the electrical resistance between the plate on the sock and the floor. We refer to the full movement cycle of the right forelimb (from the beginning of the swing to the beginning of the next swing) as a step cycle or stride and use these terms interchangeably.

Surgical Procedures

Methods of surgical preparation and recording techniques have been described in detail previously (58). Briefly, the skin and fascia were retracted from the dorsal surface of the skull. At 10 points around the circumference of the skull, stainless steel screws were implanted. The screw heads were then embedded into a plastic cast that formed a circular base. Later, this base was used for the fixation of electrical connectors, electrode microdrives, and preamplifiers, and to rigidly hold the cat's head while searching for neurons. On the left side of the head, the dorsal surface of the premotor, motor, and somatosensory cortex was exposed by removing 1.4 cm^2 of bone and dura mater (Fig. 2, A and B). The aperture was covered with a 1-mm thick acrylic plate, which was preperforated with holes of 0.36 mm in diameter spaced by 0.5 mm. The holes were filled with a bone wax and petroleum jelly mixture. This plate allowed access to the cortex in the awake animal for recording neuronal activity. In addition, in cat 1, two 26-gauge hypodermic guide tubes fitted with stainless-steel wires were implanted 7 mm above the left medullary pyramidal tract. Later, in the awake cat, a 200-μm platinum-iridium wire (A-M Systems, Carlsborg, WA) insulated with Teflon to within 0.4 mm of the tapered tip was inserted into the medullary pyramid using physiological guidance (58) to serve as a stimulation electrode for identification of premotor cortex neurons with axons descending within the pyramidal tract, the pyramidal tract neurons (PTNs). A cap covered the elements of the head base for mechanical protection and electrical shielding of recording circuits.

Single-Unit Recording and Neuron Identification

Tungsten varnish-insulated microelectrodes (120 μm; FHC Inc., Bowdoin, ME) were used to record electrical activity of neurons (impedance 1–3 MX at 1,000 Hz). A lightweight (2.5 g) manual single-axis micro-manipulator chronically mounted

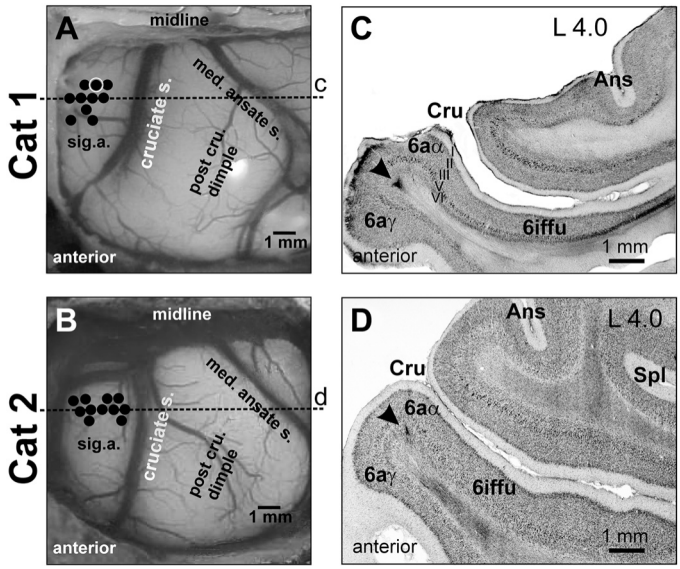

Figure 2. The region of recording in the premotor area 6. A and B: the top view of the live cortex of cat 1(A) and cat 2 (B). Microelectrode entry points into the cortex are shown by black circles. In A, the microelectrode track is highlighted by a white ring from where the PTN was recorded, the activity of which during locomotion is shown in Fig. 3. In A and B, horizontal dashed lines c and d indicate positions of the parasagittal sections shown in C and D, respectively. C: a photomicrograph of a parasagittal section of cat 1 brain at approximately 4 mm laterality. An arrowhead points to a reference lesion made in the area of recordings. Layers of the cortex are numbered. Layer V, which contains giant pyramidal cells characteristic for area 6aa, is visible. D: a photomicrograph of a parasagittal section of cat 2 brain at approximately 4 mm laterality. The arrowhead points to a reference lesion in the area of recordings. C and D: cresyl violet stain. Ans, ansate sulcus; Cru, cruciate sulcus; PTN, pyramidal tract neuron; sig.a., anterior sigmoid gyrus; Spl, splenial sulcus.

on the cat's head was used to advance the microelectrode. Signals from the microelectrode were preamplified with a preamplifier on the cat's head and further amplified and filtered (0.3–10 kHz band pass) with the CyberAmp 380 (Axon Instruments). After amplification, signals were digitized with a sampling frequency of 30 kHz and recorded using a data acquisition and analysis package (Power-1401/Spike2 System, Cambridge Electronic Design, Cambridge, UK). The Power-1401/Spike2 waveform-matching algorithm was used to initially identify and isolate spikes of single neurons. Only well-isolated neurons were used for further analyses.

The somatosensory receptive fields (RFs) of the neurons were examined in animals sitting with their head restrained. Stimulation was produced by lightly stroking fur, palpation of the muscle bellies and tendons, and passive movements around limb joints. In cat 1, each well-isolated neuron was tested for antidromic activation before, during, and after every locomotion test using 0.2 ms rectangular pulses of graded intensity in the range of 0.1–0.5 mA. The criterion for identification of antidromic responses was the test for collision of spikes (24, 59, 60). Stimulation pulses typically did not evoke any visible motor responses and never produced any signs of discomfort or distress in the cat.

Processing of Neuronal Activity

From the four or five strides that the cat took along each corridor (Fig. 1A), two or three strides in the middle were selected for the analyses. The strides were further selected so that their average duration during locomotion on the flat surface, ladder, or with barriers during each session differed by no more than 10%. Selecting strides of a similar duration minimized potential differences in the activity of neurons due to the difference in the speed of locomotion on different surfaces. Each group of selected strides contained at least 15 strides. The onset of the swing phase of the right forelimb was taken as the beginning of the step cycle. The step cycles were time normalized. The duration of each cycle was divided into 20 equal bins, and a phase histogram of the firing rate of each neuron during the cycle was generated. The phase histogram was smoothed by recalculating the value of each bin as follows: $F_n^0 = 0.25F_{n1} + 0.5F_n + 0.25F_{n+1}$, where F_n is the original value of a bin.

To quantify the modulation of firing activity of individual neurons, we used two coefficients. The "depth of modulation," dM, was calculated as dM = (Nmax − Nmin)/N 100%, where Nmax and Nmin are the number of spikes in the maximal and minimal histogram bin, and N is the total number of spikes in the histogram. The coefficient of modulation, M, was calculated as M = (1 Fmin/Fmax) 100%, where Fmax and Fmin are the maximal and minimal frequencies of discharge in the histogram. The firing of neurons with dM > 5.2% or M > 65% was judged to be modulated with the rhythm of strides. This was based on an analysis of fluctuation in the activity of neurons in the standing animal. For this analysis, the activities of 100 neurons (55 from cat 1 and 45 from cat 2) recorded while the cat was standing in front of the feeding dish were processed as if the cat was walking (24, 61). The timing of steps made by the same cat was used to construct the histogram. Supplemental Fig. S1 shows an example result (all Supplemental Material is available at https://doi.org/10.6084/m9.figshare.22270321). This analysis revealed that during standing, the value of the dM exceeded 5.2% for only five neurons, and the value of M exceeded 65% for only five neurons. Therefore, when the dM of the activity of a neuron was greater than 5.2% or the M was greater than 65% during locomotion, we assumed, with 95% confidence, that this modulation was stride-related. Two coefficients were used jointly to increase objectivity of the classification.

To characterize the variability of the discharge over the step cycle, the portion of the step cycle, in which the discharge rate exceeded the value of the minimal rate plus 25% of the difference between the maximal and minimal rates in the histogram, was defined as the "period of elevated firing" (PEF). PEFs were smoothed by removing all one-bin peaks and troughs. For neurons with a single PEF per cycle, the "preferred phase" (PrPh) of the activity was calculated using circular statistics (62–65). For neurons with more than one PEF per cycle, the phase of each PEF was assessed by visual examination of the histogram.

The following parameters of the activity were calculated for each neuron: the mean discharge rate, dM, the number of PEFs, duration of PEF(s), and, for neurons with a single PEF per step cycle, the PrPh. For each neuron, the difference in each activity parameter between locomotion on the flat surface and the ladder or with barriers was determined. For the comparison of the discharge frequency during different tasks, the Student's two-tailed t test was used. When

comparing dMs, PrPhs, and durations of PEFs, differences equal to or greater than 2%, 10%, and 20%, respectively, were considered significant. These criteria were adopted to be same as for the activity of neurons in the cat motor cortex for which they were determined based on the results of a bootstrapping analysis that compared differences in the activity parameters between various reshufflings of strides of the same locomotion task (24, 66).

For populations of neurons, the following activity parameters were calculated and compared between locomotion tasks: the percentage of neurons at their PEF during different phases of the step cycle, the distribution of the average discharge frequency of the population over the step cycle, the average dM, and the average width of PEF(s). When data were categorical, a nonparametric v^2 test (Fisher's two-tailed test) was performed. The significance level for all tests was set at 0.05. Unless noted otherwise, for all mean values, the standard error of the mean (SE) is given.

Histological Procedures

On the day of termination, cats were deeply anesthetized with pentobarbital sodium, and reference electrolytic lesions were made in the areas of recording and stimulation. Cats were perfused with 4% paraformaldehyde solution, and brains were harvested. Frozen brain sections, 40-μm thick, were cut in the regions of recording and stimulation. The tissue was stained for the Nissl substance with cresyl violet or thionine. The positions of recording tracks in premotor cortex were estimated in relation to the reference lesions (Fig. 2, C and D). In cat 1, the position of the stimulation electrode in the medullary pyramids was verified. Further details of histological examination can be found in Refs. 30 and 48.

RESULTS

Area of Recording and Neuronal Populations Studied

Recordings were obtained from the medial part of the anterior sigmoid gyrus and the most dorsal part of the proreus gyrus. Figure 2, A and B, shows the entry points of microelectrode penetrations in which neurons were recorded in cat 1 (Fig. 2A, 10 penetrations) and cat 2 (Fig. 2B, 12 penetrations). Histological examination showed that the microelectrode tracks were made through the cytoarchitectonic areas 6aa and 6ac (67–70). Photomicrographs of parasagittal sections through the area of recordings are shown in Fig. 2, C and D, and feature reference lesions next to clusters of large pyramidal cells in cortical layer V, which are characteristic for area 6aa.

The activity of 201 neurons was analyzed: 133 in cat 1 and 68 in cat 2. Between 1 and 30 neurons were recorded in a penetration, 13.3 ± 10.0 in cat 1 and 5.7 ± 3.4 in cat 2 (means ± SD). In cat 1, 33 neurons were identified as PTNs because they responded antidromically to stimulation of the pyramidal tract through an electrode implanted there in this cat. All but one penetration yielded PTNs, 1–7 per penetration. Latency of responses ranged from 0.8 to 8.0 ms. The distance between electrodes in the medullary pyramidal tract and recording sites in the cortex was estimated at 51.5 mm (which included the curvature of the pathway and the spread of current and refractory period at the site of stimulation). Thus, the conduction velocities of PTNs were estimated to range between 6.4 m/s and 64 m/s. Slightly over a half of PTNs (n ¼ 19) responded with latency of 2.5 ms or less, thus conducting faster than 21 m/s, and were identified as fast-conducting PTNs (fPTNs) according to criteria of Takahashi (71) (see also Ref. 24). Other PTNs (n ¼ 14) responded with longer delays, conducting slower than 21 m/s, and thus were identified as slow-conducting PTNs (sPTNs).

Responses of 58 neurons in cat 1 and 49 neurons in cat 2 to somatosensory stimulation were tested. The majority of these neurons (64%, 60/107) did not respond. Of the 47 neurons responding, 29 were activated by a movement of the contralateral (right) forelimb or palpation of its muscles: on the shoulder (n ¼ 6), elbow (n ¼ 9), wrist (n ¼ 5), or the entire limb (n ¼ 9). Many of these neurons were also activated by a movement of the contralateral (right) hindlimb or palpation of its muscles: on the hip (n ¼ 2), knee (n ¼ 2), ankle (n ¼ 2), or the entire limb (n ¼ 5). Two neurons were activated exclusively by palpation of body muscles and four by palpation of neck muscles or head movement.

Locomotor Activity

During recording of each neuron, the cat walked between 5 and 50 times (25 ± 10; mean ± SD) down each corridor. For each neuron, the number of strides selected for the analysis according to the criteria described in the METHODS was 41 ± 15 for the flat surface, 37 ± 10 for the ladder, and 38 ± 12 for the barriers (means ± SD).

Cat 1 walked slower than cat 2 with 794 ± 58 ms long strides (n = 3,459) on the flat surface and 822 ± 66 ms strides (n = 4,814) on the ladder compared with 668 ± 105 ms and 685 ± 100 ms long strides (n = 1,422 and n = 2,373) of cat 2, respectively (means ± SD, Fig. 1B). These stride durations corresponded to the locomotion speed of 0.6 m/s for cat 1 and 0.7 m/s for cat 2. Cat 1 walked slightly slower on the ladder than the flat surface and still slower when stepping over barriers, however, the difference was small, 3.5% and 5%, respectively. The difference in the stride duty factor, which is the proportion of the stance phase in the step cycle and characterizes the structure of the stride, was similarly small, 1.5% and 4.1%, respectively (Fig. 1C). Cat 2 walked with similar speeds and stride duty factors on the flat surface and ladder (Fig. 1, B and C).

Neuronal Firing at Rest and during Locomotion on the Flat Surface

When the cat was sitting or standing, almost all neurons 96% (193/201) were active. The average firing rate of the entire population was 7.5 ± 0.5 spikes/s. Neurons responsive to somatosensory stimulation tended to be more active than nonresponsive cells, discharging 10.1 ± 1.3 versus 7.1 ± 0.8 spikes/s, respectively (P ¼ 0.059, t test). PTNs fired at rates two and a half higher than nonidentified neurons (noIDs) recorded in the same microelectrode tracks (14.4 ± 1.6 vs. 5.4 ± 0.4 spikes/s, respectively; P < 0.001, t test). Among PTNs, fPTNs were more active than sPTNs, discharging 17.2 ± 2.2 versus 10.8 ± 2.2 spikes/s, respectively (P ¼ 0.046, t test).

When the cat walked on the flat surface, 98% (196/201) of neurons were active. The activity of an example neuron is shown in Fig. 3, A–C. This was a fPTN responding to stimulation of the pyramidal tract with a 1.5 ms delay (Fig. 3A, inset A1). In the resting cat, the neuron was activated by palpation

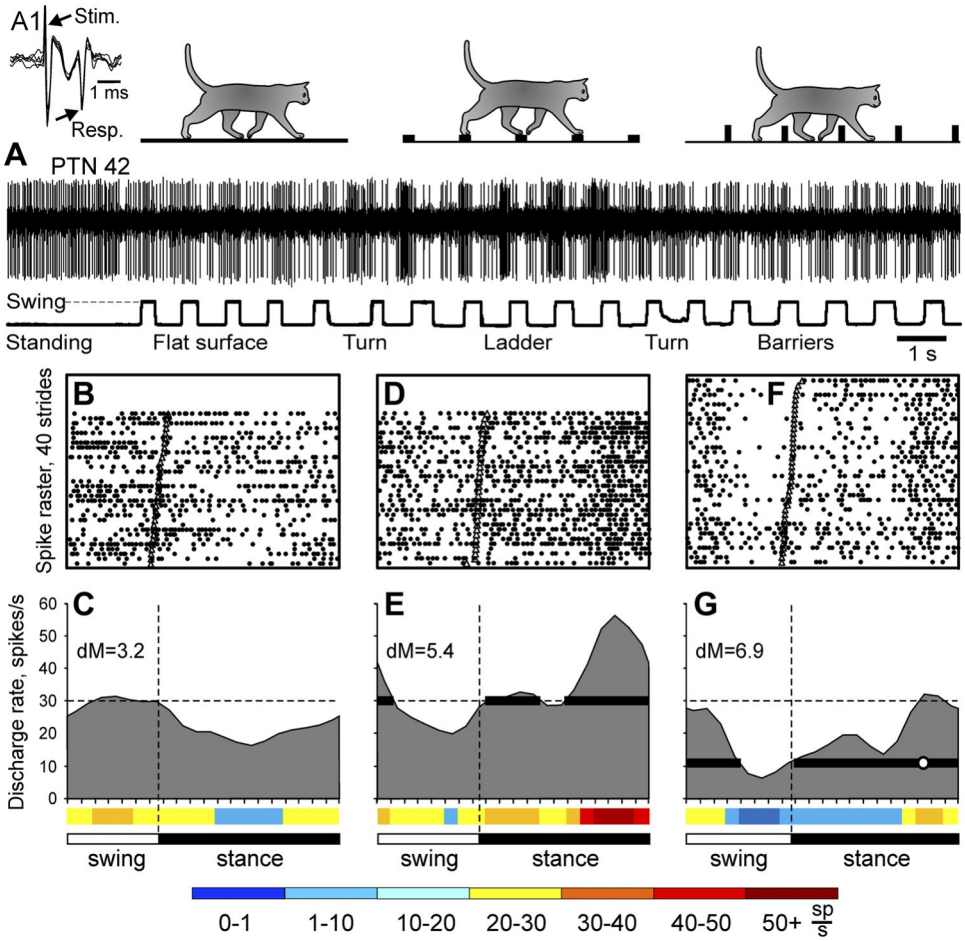

Figure 3. Example activity of a neuron during locomotion on the flat surface, along the horizontally placed ladder, and while overstepping barriers. A–G: the activity of a pyramidal tract neuron (PTN #42) during locomotion. This PTN recorded in cat 1was located medially in the anterior sigmoid gyrus. In Fig. 2A, the track where it was encountered is highlighted with a white ring. The neuron responded to electrical stimulation of the medullary pyramid with a 1.5 ms delay. Insert A1 shows five superimposed traces of the PTN's response (Resp.) to stimulation of the medullary pyramidal tract (Stim.). A: the firing of the PTN during standing and one round of locomotion. Silhouettes of cats at top indicate locomotion on the flat surface (left), the ladder (middle), and with bar-riers (right). Bottom trace shows the swing (deflection up) and stance (deflection down) phases of the step cycle of the right forelimb. The first 2.5 s of the record (far left) show the firing of the neuron while the cat was standing. The variability of the discharge during standing is illustrated in the Supplemental Fig. S1. B and C: the activity of the same PTN during locomotion on the flat surface is presented as a raster of 32 step cycles (B) and a histogram (C). In the raster, the duration of step cycles is normalized to 100%, and the cycles are rank-ordered according to the duration of the swing phase. The beginning of the stance phase in each step cycle is indicated by an open triangle. In the histogram, the horizontal interrupted line shows the firing rate of the neuron dur-ing standing. The value of the depth of modulation (dM) is indicated. The rainbow bar below the histogram shows the average discharge frequency of the neuron in each 1/20th portion of the step cycle, color-coded according to the scale at bottom of the figure. Periods of the swing and stance phases of the stride of the right forelimb are indicated by white and black horizontal bars, respectively. D and E: activity of the same PTN during locomotion on the ladder. In E, the horizontal black bar indicates the period of elevated firing (PEF). F and G: activity of the same PTN during stepping over barriers. In G, the horizontal black bar indicates the PEF, and the white circle shows the preferred phase of the activity (PrPh). PTN, pyramidal tract neuron.

of the elbow, wrist, hip, and knee. During standing, it fired with an average frequency of 30 spikes/s. During locomotion on the flat surface, the frequency of discharge was slightly lower, 24 spikes/s, and tended to be weakly modulated with the rhythm of strides (Fig. 3A, left). The raster in Fig. 3B shows that the activity of the neuron varied somewhat between strides and generally was higher during the swing phase. The activity is summed in Fig. 3C, which shows distribution of the firing rate across 32 strides. The discharge peaked at 31 spikes/s in the middle of the swing phase and was 15–18 spikes/s in the middle of stance. The depth of modulation dM was 3.2, and the coefficient of modulation M was 53.3, which classified the firing of the neuron as stride rhythm unmodulated (see METHODS for the dM and M definitions and activity classification criteria). The firing during locomotion on the ladder or with barriers was classified as stride-modulated (Fig. 3, D–G), and the period of elevated firing (PEF) was indicated by a black horizontal bar (Fig. 3, E and G). When there was only one PEF per cycle as during locomotion with barriers, the preferred phase of the activity (PrPh) was depicted with a white circle on the PEF bar (Fig. 3G). Rainbow bars below the firing rate distribution graphs show the average firing rate in each 1/20th portion of the step cycle, color coded according to the scale at the bottom of the figure.

The average discharge across all neurons during locomotion on the flat surface was similar to that during sitting or standing, 7.6±0.5 spikes/s (P ¼ 0.6, t test). However, the firing rate of individual neurons often changed when locomotion began. In Fig. 4A, the mean firing rate of individual

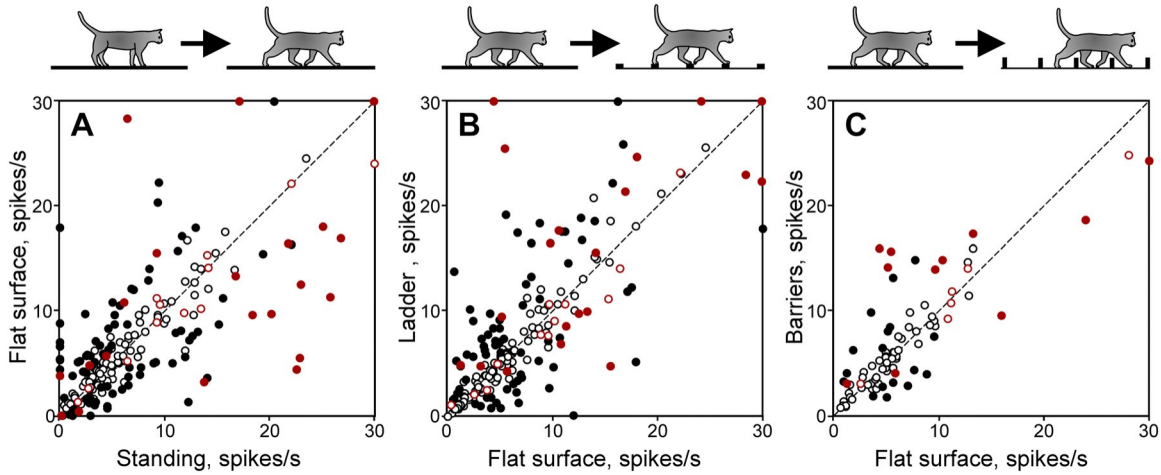

Figure 4. Comparison of the firing rate of individual neurons between different tasks. A: comparison of the firing rate of individual PTNs (red symbols) and noIDs (black symbols) during sitting or standing and locomotion on the flat surface. The x- and y-axes of each point show the firing rate of a neuron during sitting or standing and locomotion, respectively. Neurons whose firing rate was statistically significantly different between the tasks are shown by filled symbols, the others are shown by open symbols. B: comparison of the firing rate of neurons during locomotion on the flat surface and ladder. C: comparison of the firing rate of neurons during locomotion on the flat surface and with barriers. noIDs, nonidentified neurons; PTNs, pyramidal tract neurons.

neurons during locomotion is plotted against that during sitting or standing. The firing rate of a quarter of neurons (50/201) was greater during locomotion than sitting or standing. These typically were cells with a low firing rate at rest (5.4±0.8 spikes/s), and their population firing doubled during locomotion (to 11.2±1.4 spikes/s, P ¼ 0.001, t test). The firing rate of another quarter of neurons (52/201) was lower during locomotion than sitting or standing. Typically, these were cells with a relatively high firing rate at rest (10.2±1.1 spikes/s), and their population firing halved during locomotion (to 5.4±0.7 spikes/s, P < 0.001, t test). PTNs did not have a preference for increasing, decreasing, or not changing the discharge rate between sitting or standing and locomotion, and the same was true for somatosensory responsive cells.

During locomotion, the discharge of 59% (115/196) of neurons was modulated with the rhythm of strides, i.e., their firing rate was higher during some phase(s) of the stride and lower during other phase(s). Neurons had either one period of elevated firing (1-PEF) or two periods (2-PEF). Three cells had three PEFs, and they will be considered jointly with the 2-PEF group. The 1-PEF pattern was more common, expressed by 39% (77/196) of cells, whereas 19% (38/196) had two PEFs (P < 0.001, v^2 test). The average dM was similar between the 1-PEF and 2-PEF groups, 8.5±0.3 across all. The average duration of the PEF in the 1-PEF group was 63±2.5% of the cycle, and the combined duration of the PEFs in the 2-PEF group was similar, 62±2.0%. In both subpopulations, PEFs of different neurons were distributed over the step cycle and overlapped (Fig. 5, A1 and B1). Around 60% of neurons were at their PEF at every phase of the cycle (Fig. 5, A3 and B3). Excluding one unusually active 2-PEF neuron, the 1-PEF population was 3 spikes/s more active throughout the cycle compared with the 2-PEF group (P < 0.05, t test; Fig. 5, A2 and A4, and Fig. 5, B2 and B4).

Among neurons with a somatosensory receptive field (RF), the firing of 70% (26/37) was modulated with strides, whereas the discharge of only 47% (32/68) of neurons without an RF was modulated (P < 0.022, v^2 test). However, the average dM was similar between the groups, 10.0±0.9 and 8.8±0.7, respectively (P ¼ 0.3, t test), and in either group, the majority of cells with stride-related firing had a 1-PEF discharge pattern, 81% (21/26) and 59% (19/32), respectively. PrPhs of 1-PEF neurons with an RF were distributed across the step cycle, whereas those of cells without an RF concentrated in the swing phase (P ¼ 0.022, t test; not illustrated).

Among PTNs, the discharge of 66% (21/32) was modulated with the rhythm of strides. The majority of these neurons had a 1-PEF pattern (57%, 12/21), which was 38% of the PTN population (12/32). Compared with noIDs recorded in the same microelectrode tracks, PTNs tended to have the 1-PEF pattern more often while showing the 2-PEF pattern less often (P ¼ 0.067, v^2 test). In both subpopulations, PEFs were distributed across the step cycle (Fig. 6, A1, B1 and C1), however, several fPTNs were preferentially active at the end of the stance and the beginning of the swing phase, so the entire fPTN population was more active during transition from stance to swing (Fig. 6, A2 and A4). In contrast, the firing of the sPTN group had a small peak in the opposite phase, the end of the swing, and the first half of the stance phase (Fig. 6, B2 and B4). The discharge of the noID population was steady over the step cycle (Fig. 6C4). The dM of PTNs at 8.8±0.5 tended to be higher than that of noIDs (P ¼ 0.069, t test).

Neuronal Firing during Locomotion on the Ladder

The ladder added a requirement for visual control of strides. The cat had to constrain its paw placement during locomotion to the raised crosspieces of the ladder. When the cat walked on the ladder, all cells that were active on the flat surface were also active (98%, 196/201). The firing of an example neuron is shown in Fig. 3, A, D, and E. This is the same fPTN whose activity during locomotion on the flat surface is shown in Fig. 3, A–C. On the ladder, the average firing rate of the neuron was 34 spikes/s, 10 spikes/s higher than on the flat surface. The firing was now fairly consistent stride to stride and was strongly modulated with their rhythm with a peak at the end of stance (Fig. 3A, middle; Fig. 3D). Figure 3E sums the distribution of the firing frequency across all 32

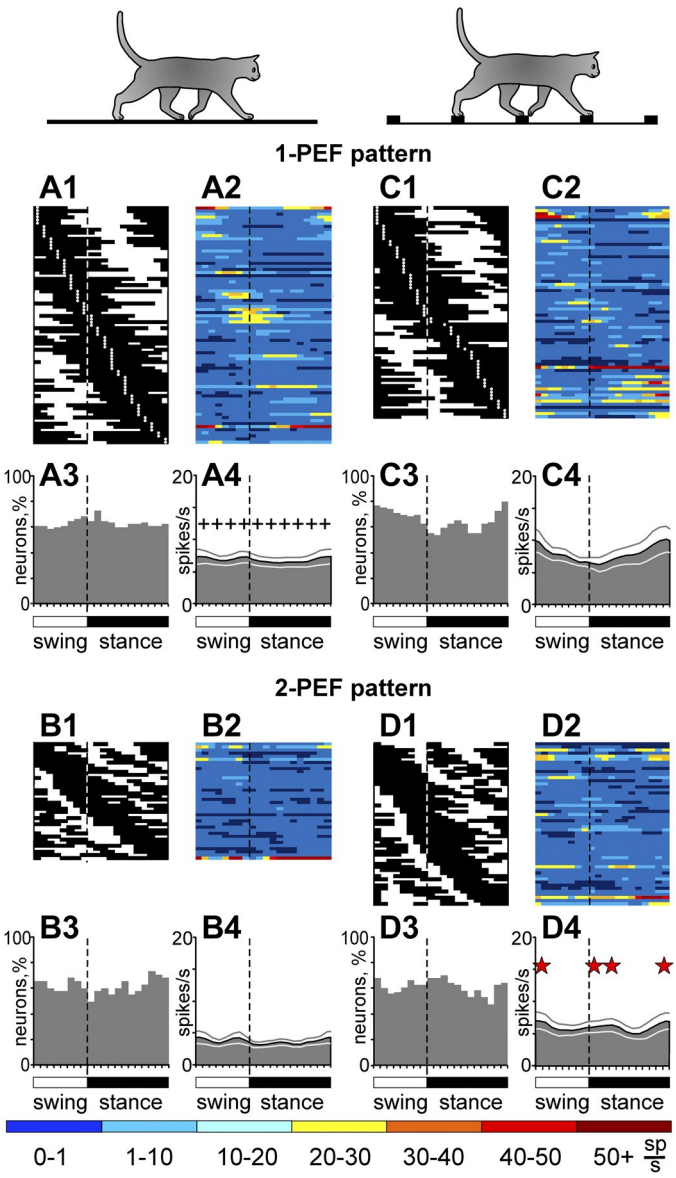

Figure 5. Population characteristics of neurons with 1-PEF and 2-PEF discharge pattern during locomotion on the flat surface and ladder. Activity of neurons discharging with a 1-PEF (A and C) or 2-PEF (B and D) pattern during locomotion on the flat surface (A and B) and ladder (C and D). A1, B1, C1, and D1: phase distribution of PEFs. Each horizontal bar represents the PEF of one neuron. Neurons are rank ordered so that those active earlier in the cycle are plotted at top of the graph. In A1 and C1, circles indicate the preferred phase of the activity (PrPh). A2, B2, C2, and D2: corresponding phase distribution of firing frequencies. Average firing frequency in each 1/20th portion of the cycle is color-coded according to the scale shown at bottom of the figure. A3, B3, C3, and D3: proportion of active neurons (neurons in their PEF) in different phases of the step cycle during flat surface (A3 and B3) and ladder (C3 and D3) locomotion. A4, B4, C4, and D4: the distribution of the mean firing rate across the step cycle of the flat surface (A4 and B4) and ladder (C4 and D4) locomotion. Thin lines show SEM. Crosses indicate periods of the stride when the activity of the 1-PEF population was statistically significantly greater than that of the 2-PEF population ($P < 0.05$, t test). Red stars show periods of the stride when the activity during ladder locomotion was statistically significantly greater than during flat surface locomotion ($P < 0.05$, t test). In each panel, a vertical interrupted line denotes the average end of the swing and be-ginning of the stance phase of the right forelimb. PEF, period of elevated firing.

strides. The neuron had two PEFs: a prominent peak of 56 spikes/s at the end of the stance and a smaller peak at the beginning of the stance. The dM was 5.4, and the M was 71.6, which classified the firing of the neuron as stride rhythm-modulated.

The average discharge across all neurons was similar to that on the flat surface, 8.7 ± 0.6 spikes/s ($P = 0.193$, t test). However, the firing rate of individual neurons often changed on transition to the ladder (Fig. 4B). The firing rate of 28% (54/196) of neurons was about twice higher than on the flat surface, while that of 17% (33/196) was half smaller ($P = 0.002$ and $P = 0.008$, respectively, t test). The firing rate of half of neurons with a somatosensory RF (59%, 22/37) changed on the transition, either increasing (2–2.5 times) or decreasing (by 1/3). The firing rate of half of the neurons without an RF (51%, 35/68) also changed. In this group, the decreases were more substantial than the increases (by 1/ 2, $P = 0.006$, t test). The firing rate of fPTNs usually changed (83%, 15/18), either increasing or decreasing, whereas that of sPTNs usually did not change ($P = 0.006$, v^2 test for proportions).

The discharge of 62% (122/196) of neurons was modulated with the rhythm of strides, a similar proportion as on the flat surface. These included 34 cells, the firing of which became stride-modulated only on the ladder. On the other hand, the activity of 28 neurons lost the stride-related modulation upon the transition. Neurons with a stride-modulated firing had either 1-PEF or 2-PEF pattern (3 cells had three PEFs, and they will be considered jointly with the 2-PEF group). The majority of neurons with modulated firing still had a 1-PEF pattern (57%, 69/122), comprising 35% (69/196) of the entire population. However, the proportion of neurons with a 2-PEF pattern at 27% (53/196) tended to be higher than on the flat surface (by 8%, $P = 0.073$, v^2 test).

The average duration of the PEF in the 1-PEF group and the combined duration of PEFs in the 2-PEF group remained similar to those seen on the flat surface ($P = 0.6$, t test). However, the duration of PEF(s) of 24% of individual neurons (21 of 88 with stride-modulated firing during both tasks) changed, decreased ($n = 11$), or increased ($n = 10$) by 25–50% of the cycle. In both 1-PEF and 2-PEF subpopulations, PEFs of different neurons were distributed over the step cycle and overlapped (Fig. 5, C1 and D1). In 1-PEF group, 55–80% of neurons were at their PEF at every phase of the step cycle, with slightly more cells active during the swing and late stance phases ($P = 0.001$, v^2 test; Fig. 5C3). This was different from the steady recruitment of 1-PEF neurons on the flat surface (Fig. 5A3) and resulted in a slight one-peak modulation of this group's discharge ($P < 0.001$, t test; Fig. 5C4). The recruitment of 2-PEF neurons was at 50–70% throughout the cycle, with the smallest proportion of active cells in the second half of the stance ($P = 0.018$, v^2 test; Fig. 5D3). This was also different from the rather steady recruitment of 2-PEF neurons on the flat surface (Fig. 5B3) and resulted in a weak two-peak modulation of this group's discharge ($P < 0.01$, t test; Fig. 5D4). Overall, the 2-PEF group was slightly more active across the cycle than on the flat surface ($P = 0.040$, t test) and was now as active as the 1-PEF group ($P = 0.5$, t test).

The average dM was still similar between the 1-PEF and 2-PEF groups, 8.8 ± 0.4 across both, and for both, it was

similar to that on the flat surface (P ¼ 0.5, t test). However, the dM of 43% (85/196) of individual neurons changed upon the flat-to-ladder transition (Fig. 7A). Among neurons whose firing was stride-modulated during both tasks (n ¼ 88), the dM of 28% (25/88) was greater on the ladder, whereas that of a similar proportion (20/88) was smaller. In half of the neurons with an altered dM (25/45), the average firing rate did not change. This indicates that the dM of the activity of these neurons changed due to a matched increase of the peak firing and a decrease of the inter-PEF discharge. For the other half of the dM changers, there was a negative correlation between the alterations of the dM and average firing rate. Namely, an increase of the dM was associated with a decrease in the firing rate, whereas the decrease of the dM was accompanied by an increase in firing (r ¼ 0.5, P ¼ 0.033; Fig. 7B). This indicates that the change of the dM of these neurons was chiefly caused by a change of the inter-PEF firing rate: the dM increase was due to a reduction of it, whereas the dM decrease was due to its raise. Concurrent increases of the dM and the average firing rate, which signify an increase of the peak discharge, were observed in only 11% (5/45) of neurons (Fig. 7B, right top quadrant).

The stride phase preference of the discharge typically changed on the flat-to-ladder transition. In the group of neurons that had one PEF during both tasks, the PrPh of the majority (59%, 24/41) changed by 10–45% of the step cycle, 24±2% on average (Fig. 7C). Among these, the PrPh of 4 (17%) cells shifted from swing to stance and that of 4 (17%) changed from stance to swing (shaded areas in Fig. 7C). In addition, for 73% (11/15) of neurons that had two PEFs during both tasks, the phase of one or both PEF(s) was different between the tasks.

Across the five activity parameters that we assessed: the average firing rate, pattern of firing, width of the PEF, dM, and PrPh, the value of at least one changed for 83% (162/196) of neurons on the flat-to-ladder transition and the value of more than one changed for 69% (136/196) of cells.

Among neurons with a somatosensory RF, the firing of a great majority (81%, 30/37) was modulated with the rhythm of strides, whereas the discharge of only 57% (39/68) of cells without an RF was modulated (P ¼ 0.014, v^2 test). In addition, there tended to be more cells in the no RF group whose firing lost the stride-related modulation on transition to the ladder than among cells with an RF (P ¼ 0.078, v^2 test). Nevertheless, in the groups with and without an RF, similar proportions of cells discharged one PEF per stride (51% and 34%, respectively; P ¼ 0.08, v^2 test) and similar proportions had two PEFs (30% and 24%, respectively). The average dM was also similar (9.6±0.9 and 8.9±0.8, respectively, P ¼ 0.7, t test), and the dM of individual neurons in both groups had similar propensity to change on the transition (P ¼ 0.6, v^2 test). These mixed data suggest that while the somatosensory responsiveness of the neurons observed in the resting animal may have contributed to the modulation of their firing

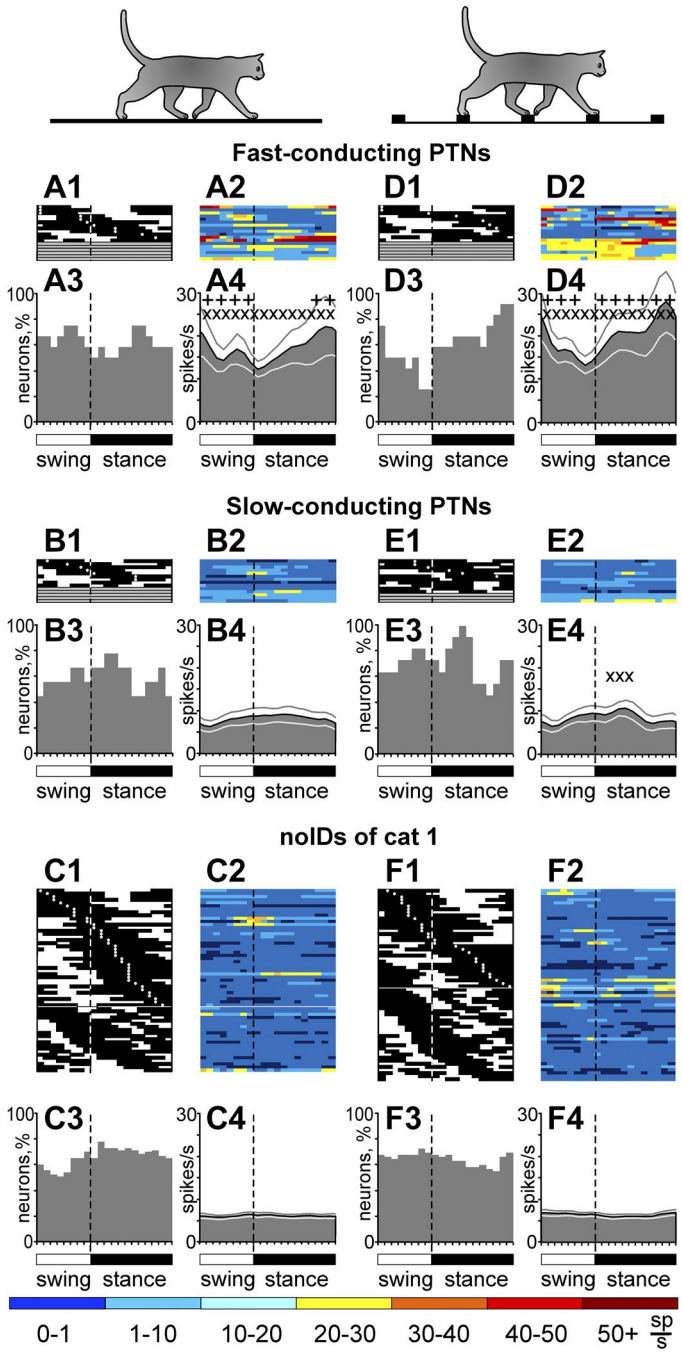

Figure 6. Population characteristics of fast- and slow-conducting PTNs and noID neurons during locomotion on the flat surface and ladder. Activity of fPTNs (A and D), sPTNs (B and E), and noIDs (C and F) during locomotion on the flat surface (A, B, and C) and ladder (D, E, and F). A1–F1: phase distribution of PEFs. Neurons discharging with a 1-PEF and 2-PEF pattern are rank ordered separately. 1-PEF neurons are shown at top of the graph, 2-PEF neurons are shown at bottom. Within each group, cells firing earlier in the cycle are plotted at top of the group. Gray bars in A1, B1, D1, and E1 designate neurons whose activity was not stride-modulated. In C1 and F1, noIDs whose firing was not stride-modulated are not shown. A2–F2: corresponding phase distribution of firing frequencies. A3–F3: proportion of neurons in their PEF in different phases of the step cycle during flat surface (A3–C3) and ladder (D3–F3) locomotion. A4–F4: the distribution of the mean firing rate during the stride of the flat surface (A4–C4) and ladder (D4–F4) locomotion. In C4 and F4, noIDs whose firing was not stride-modulated are also included. Crosses indicate periods of the stride when the activity of fPTNs was statistically significantly greater than that of sPTNs (P < 0.05, t test). X-es indicate periods of the stride when the activ-ity of PTNs was statistically significantly greater than the activity of noIDs (P < 0.05, t test). Other designations as in Fig. 5. fPTN, fast-conducting PTN; noIDs, nonidentified neurons; PTN, pyramidal tract neuron; sPTN, slow-conducting PTN.

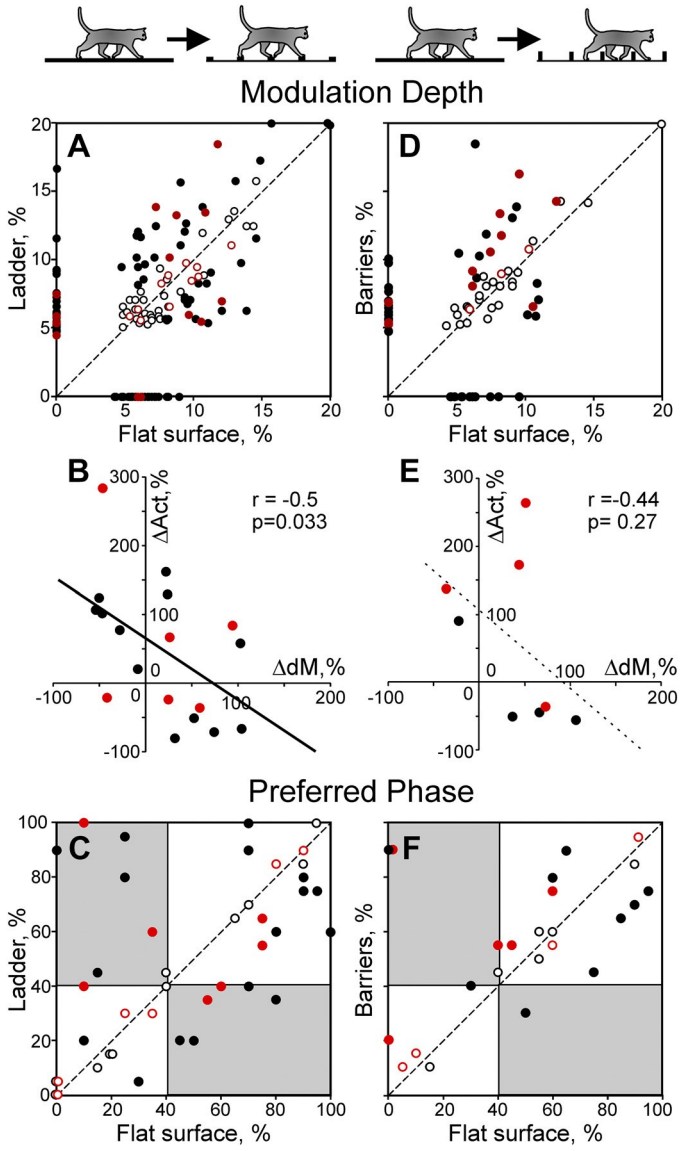

Figure 7. Comparison of the depth of stride-related activity modulation (dM) and preferred phase of the activity (PrPh) of individual neurons between locomotion on the flat and uneven surface. A and D: comparison of the dMs of the activity of individual PTNs (red symbols) and noID neurons (black symbols) during locomotion on the flat surface and ladder (A) and on the flat surface and with barriers (D). The x- and y-axes of each point show the dM of the activity of a neuron during locomotion of the flat and uneven surface, respectively. Neurons whose dMs were statistically significantly different between the tasks are shown by filled symbols, the others are shown by open symbols. B and E: negative correlation between the change of the dM (DdM) and mean discharge frequency (DAct) of neurons, in the activity of which both of these parameters altered between locomotion on the flat and uneven surface. The x-axis and y-axis of each point show the difference of the discharge characteristic of a neuron between locomotion on the flat surface and ladder (B), and on the flat surface and with barriers (E). The dif-ference is positive if the value of the parameter was larger during locomo-tion on an uneven surface. Red symbols designate PTNs, black symbols show noIDs. The coefficient of correlation (r) and probability (p) are indi-cated. For the flat-to-barriers transition, the correlation did not reach the level of statistical significance. C and F: comparison of PrPhs of the activity of neurons with a 1-PEF discharge pattern during both locomotion on the flat surface and ladder (C) and during both locomotion on the flat surface and with barriers (F). Areas that correspond to the swing phase during one task but stance phase during the other task are shaded. Other designations as in A and D. PEF, period of elevated firing.

during locomotion on the ladder, it was not the main driving force behind it.

Among PTNs, the firing of 72% (23/32) was modulated with the rhythm of strides on the ladder, a similar proportion to that on the flat surface. More cells had a 1-PEF than 2-PRF pattern: 47% (15/32) and 25% (8/32) of neurons, respectively, and these proportions were also similar to those seen on the flat surface. This distinguished PTNs from the general population where the proportion of neurons with a 2-PEF pattern tended to be higher on the ladder than on the flat surface. PTNs more often retained the 1-PEF pattern between the two tasks than noIDs recorded in the same microelectrode tracks (P ¼ 0.044, v^2 test). The group of fPTNs was still preferentially active during the stance-to-swing transition (Fig. 6D4) while the activity of the sPTN group peaked in the opposite phase (Fig. 6E4). The activity profiles of both groups were similar to those on the flat surface (Fig. 6, D4 and E4, versus Fig. 6, A4 and B4), and the firing of the noID population was still steady over the step cycle (Fig. 6F4). The dM of 42% (8/19) of PTNs with stride-related firing during both tasks changed on transition to the ladder by either increasing or decreasing (Fig. 7A), while the average dM at 8.7±0.7 remained similar to that on the flat surface.

To summarize, the transition from locomotion on the flat surface to vision-guided stepping on the ladder was associated with: 1) a change in the firing rate of 44% of the neurons, including 83% of fPTNs; 2) an activity increase of cells with a 2-PEF pattern and a tendency for the proportion of 2-PEF cells to increase; 3) a change of the dM of 51% of neurons, including 42% of PTNs; 4) a 10–45% of the cycle shift of the PrPh of 59% of neurons with a 1-PEF pattern and a phase change of one or both PEFs of 73% of neurons with a 2-PEF pattern; and 5) 25–50% change of the duration of the PEF(s) of 24% of neurons.

Neuronal Firing during Stepping over Barriers

Negotiating barriers added a requirement to lift a limb higher while stepping under visual guidance on a complex pathway. By comparing discharges of neurons during locomotion on the ladder and with barriers one can assess how the additional lifting of limbs during visually guided stepping is reflected in neuronal activity.

The activity of 84 neurons of cat 1 that were recorded during locomotion on the flat surface and ladder was also recorded as the cat stepped over a series of barriers. An example is shown in Fig. 3, A, F, and G. This is the same fPTN whose activity during locomotion on the flat surface and ladder is shown in Fig. 3, A–E. While stepping over barriers, the average firing rate of the neuron was 19 spikes/s, the same as on the flat surface and 15 spikes/s lower than on the ladder. The firing was fairly consistent between strides (Fig. 3A, right side; Fig. 3F) and was strongly modulated with their rhythm. The discharge was 27–28 spikes/s at the beginning of the swing phase, dropped to 6 spikes/s in its second half, and then fluctuated upward during stance to peak at 32 spikes/s (Fig. 3G). One long PEF covered the stance and the first half of the swing phases. The PrPh was at the end of stance. The dM was 6.9, not statistically significantly different from that on the ladder.

All neurons were active during stepping over barriers. The average discharge was 7.2±0.6 spikes/s, similar to that on

the flat surface or the ladder (P ¼ 0.7, t test). However, as on the flat-to-ladder transition, the firing rate of individual neurons often changed upon transition from the flat surface to stepping over barriers (Fig. 4C). The firing rate of 19% (16/84) approximately doubled, while that of 14% (12/84) became half smaller (P ¼ 0.002 and P ¼ 0.031, respectively, t test). The proportions of neurons increasing and decreasing the firing and the magnitude of these changes were similar to those seen on the flat-to-ladder transition. The proportion of neurons with somatosensory RFs (63%, 15/24) that changed the firing rate was similar to that observed on the flat-to-ladder transition (P ¼ 0.8, v^2 test). Same was true for PTNs the discharge rate of 65% (11/17) of which changed, including 70% (7/10) of fPTNs. However, unlike on the flat-to-ladder transition when fPTNs usually altered their firing rate while sPTNs usually did not, on the flat-to-barriers transition fPTNs and sPTNs had similar propensity to increase, decrease, or not change the firing rate.

The discharge of 76% (64/84) of neurons was modulated with the rhythm of strides, a greater proportion than on the flat surface or ladder (P < 0.024, v^2 test). The proportion of cells whose activity became stride-modulated upon the flat-to-barriers transition (21%, 18/84) tended to be higher than that of cells whose activity lost its stride-related modulation on the transition (11%, 9/84; P ¼ 0.059, v^2 test). This was different from the flat-to-ladder transition, upon which these proportions were similar. During walking with barriers, the great majority of neurons still had either 1-PEF or 2-PEF discharge pattern, with only three cells showing three PEFs. The 1-PEF pattern was again more common, expressed by 52% (44/84) of cells, while 24% (20/84) had 2-PEFs. The proportion of 1-PEF cells exceeded those on the flat surface or ladder (P < 0.043, v^2 test), whereas the proportion of 2-PEF cells was the same as during other tasks (P > 0.4, v^2 test).

Similar to the flat-to-ladder transition, the average duration of the PEF in the 1-PEF group and the combined duration of PEFs in the 2-PEF group was the same as on the flat surface (P > 0.162, t test). However, the duration of PEF(s) of 28% of individual neurons (13 of 46 with stride-modulated firing during both locomotion on the flat surface and with barriers) changed on the flat-to-barriers transition, increasing (n ¼ 7) or decreasing (n ¼ 6) by 25–50% of the cycle. In both 1-PEF and 2-PEF subpopulations, PEFs of different neurons were distributed over the step cycle and overlapped (Fig. 8, C1 and D1). In the 1-PEF group, between 60–70% of neurons were at their PEF at every phase of the cycle (Fig. 8C3), whereas the recruitment of 2-PEF neurons fluctuated between 45–75% (Fig. 8D3). During the midswing and midstance phases, the group of 2-PEF neurons was more active than on the flat surface (P < 0.05, t test; Fig. 8D4). This was different from the flat-to-ladder transition when the firing of the 2-PEF group was higher during the swing-to-stance and stance-to-swing transition phases (Fig. 5D4). Supplemental Fig. S2 shows the activity of 1-PEF and 2-PEF neurons recorded during locomotion with barriers side-by-side with their activity on the ladder.

The average dM was still similar between 1-PEF and 2-PEF groups, 8.8±0.5 across both, and for both groups was similar to that seen on the flat surface or the ladder (P > 0.3, t test). However, like on the flat-to-ladder transition, the dM of 43% (36/84) of individual neurons changed compared with the

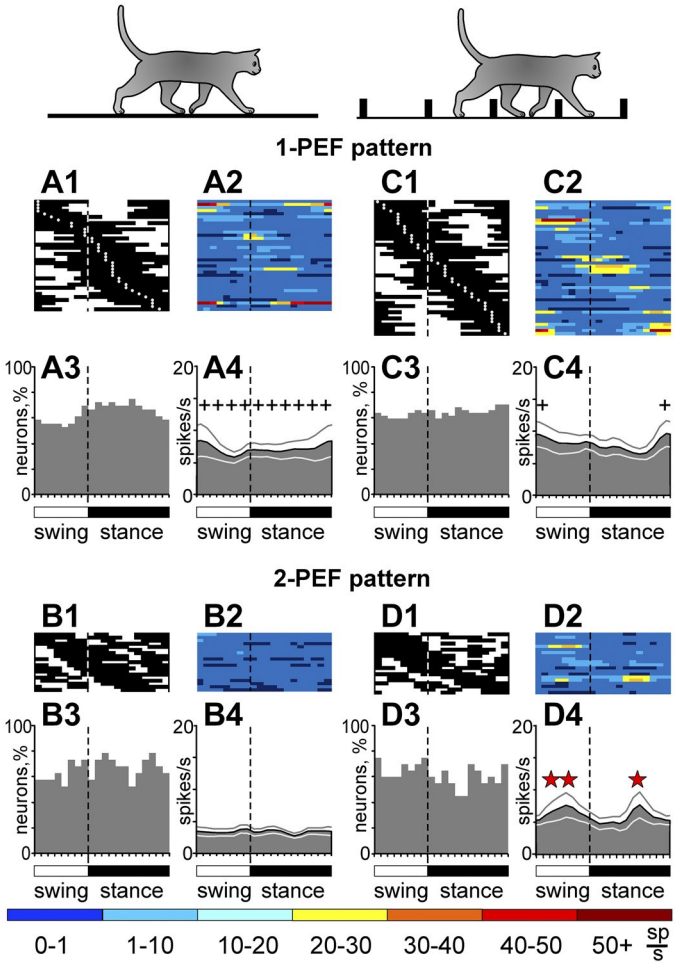

Figure 8. Population characteristics of neurons with 1-PEF and 2-PEF pattern during locomotion on the flat surface and with barriers. The activity of neurons discharging with a 1-PEF (A and C) or 2-PEF (B and D) pattern during locomotion on the flat surface (A and B) and with barriers (C and D). A1–D1: phase distribution of PEFs. A2–D2: corresponding phase distribution of discharge frequencies. A3–D3: proportion of active neurons (neurons in their PEF) in different phases of the step cycle during locomotion on the flat surface (A3 and B3) and with barriers (C3 and D3). A4–D4: the distribution of the mean firing rate during the stride on the flat surface (A4 and B4) and with barriers (C4 and D4). Designations as in Fig. 5. PEF, period of elevated firing.

flat surface (Fig. 7D). Among cells whose firing was stride-modulated during both locomotion on the flat surface and with barriers, the dM of 30% (14/46) was greater on barriers, while that of only 11% (5/46) was smaller (P ¼ 0.020, v^2 test). In analogy to the flat-to-ladder transition, for a half of neurons whose dM changed on the flat-to-barriers transition (58%, 11/19), this change occurred without a change of the average firing rate. This indicates that the dM of the discharge of these neurons changed due to a matched increase of the peak firing and a decrease of the inter-PEF discharge. For the other half of the dM changers, there was a tendency for a negative correlation between the alterations of the dM and the average firing rate, analogous to the correlation seen on the flat-to-ladder transition (Fig. 7E). This suggests that in the activity of these neurons, the dM change tended to be caused by a change of the inter-PEF discharge: the dM increase occurring due to a reduction of it, whereas the dM

decrease occurring due to its raise. Concurrent increases of the dM and the average firing rate, which signify an increase of the peak discharge, was observed in only 11% (2/19) of cells (Fig. 7E, right top quadrant), and this was similar to the flat-to-ladder transition (8%, Fig. 7B, right top quadrant).

In the group of neurons that had one PEF during both locomotion on the flat surface and with barriers, the majority (58%, 14/24) changed the PrPh on transition to barriers. The change ranged between 10% and 30% of the cycle (Fig. 7F) and was 18±1.5% on average, slightly smaller than on the flat-to-ladder transition ($P = 0.026$, t test). The PrPh of 4 (17%) cells shifted from swing to stance while that of one cell (4%) changed from stance to swing (shaded areas in Fig. 7F). These were similar proportions to those seen for the flat-to-ladder transition (Fig. 6C; $P > 0.4$, v^2 test). Also, similarly to that transition, for 71% (5/7) of neurons with two PEFs during both flat surface and barriers locomotion, the phase of one or both PEFs was different between the two tasks.

Across the five activity parameters that we assessed: the average firing rate, pattern of firing, width of the PEF, dM, and PrPh, the value of at least one changed for 86% (72/84) of neurons on the flat-to-barriers transition, and the value of more than one changed for 70% (59/84) of neurons.

Among cells with a somatosensory RF, the firing of 88% (21/24) was modulated with the rhythm of strides over barriers. The 67% (16/24) majority discharged with a 1-PEF pattern, which was the same proportion as on the flat surface or ladder (where it was 81% and 51%, respectively; $P > 0.2$, v^2 test). The firing of neurons with an RF that in addition to the contralateral (right) forelimb also included the body, head, or the right hindlimb was more stride-modulated on average than that of cells with an RF confined to the right forelimb (11.9±1.8 vs. 7.4±0.7, respectively; $P = 0.033$, t test). In addition, the average duration of the PEF(s) in this group was shorter (54.5±5% vs. 70±5%, respectively; $P = 0.040$, t test), so that their firing was more stride phase-specific. Neither of these differences was present during locomotion on the flat surface or ladder. The differences emerged on barriers because the dM of cells whose RF included more than just the forelimb typically increased, whereas that of cells with a forelimb-only RF usually did not change.

Among PTNs, the firing of 88% (15/17) was modulated with the rhythm of strides, same proportion as on the flat surface or ladder ($P > 0.09$, v^2 test). The majority (71%, 12/17) had a 1-PEF pattern, a greater proportion than on the flat surface and similar to that on the ladder ($P = 0.027$ and $p = 0.112$, respectively, v^2 test). All but one 1-PEF PTN (90%, 9/10) retained the 1-PEF pattern between the flat surface and barriers locomotion. This, in analogy with the flat-to-ladder transition, tended to be a greater proportion of such cells than among noIDs recorded in the same microelectrode tracks ($P = 0.066$, v^2 test). The group of fPTNs was still preferentially active during the stance-to-swing transition phase (Fig. 9D4) while the activity of sPTNs peaked in the opposite phase (Fig. 9E4). The activity profiles of both groups were similar to those seen on the flat surface (Fig. 9, D4 and E4,

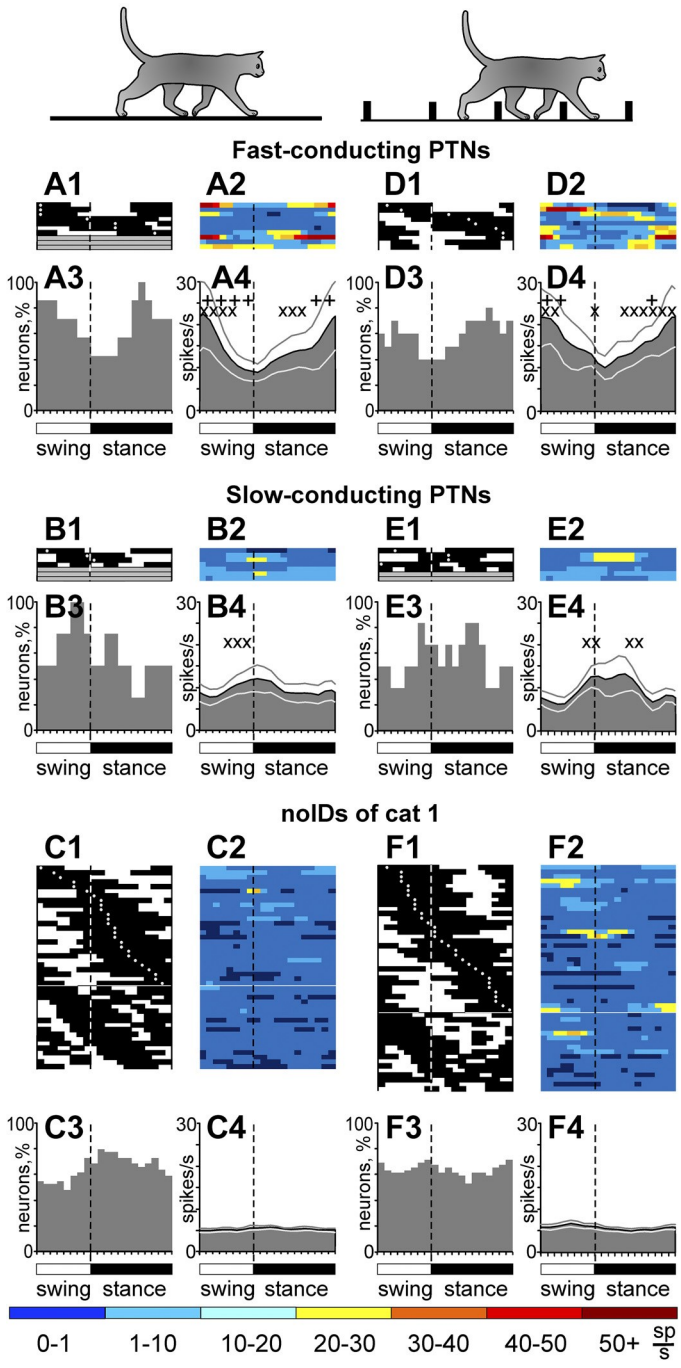

Figure 9. Population characteristics of fast- and slow-conducting PTNs and noID neurons during locomotion on the flat surface and with barriers. Activity of fPTNs (A and D), sPTNs (B and E), and noIDs (C and F) during locomotion on the flat surface (A, B, and C) and with barriers (D, E, and F). A1–F1: phase distribution of PEFs. Neurons with a 1-PEF and 2-PEF pattern are rank ordered separately. 1-PEF neurons are shown at top of the graph. 2-PEF neurons are shown at bottom. Within each group, cells firing earlier in the cycle are plotted at top of the group. Gray bars in A1, B1, and E1 designate neurons whose activity was not stride-modulated. In C1 and F1, noIDs whose firing was not stride-modulated are not shown. A2–F2: corresponding phase distribution of firing frequencies. A3–F3: proportion of neurons in their PEF in different phases of the step cycle during locomotion on the flat surface (A3–C3) and with barriers (D3–F3). A4–F4: the distribution of the mean firing rate during the stride on the flat surface (A4–C4) and over barriers (D4–F4). In C4 and F4, noIDs whose firing was not stride-modulated are also included. Designations as in Figs. 5 and 6. fPTN, fast-conducting PTN; noIDs, nonidentified neurons; PEF, period of elevated firing; PTN, pyramidal tract neuron; sPTN, slow-conducting PTN.

vs. Fig. 9, A4 and B4) or ladder (Fig. 6, D4 and E4). The discharge of the noID population was vaguely modulated with a low peak in the swing phase (Fig. 9F4). Supplemental Fig. S3 shows the activity of fPTNs, sPTNs, and noIDs recorded in the same microelectrode tracks side-by-side with their activity during ladder locomotion. The dM of 73% (8/11) of PTNs with stride-related firing during both tasks changed on the flat-to-barriers transition by either increasing or decreasing (Fig. 7D), which was a statistically similar proportion to that of PTNs changing the dM on the flat-to-ladder transition. The average dM at 9.3±0.9 was also similar to that seen on the flat surface or ladder ($P > 0.4$, t test).

To summarize, the transition from locomotion on the flat surface to stepping over barriers was associated with 1) a change of the firing rate of 33% of neurons, including 65% of PTNs; 2) an increase of the proportion of cells with stride-modulated firing, particularly those with a 1-PEF pattern; 3) an increase of firing of 2-PEF cells during the midswing and midstance phases; 4) a change of the dM of 43% of neurons, including 73% of PTNs; 5) a 10–30% of the cycle shift of the PrPh of 58% of neurons with a 1-PEF pattern during both tasks, and a phase change of one or both PEFs of 71% of neurons with a 2-PEF pattern; and 6) 25–50% change of the duration of the PEF(s) of 28% of neurons. Although many of these changes were similar to those seen on the flat-to-ladder transition, neuronal activity during locomotion with barriers was distinguished from that on the ladder by 1) a greater proportion of cells with stride-modulated firing, 2) a focused increase of firing of 2-PEF cells during the midswing and midstance phases, the latter being also the swing phase of the other (left) forelimb, and 3) a relatively higher stride-related modulation of firing of cells with RFs that in addition to the contralateral (right) forelimb included the body, head, or the right hindlimb. Thus, in the population activity of areas 6aa and 6ac neurons, additional lifting of limbs during vision-guided stepping was reflected in these three characteristics and several other aspects as described in this section.

Comparison of the Activity of Individual Neurons during Locomotion on the Ladder and with Barriers

We next examined how the requirement for additional lifting of limbs during vision-guided stepping is reflected in the activity of individual neurons. In Fig. 10A, the mean firing of each neuron during locomotion with barriers is plotted against that during locomotion on the ladder. The firing rate of 14% (12/84) of neurons was greater during stepping over barriers than on the ladder, by 56±19%, and that of 15% (13/84) was smaller, by 35±6%. The firing rate of 46% (11/24) of cells with a somatosensory RF changed upon the ladder-to-barriers transition, either increased or decreased. Among PTNs, the firing rate of 65% (11/17) changed, either increased or decreased, whereas the firing of only 21% (14/67) of noIDs altered ($P < 0.001$, v^2 test).

The discharge of 86% (72/84) of neurons was stride-modulated during at least one of the two locomotion tasks. In Fig. 10B, the dM of the activity of these neurons during locomotion with barriers is plotted against that on the ladder. The firing of a quarter of neurons (25%, 18/72) was only stride-modulated during stepping over barriers, whereas the firing of only 10% (7/72) lost the modulation on the ladder-to-barriers transition, a smaller proportion ($P = 0.016$, v^2 test). The dM of 15% (11/72) of neurons with stride-modulated firing during both tasks was greater on barriers, whereas that of 13% (9/72) was smaller. The dM of 58% (14/24) of cells with a somatosensory RF altered, either increased or decreased. Among PTNs, the dM of 65% (11/17) changed, either increased or decreased, and the dM of a similar proportion of noIDs also altered (51%, 34/67; $P = 0.30$, v^2 test).

Among neurons with a 1-PEF pattern during both tasks, the PrPhs of 39% (7/18) were different between the tasks (Fig.

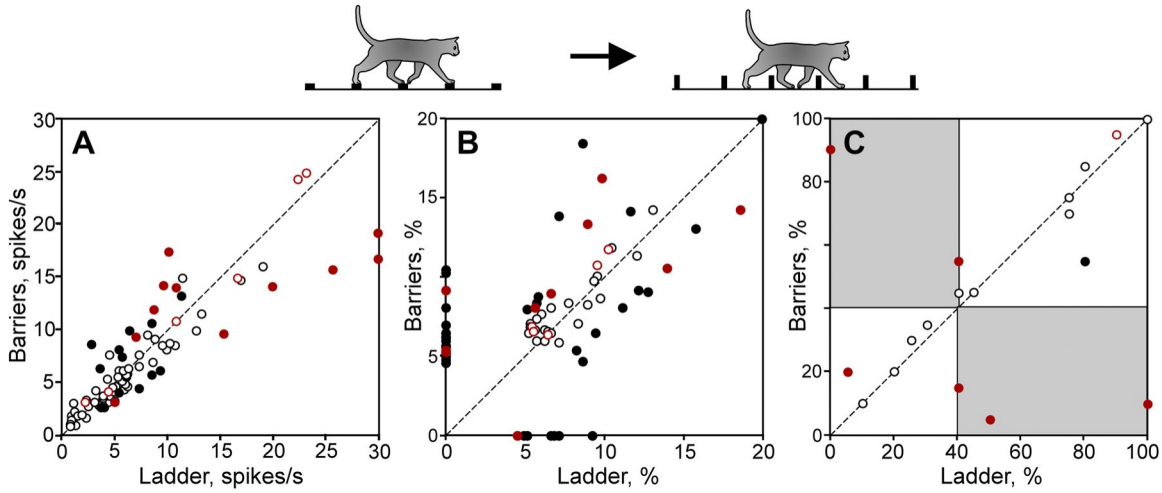

Figure 10. Comparison of the average firing rate, depth of stride-related activity modulation (dM), and preferred phase of the activity (PrPh) of individual neurons between locomotion on the ladder and with barriers. A: comparison of the average firing rates of individual PTNs (red symbols) and noID neurons (black symbols) during locomotion on the ladder and with barriers. The x- and y-axes of each point show the firing rate of a neuron during locomo-tion of the ladder and with barriers, respectively. Neurons whose firing rates were statistically significantly different between the tasks are shown by filled symbols, the others are shown by open symbols. B: comparison of the dMs of the activity of neurons during locomotion on the ladder and with bar-riers. C: comparison of the PrPhs of the activity of neurons with a 1-PEF discharge pattern during both locomotion tasks during locomotion on the ladder and with barriers. Areas that correspond to the swing phase during one task but stance phase during the other task are shaded. noIDs, nonidentified neurons; PEF, period of elevated firing; PTN, pyramidal tract neuron.

10C). All but one of such PTNs had different PrPhs, while all but one noIDs had similar PrPhs ($P = 0.001$, χ^2 test). The PrPhs of 63% (5/8) of neurons with a somatosensory RF that had a 1-PEF pattern during both tasks changed on the ladder-to-barriers transition. Among neurons with a 2-PEF pattern during both tasks ($n = 5$), the phase of one or both PEFs of all of them was different between the tasks.

Across the five activity parameters that we assessed: the average firing rate, pattern of firing, width of the PEF, dM, and PrPh, the value of at least one changed for 80% (67/84) of neurons on the ladder-to-barriers transition, and the value of more than one changed for 57% (48/84) of neurons. The groups of neurons whose firing rate, dM, and PrPh changed between locomotion on the ladder and with barriers overlapped only partly. Among cells with a somatosensory RF, 13% (3/24) had a similar firing rate, dM, and PrPh between these two tasks, while showing differences in one or more of these parameters between locomotion on the flat surface and on one or both complex surfaces.

Thus, additional lifting of limbs during vision-guided stepping was associated with a change of the PrPh of firing of nearly all PTNs and a change of the firing rate and dM for most of them. The firing rate of noIDs altered less frequently and the PrPh of 1-PEF noIDs nearly never changed. This suggests that PTNs of areas 6aa and 6ac contribute to additional lifting of limbs during vision-guided stepping. Because in the activity of only half of the neurons with a somatosensory RF the firing rate and dM changed on the ladder-to-barriers transition, we concluded that although the responsiveness to somatosensory stimulation that is detected in the resting cat may have influenced responses of areas 6aa and 6ac neurons to additional lifting of limbs during vision-guided stepping it was not the only driving force behind these responses.

DISCUSSION

The main findings of this study are that the firing activity of the majority of neurons in the premotor areas 6aa and 6ac is modulated with the rhythm of strides and changes when the animal transitions from vision-nonrequiring locomotion on the flat surface to vision-guided stepping on the ladder or over barriers. This includes changes in the firing rate and the depth of the stride-related activity modulation of 33–44% of the neurons and changes in the stride phase(s) of firing of 58–73% of neurons. Our findings suggest that premotor areas 6aa and 6ac are involved in the control of visually guided stepping on the complex surface. Below we compare this involvement with that of other rostral cortical areas, shortly discuss the role of somatosensory responsiveness of neurons in their locomotion-related responses, and consider the potential influence of areas 6aa and 6ac PTNs on downstream neuronal networks supporting vision-guided stepping on the complex surface.

Comparison with Neuronal Activity in Other Premotor Cortex Areas of the Cat

The activity of neurons in feline premotor cortex areas 6iffu, 4fu, and 4d during treadmill locomotion with stepping over an obstacle was analyzed by Nakajima and colleagues (12). They found that the activity of only 54% (136/254) of neurons in these premotor cortex areas was related to the locomotion task, e.g., the strides leading up to the obstacle or taking the limbs over the obstacle. This is a substantially smaller proportion of stride-modulated cells than we found in areas 6aa and 6ac during locomotion with barriers (76%; $P < 0.001$, t test). Particularly striking is the difference with area 6iffu where during unobstructed locomotion only 23% of cells had stride-modulated discharges (12), whereas there were 59% of such cells in areas 6aa and 6ac. This difference could be partially due to a larger proportion of PTNs in our sample, 25% (33/133, cat 1) compared with 19% (48/254) in the sample of Nakajima et al. (12) ($P = 0.005$, χ^2 test). However, the 6% difference in the proportion of PTNs cannot account for the 22–36% difference in the proportion of stride-modulated cells between the two groups of premotor cortex areas. It is more likely that, in addition to anatomical differences between the areas, the difference in the proportion of stride-modulated cells is related to the difference between locomotion tasks used. Natural overground locomotion that we studied may have engaged a larger proportion of neurons than locomotion on the treadmill, a task that terrestrial animals almost never face in nature.

Nakajima et al. (12) reported that the firing rate of 40–70% of cells in areas 6iffu, 4fu, and 4d increased while that of 10–20% of cells diminished compared with unobstructed walking when the contralateral forelimb was taken over the obstacle (Fig. 4C in Ref. 12). Although these observations are not directly comparable with our data on the firing activity averaged over the entire step cycle, our analysis suggests that the proportions of neurons the firing of which increases or decreases during stepping over an obstacle, are similar in areas 6iffu, 4fu, and 4d and areas 6aa and 6ac. Also similar is the proportion of neurons whose firing rate changes with the obstacle height, and thus the height of the limbs lifting: 30% (25/84) in our study (Fig. 10A) and 21% (24/114) in the study of Nakajima et al. (12) ($P = 0.871$, χ^2 test). The mixed effect of the size of the obstacle that Nakajima et al. (12) observed when the firing of two-thirds of cells increased during stepping over a taller obstacle while that of one-third decreased is analogous to the mixed responses to limbs lifting of neurons in areas 6aa and 6ac whose firing could either increase or decrease during stepping over barriers compared with locomotion on the ladder (Fig. 10A).

It appears that neurons across all areas of the cat premotor cortex participate in control of visually guided stepping on a complex surface. However, the extent of this participation varies by the locomotion task and cortical area. The latter dissimilarity likely results from the differences in the inputs and efferent connections of the areas (37, 69, 72–78).

Comparison with the Adjacent Primary Motor Cortex

Several reports from our laboratory described activity of neurons in the primary motor cortex (area 4c, M1) as the cat walked on the flat surface, horizontally placed ladder, and stepped over a series of barriers (17, 22, 23, 26). Constructions of the walkway, ladder, and barriers were consistent across all studies. To facilitate comparison of the activity of neurons in areas 6aa and 6ac with that of area 4c, we have compiled a table (Fig. 11) that shows side by side the values of selected locomotion-related activity characteristics of neurons in these areas. The four reports on M1 indicated above are abbreviated in Fig. 11 as B&S,1993a (17); B&al,2010 (22); S&B,2012 (23); and F&al,2015 (26), respectively. Values for areas 6aa and 6ac that

Flat Surface

Source, n of neurons	1. Average Firing rate spikes/s	2. Prop. Modul. %	3. Depth of Modul. dM, %	4.Duration of PEF % cycle	5.Prop. 1-PEF %
a.6aα,6aγ n=196	7.6 ± 0.5	59	8.5 ± 0.3	62.5 ±1.5	39
B&S,1993 n=98	**15.4 ± 1.1** *p*<0.0001	–	**11.5 ± 0.8** *p*<0.0001	–	–
B&al,2010 n=63	–	**94** *p*<0.0001	–	60 ± 1.5	–
S&B,2012 n=145	**17.4 ± 0.9** *p*<0.0001	**97** *p*<0.0001	**10.2 ± 0.4** *p*<0.001	**55** – 60 for 55-58, *p*<0.05	**79** *p*<0.0001
F&al,2015 n=114	**15.1 ± 1.0** *p*<0.0001	**96** *p*<0.0001	9.3-**11± 0.6** for 9.7-11, *p*<0.05	**55 ±1.5** *p*=0.001	**73** *p*<0.0001

Sitting or Standing

Source, n of neurons	1.Average Firing rate spikes/s
a.6aα,6aγ n=196	7.5 ± 0.5
B&S,1993a n=98	**10.2 ± 1.0** *p*=0.007
B&al,2010 n=63	**12.7 ± 1.0** *p*<0.0001
S&B,2012 n=145	**13.0 ± 0.7** *p*<0.0001

Ladder — **Change between locomotion on the Flat surface and Ladder**

Source, n of neurons	1. Average Firing rate spikes/s	2. Prop. Modul. %	3. Depth of Modul. dM, %	4.Duration of PEF % cycle	5. Prop. 1-PEF %	6. Discharge rate Proportion of cells		7. dM Proportion of cells		8. PEF Proportion of cells		9. PrPh Proportion of cells	
						Incr.,%	Decr.,%	Incr.,%	Decr.,%	Incr.,%	Decr.,%	Incr.,%	Decr.,%
a.6aα,6aγ n=196	8.7 ± 0.6	62	8.8 ± 0.4	63.5 ± 1.5	35	28	17	28 (n=88)	23 (n=88)	11 (n=88)	13 (n=88)	24 (n=41)	34 (n=41)
B&S,1993a n=108	**15.7 ± 1.7** *p*<0.0001	**96** *p*<0.0001	**13.4 ± 0.7** *p*<0.0001	–	–	**47** *p*<0.001	14	**45** *p*=0.014	27	–	–	**4** *p*<0.001	**7** *p*<0.0001
B&al,2010 n=63	–	–	–	–	–	38	22	**60** *p*<0.0001	23	*20**	***40**** *p*<0.001	*11**	***11**** *p*=0.004
S&B,2012 n=145	**19.1 ± 0.1** *p*<0.0001	–	–	**51**-63 ± 2.5 for 51 – 58, *p*<0.05	–	27 – **42** for 38.5-42, *p*<0.05	15 – **40** for 26 - 40, *p*<0.05	40 – **51** (n=115) for 41.5 - 51, *p*< 0.05	20 – 25	–	**31 – 33** (n=85) *p*<0.004	**11** – 23 (n=115) for 11, *p*=0.042	**11** – 23 (n=115) for 11-19, *p*<0.05

Barriers — **Change between locomotion on the Flat surface and Barriers**

Source, n of neurons	1. Average Firing rate spikes/s	2. Prop. Modul. %	3. Depth of Modul. dM, %	4.Duration of PEF % cycle	5. Prop. 1-PEF %	6. Discharge rate Proportion of cells		7. dM Proportion of cells		8. PEF Proportion of cells		9. PrPh Proportion of cells	
						Incr.,%	Decr.,%	Incr.,%	Decr.,%	Incr.,%	Decr.,%	Incr.,%	Decr.,%
a.6aα,6aγ n=84	7.2 ± 0.6	76	8.8 ± 0.5	63.5 ± 2.0	52	19	14	30 (n=46)	11 (n=46)	15 (n=46)	13 (n=46)	38 (n=24)	21 (n=24)
B&S,1993a n=98	**16.2 ± 1.2** *p*<0.0001	_	**12.6 ± 1.0** *p*=0.002	_	_	**39** *p*=0.003	**27** *p*=0.032	42	19	_	_	**1** *p*<0.0001	8

statistically significantly smaller than in M1 by t test or χ^2 test

statistically significantly greater than in M1 by t test or χ^2 test

The value of *p* is shown below the values observed in M1.

20, 40*, 11** - these values are not explicitly stated in the publication

Figure 11. Selected parameters of locomotion-related activity of areas 6aa and 6ac population compared with published data on primary motor cortex, area 4c. Publications from our laboratory on the activity of M1 are abbreviated as follows: B&S, Beloozerova and Sirota, 1993 (17); B&al, Beloozerova et al. (22); S&B, Stout and Beloozerova (23); F&al, Farrell et al. (26). For averages, the mean value and the error of the mean are shown (mean ± SE). For proportions, when a proportion was calculated for a subset of neurons, the number of neurons in the subset, n, is given in brackets under the value.

are significantly smaller than the corresponding values for M1 (Student's or v^2 test) are highlighted by a blue background. Greater values are highlighted by orange background. The probability for the difference is stated under the value for M1 from which the value for areas 6aa and 6ac differs.

A fast glance at Fig. 11 shows that many values for areas 6aa and 6ac have a blue background. Indeed, we found that the firing rates of neurons in these areas during standing or sitting, as well as locomotion on the flat surface, ladder, or with barriers are about half smaller than in M1 (Fig. 11, column 1). The proportion of cells with a stride-modulated firing is smaller (Fig. 11, column 2). The dM of the stride-modulated activity is substantially smaller, whereas the PEF (s) are longer (Fig. 11, columns 3 and 4). In addition, the proportions of cells whose firing rate, dM, and duration of PEF (s) change on transition from the flat surface to vision-guided stepping on the ladder or over barriers are often smaller than in M1, particularly the proportions of neurons

whose firing rate changes upon the transition (Fig. 11, columns 6–8). At the same time, the proportion of cells whose PrPh of the activity changes on the transition tends to be larger (Fig. 11, column 9). This suggests that the neuronal population of areas 6aa and 6ac participates less in the locomotion-related activity than the M1 population. Particularly, the involvement of the neuronal population of areas 6aa and 6ac in adjustment of strides based on visual information about the walkway is lower than in M1.

We next compared the involvement of areas 6aa and 6ac and M1 PTNs, the neurons whose axons pass within the pyramidal tract in the medulla and may descend to the spinal cord. The table in Fig. 12 lists selected locomotion-related activity characteristics of fPTNs and sPTNs in a format similar to that of published M1 data (Table 2 in Ref. 24). Highlighting is as in Fig. 11. We found that the average firing rates of both fPTNs and sPTNs in areas 6aa and 6ac during standing or sitting, as well as locomotion on the flat surface or ladder (for locomotion with barriers, there is no M1 data on the activity of fPTN and sPTN separately) were similar to those of the respective PTN groups in M1 (Fig. 12, column 1). Like in M1, the firing rates of fPTNs in areas 6aa and 6ac were significantly higher than those of sPTNs during every task. However, the variability of the discharge of fPTNs during locomotion on the flat surface was higher than in M1 (Fig. 12, column 2), whereas the proportion of both fPTNs and sPTNs with stride-modulated firing was smaller than in M1 during both the flat surface and ladder locomotion (Fig. 12, column 3). Mean peak rates of sPTNs were smaller than in M1 during both tasks (Fig. 12, column 5). The average magnitude of the stride-related activity modulation of sPTNs during ladder locomotion was smaller than in M1 by both the dM and M measures, whereas the PEF(s) were longer (Fig. 12, columns 6–8). The proportion of sPTNs discharging with a 1-PEF pattern on the flat surface or ladder was smaller than in M1, and that of fPTNs was smaller on the flat surface (Fig. 12, column 9).

The results of the comparison suggest that both fPTN and sPTN populations of areas 6aa and 6ac participate less in the locomotion-related activity than their counterparts in M1 and, due to lower discharge rates and depths of activity modulation of sPTNs, may have a lesser influence on the information processing in the downstream neuronal networks, including the locomotion-related networks in the spinal cord. In M1, sPTNs have vigorous and concerted changes to their activities on transition from locomotion on the flat surface to vision-guided stepping on the ladder (24). This lesser influence of areas 6aa and 6ac efferents may still be important for adjusting limb movement for accurate stepping on a complex surface. It was indeed shown that electrical microstimulation in these areas readily produces contractions of forelimb muscles (69, 72), and the intensity of these contractions depends on the phase of the step cycle when the stimulation is applied (79).

Comparison with the Primary Somatosensory and Posterior Parietal Cortex of the Cat

We have previously described the activity of neurons in the cat primary somatosensory cortex (areas 1 and 2, S1) as the cat walked on the flat surface and horizontally placed ladder in a setting identical to that used in this study (30). Selected parameters of locomotion-related activity of neurons in areas 6aa and 6ac are compared with those in areas 1

fPTNs, n=18 sPTNs, n=14	1. Aver.Firing rate, spikes/s	2. Discharge Variabil., CV	3.Prop.Mod. %	4.Prop.with zero spikes in any bin, %	5. Mean Peak rate, spikes/s	6. Depth of Mod., dM, %	7. Coef. of Mod., M, %	8. Duration of PEF, % cycle	9. Prop. with one PEF, %
Standing/ Sitting	**17.2 ± 2.2** **10.8 ± 2.2**	1.6 ± 0.4 1.1 ± 0.3							
		p=0.048	*p*<0.0001						
Flat Surface Locomotion	**16.9 ± 3.2** **8.3 ± 1.4**	**2.5 ± 0.4** 2.0 ± 0.6	**67** **64**	0 7.1	**36.9 ± 9.9** **11.6 ± 2.5**	9.2 ± 0.7 8.2 ± 0.6	88.0 ± 2.4 82.3 ± 3.4	62.5 ± 5.0 59.5 ± 5.0	61 **50**
			p<0.001		*p*=0.008				*p*=0.01
			p<0.0001						*p*=0.004
Ladder Locomotion	**19.9 ± 3.6** 8.6 ± 1.4	2.0 ± 0.2 1.8 ± 0.3	**67** **79**	5.6 0	**38.1 ± 9.9** **11.9 ± 2.0**	9.8 ± 1.1 **7.5 ± 0.8**	86.2 ± 3.5 **79.8 ± 3.1**	60.5 ± 4.0 **71.5 ± 5.0**	**44** **50**
			p=0.021		*p*=0.005	*p*=0.002	*p*<0.001	*p*=0.03	*p*<0.001
fPTNs, n=10 Barriers Locomotion sPTNs, n=7	**15.9 ± 2.0** **9.7 ± 1.7**	2.2 ± 0.4 1.2 ± 0.2	100 71	10 0	**37.1 ± 6.9** **17.3 ± 5.3**	10.0 ± 1.2 7.8 ± 1.2	86.5 ± 4.2 80.4 ± 5.0	58.5 ± 4.5 67.0 ± 8.5	80 57

statistically significantly smaller than in M1 by t test or χ^2 test (see Table 2 in Stout and Beloozerova, 2013)

statistically significantly greater than in M1 by t test or χ^2 test (see Table 2 in Stout and Beloozerova, 2013)

The value of *p* is shown above the values for fPTNs and below the values for sPTNs.

Figure 12. Selected parameters of locomotion-related activity of areas 6aa and 6ac fast- and slow-conducting PTN populations compared with published data on primary motor cortex, area 4c (24). Values for fast-conducting PTNs (fPTNs) are shown in red font, values for slow-conducting PTNs (sPTNs) are shown in blue font. The mean value and the error of the mean are shown (mean ± SE). Underlined values in bold are statistically significantly different between fast- and slow-conducting PTNs according to Student's unpaired t test for averages. CV, the coefficient of variability of discharge rate is defined as CV ¼ s/m, where s is standard deviation and m is mean firing rate. PTN, pyramidal tract neuron.

and 2 in the table in Fig. 13. Designations are as before. One can see that the firing rates of neurons in areas 6aa and 6ac during standing or sitting, as well as locomotion on the flat surface and ladder are about half smaller than in S1 (Fig. 13, column 1). The proportion of cells with a stride-modulated firing is substantially smaller and the dM of the stride-modulated activity is much smaller (Fig. 13, columns 2 and 3). At the same time, the PEF(s) tend to be longer, at least for the flat surface locomotion for which there are published S1 data (Fig. 13, column 4). The proportion of neurons with a 1-PEF discharge pattern is much lower than in S1 (Fig. 13, column 5), and the proportion of 1-PEF cells with the PrPh shifting earlier in the cycle on the flat-to-ladder transition is larger (Fig. 13, column 9). These results show that the relationship of neuronal activity in areas 6aa and 6ac with the rhythm of strides is not as clear cut as in S1. They suggest that neuronal populations of these areas participate less in the locomotion-related activity than S1 populations and differ from them by a greater propensity to change the stride phase of the discharge when visual control of strides is required.

Two of our previous reports described the activity of neurons in the cat posterior parietal cortex area 5 as the cat walked on the flat surface, horizontally placed ladder, and stepped over a series of barriers in a setting identical to that of this study (31, 36). Selected parameters of locomotion-related activity of neurons in areas 6aa and 6ac are compared with those in area 5 in the table in Fig. 14. Designations are as before. Although the two studies of area 5 sampled slightly different regions of the area and thus yielded somewhat different data, the activity of neurons in areas 6aa and 6ac is distinct from that in either of these area 5 regions. Namely, the firing rates during standing or sitting, as well as locomotion on the flat surface, ladder, and with barriers are by far lower in areas 6aa and 6ac than in area 5 (Fig. 14, column 1). The proportion of cells with the stride-modulated firing on the flat surface and ladder is smaller, and the dM of the activity is substantially smaller across all tasks (Fig. 14, columns 2 and 3). This is while the PEF(s) are longer on both the flat surface and ladder, the two tasks for which area 5 data are available (Fig. 14, columns 4). Also, during locomotion on the flat surface, the proportion of neurons with a 1-PEF pattern is smaller, whereas during stepping over barriers it is larger (Fig. 14, columns 5). In addition, the proportions of cells the discharge rate of which increases on transition from the flat surface to vision-guided stepping on the ladder or over barriers are both smaller than in area 5 (Fig. 14, column 6). For the flat-to-ladder transition, the proportion of cells for which the dM increases is smaller, whereas the proportion for which it decreases is larger (Fig. 14, column 7). Also, the proportion of 1-PEF neurons, the PrPh of the activity of which shifts earlier in the cycle on transition to vision-guided stepping, is substantially larger (Fig. 14, column 9). Jointly, these results suggest that the neuronal population of areas 6aa and 6ac participates less in the locomotion-related activity than the area 5 population and that its firing behavior related to vision-guided locomotion is distinct from that of neurons in area 5.

The Origin of the Locomotion-Related Activity in Areas 6aa and 6ac

The stride rhythm-modulation of the firing during locomotion on the flat surface of 47% of neurons that do not have a

Flat Surface

Source, n of neurons	1. Average Firing rate spikes/s	2. Prop. Modul. %	3. Depth of Modul. dM, %	4. Duration of PEF % cycle	5. Prop. 1-PEF %
a.6aα,6aγ n=196	7.6 ± 0.5	59	8.5 ± 0.3	62.5 ± 1.5	39
F&al.2015 n=82	16.6 ± 1.4 $p<0.0001$	100 $p<0.0001$	10-13 ± 0.7 $p\leq 0.021$	52-57 ± 4.0 for 52–55.5, $p<0.05$	78 $p<0.0001$

Sitting or Standing

Source, n of neurons	1.Average Firing rate spikes/s
a.6aα,6aγ n=196	7.5 ± 0.5
F&al.2015 n=82	13.3 ± 1.2 $p<0.0001$

Ladder

Change between locomotion on the Flat surface and Ladder

Source, n of neurons	1. Average Firing rate spikes/s	2. Prop. Modul. %	3. Depth of Modul. dM, %	4. Duration of PEF % cycle	5. Prop. 1-PEF %	6. Discharge rate Proportion of cells Incr.,%	6. Discharge rate Proportion of cells Decr.,%	7. dM Proportion of cells Incr.,%	7. dM Proportion of cells Decr.,%	8. PEF Proportion of cells Incr.,%	8. PEF Proportion of cells Decr.,%	9. PrPh Proportion of cells Incr.,%	9. PrPh Proportion of cells Decr.,%
a.6aα,6aγ n=196	8.7 ± 0.6	62	8.8 ± 0.4	63.5 ± 1.5	35	28	17	28 (n=88)	23 (n=88)	11 (n=88)	13 (n=88)	24 (n=41)	34 (n=41)
F&al.2015 n=81	17.5 ± 1.5 $p<0.0001$	100 $p<0.0001$	16.4 ± 1.7 $p<0.0001$	_	72 $p<0.0001$	27	27	28	17	10	6	13 (n=52)	13 (n=52) $p=0.016$

statistically significantly smaller than in S1 by t test or χ^2 test

statistically significantly greater than in S1 by t test or χ^2 test

The value of p is shown below the values observed in S1.

Figure 13. Selected parameters of locomotion-related activity of areas 6aa and 6ac population compared with published data on primary somatosensory cortex areas 1and 2 (30). The mean value and the error of the mean are shown (mean ± SE). When a proportion was calculated for a subset of neurons, the number of neurons in the subset, n, is given in brackets under the value. Color designations are as in Fig. 11. F&al, Favorov et al. (30).

Flat Surface

Source, n of neurons	1. Average Firing rate spikes/s	2. Prop. Modul. %	3. Depth of Modul. dM, %	4. Duration of PEF % cycle	5. Prop. 1-PEF %
a.6aα,6aγ n=196	7.6 ± 0.5	59	8.5 ± 0.3	62.5 ± 1.5	39
B&S,2003 n=137	21.6 ± 1.7 p<0.0001	66	*8.5-10.6±0.5**	30-55 ± 3 p<0.02	46
B&al,2023 n=229	10.9 ± 0.7 p<0.0001	87 p<0.0001	9.7-11 ± 0.5 p<0.05	59 ± 1.3	64 p<0.0001

Sitting or Standing

Source, n of neurons	1. Average Firing rate spikes/s
a.6aα,6aγ n=196	7.5 ± 0.5
B&S,2003 n=137	16.3 ± 1.2 p<0.0001
B&al,2022 n=229	6.9 ± 0.6

Ladder

Source, n of neurons	1. Average Firing rate spikes/s	2. Prop. Modul. %	3. Depth of Modul. dM, %	4. Duration of PEF % cycle	5. Prop. 1-PEF %	Change between locomotion on the Flat surface and Ladder: 6. Discharge rate Proportion of cells Incr.,%	6. Discharge rate Proportion of cells Decr.,%	7. dM Proportion of cells Incr.,%	7. dM Proportion of cells Decr.,%	8. PEF Proportion of cells Incr.,%	8. PEF Proportion of cells Decr.,%	9. PrPh Proportion of cells Incr.,%	9. PrPh Proportion of cells Decr.,%
a.6aα,6aγ n=196	8.7 ± 0.6	62	8.8 ± 0.4	63.5 ± 1.5	35	28	17	28 (n=88)	23 (n=88)	11 (n=88)	13 (n=88)	24 (n=41)	34 (n=41)
B&S,2003 n=137	29.1 ± 2.6 p<0.0001	93 p<0.0001	*11.7 ± 0.6** p<0.0001	60 ± 1.0	22 p=0.011	55 p<0.0001	–	–	–	–	–	–	–
B&al,2023 n=229	13.5 ± 0.8 p<0.0001	98 p<0.0001	11.7 ± 0.4 p<0.0001	55 ± 1.2 p<0.0001	60 p<0.0001	41 p=0.005	14	*51*** p<0.001	*12*** p=0.014	*7***	*11***	17**	18** p=0.019

Barriers

Source, n of neurons	1. Average Firing rate spikes/s	2. Prop. Modul. %	3. Depth of Modul. dM, %	4. Duration of PEF % cycle	5. Prop. 1-PEF %	Change between locomotion on the Flat surface and Barriers: 6. Discharge rate Proportion of cells Incr.,%	6. Discharge rate Proportion of cells Decr.,%	7. dM Proportion of cells Incr.,%	7. dM Proportion of cells Decr.,%	8. PEF Proportion of cells Incr.,%	8. PEF Proportion of cells Decr.,%	9. PrPh Proportion of cells Incr.,%	9. PrPh Proportion of cells Decr.,%
a.6aα,6aγ n=84	7.2 ± 0.6	76	8.8 ± 0.5	63.5 ± 2.0	52	19	14	30 (n=46)	11 (n=46)	15 (n=46)	13 (n=46)	38 (n=24)	21 (n=24)
B&S,2003 n=47	24.4 ± 3 p<0.0001	87	11.5 ± 0.7 p=0.002	_	30 p=0.015	43 p=0.003	–	–	–	–	–	–	–

Beloozerova and Sirota, 2003: *8.5-10.6 ± 0.5** - M stated in the paper is recalculated to dM for this comparison

Beloozerova et al., 2023: ** - unpublished observations

Figure 14. Selected parameters of locomotion-related activity of areas 6aa and 6ac population compared with published data on posterior parietal cor-tex area 5 (31, 36). The mean value and the error of the mean are shown (mean ± SE). When a proportion was calculated for a subset of neurons, the number of neurons in the subset, n, is given in brackets under the value. Color designations are as in Fig. 11.

somatosensory RF suggests that the somatosensory stimulation is not the cause of this modulation. RFs determined in the resting animal are likely to be modified by locomotion, as active movements modulate somatosensation (reviewed in, e.g., Ref. 5), however, it is unlikely that they are modified to a degree necessary to explain the entire range of the observed stride-related activity modulation. We have earlier found that even in the S1 cortex the somatosensory responsiveness of neurons often is not the leading source of the stride-related modulation of their activity (30). For neurons in the M1, S1, and posterior parietal area 5, we have previously suggested that the stride-related modulation of their firing during locomotion on the flat surface is chiefly determined by the efference copy signals from the spinal cord locomotor central pattern generator (CPG, general review in, e.g., Ref. 80) (17, 23, 30, 31, 36). This suggestion is an extrapolation of the hypothesis introduced by Arshavsky and colleagues (81) that all commands addressed from the brain to the spinal mechanisms during locomotion take into account the current state of the spinal mechanisms, that is, the phase of the locomotor cycle. This is accomplished by modulating the activity of all the brain structures involved in the control of locomotion in the rhythm of strides. Such modulation allows the brain centers adjust stepping movements without disturbing the basic locomotor pattern. We want to extend this hypothesis to premotor areas 6aa and 6ac. The signals from the spinal locomotor CPG can reach these areas via the ventrolateral thalamus (VL), which in turn obtains them from the cerebellum as discussed by Marlinski et al. (61). Several studies showed that the premotor cortex, including areas 6aa and 6ac, receives projections from the VL (82–85). However, these projections are not as numerous as those to the M1, and this may explain the smaller average depth of the stride-related modulation of the firing of neurons in areas 6aa and 6ac compared with that of cells in M1 (Fig. 11, column 3).

When the animal transitions from vision-independent locomotion on the flat surface to visually guided stepping on

a complex surface, the firing of 83–86% of neurons in areas 6aa and 6ac changes. What is the source of this change? For the flat-to-ladder transition, factors other than stimulation of somatosensory RFs of the neurons must be at play. This is because mechanical characteristics of locomotion along the convenient ladder that we use in our studies differ only slightly from mechanics of locomotion on the flat surface (22). Specifically, during locomotion on such a ladder compared with the flat surface, cats only rotate the neck and head down, increase flexion of the distal joints, reduce the wrist flexion moment during stance, and modify several hindlimb joint moments during stance. Other mechanical variables, out of 229 examined, are similar between the tasks. This similarity makes it likely that the somatosensory afferent signals arriving to the premotor cortex during these tasks do not differ very much. We feel that the differences in mechanics are insufficient to cause the observed differences in discharges of areas 6aa and 6ac neurons between the flat surface and ladder locomotion, especially given that the majority of neurons in our sample did not have a somatosensory RF.

On the other hand, cats move their eyes and look at the walking pathway in a very different manner during flat surface and ladder locomotion (22, 50), and the coordination of the gaze with strides increases on complex surfaces (52). Considering the rather similar motor patterns of the two locomotion tasks but dramatically different gaze behaviors and the need for vision, we have previously suggested that during locomotion on the ladder, which requires visual guidance of stepping, processed visual information is transmitted by neurons in M1 and posterior parietal area 5 by the way of modulating their locomotion rhythm-related discharges (22, 36). We suggest that same is true for neurons in areas 6aa and 6ac, albeit they discharge with lower rates and smaller magnitudes of the stride-related modulation. Among all the cat frontal, prefrontal, and prelimbic cortices, the premotor area 6aa is the most visually responsive (37). Most neurons here have large RFs and respond briskly to visual stimulation. They prefer moving stimuli and some have directional preference for centripetal movement. Area 6ac cells less often respond briskly to visual stimulation but also have large RFs and prefer moving stimuli. These visual response properties of neurons in areas 6aa and 6ac make them well suited for detecting large approaching objects such as crosspieces of the ladder or barriers. These visual responses are likely to superimpose on and modify the stride rhythm modulation of the activity of the neurons during locomotion on the complex surface.

For the flat-to-barriers and ladder-to-barriers transitions, the high lifting of limbs during stepping over barriers certainly activated somatosensory receptors more than locomotion on the flat surface or ladder. The altered firing of area 6aa and 6ac neurons likely reflected this activation. However, the fact that the discharge rate, dM, and PrPh of 13% of cells with a somatosensory RF were similar between locomotion with barriers and on the ladder while differing from those on the flat surface indicates that factors other than stimulation of somatosensory RFs during the higher than normal lifting of limbs drove their responses. We believe that similarly to the visually guided stepping on the ladder, added responses to visual stimuli from the obstructed walkway modulated discharges of neurons in areas 6aa and 6ac during locomotion with barriers.

How Does the Integrated Visuomotor Activity of Neurons in Areas 6aa and 6ac Assist Accurate Stepping on the Complex Surface?

Low-current electrical microstimulation (<50 lA, 300 Hz) within areas 6aa and 6ac applied during locomotion modulates muscle activity in both fore- and hindlimbs, as well as the neck and face (79). This suggests that spontaneous discharges of neurons in these areas also influence muscle activity during locomotion. Accurate stepping on the ladder requires that strides are of a specific length, 50 cm. For a paw 3 cm in diameter stepping on a 5-cm-wide crosspiece, only ±1 cm (2%) variability of the stride length is allowed. It must be that the selective increases and decreases of firing of 44% of area 6aa and 6ac neurons during locomotion on the ladder result in selective increases and decreases of the activity of particular muscles and translate into this specific size of the stride. Of course, areas 6aa and 6ac neurons are not the sole controllers as cells in M1, S1, and posterior parietal area 5 also contribute (17, 22–24, 30, 31, 36). Across all these areas, both increases and decreases of neuronal activity on the flat-to-ladder transition take place, suggesting that for making a stride of a certain length some muscles need to be additionally activated, while the activity of others is reduced.

The precision of the stride length, i.e., the consistency of the needed length, is likely to be encoded by the magnitude of the stride-related firing, the dM and M. These parameters signify the salience of the stride-related signal, as greater they are as greater is the salience. A more salient signal from 28% of areas 6aa and 6ac neurons during locomotion on the ladder likely results in more precise strides. In M1, 60–80% of neurons use this mechanism (17, 22). The precision of the stride length can be also regulated by the duration of the PEFs. For example, in M1, the duration of the PEF of 30% of neurons decreases with increasing requirement for precision of strides; a lower variability of the stride length is associated with a shorter firing of the neurons (22). However, in areas 6aa and 6ac, the duration of PEF(s) of only 13% of neurons decreases on the flat-to-ladder transition, indicating that only a small proportion of the population contributes to the precision of the stride length using this mechanism.

It should be noted, however, that no change in the discharge rate, dM, or duration of PEF(s) was the most frequent response of areas 6aa and 6ac neurons to the demand for visuomotor coordination on the ladder. Rather it was a change of the stride phase during which neurons fired. This could be a change of the discharge pattern from 1-PEF to 2-PEF (more often) or vice versa (less often), or a shift of the PrPh of firing of neurons with a 1-PEF pattern or a phase shift of one or both PEFs of neurons with a 2-PEF pattern. The change of the stride phase where areas 6aa and 6ac neurons discharged was probably reflected in the small change of the phase of the activity of selected limb muscles [e.g., lateral gastrocnemius muscle (22)] and potentially some neck muscles (53). Overall, however, this change appears to be more substantial than phase changes in the activity of muscles, at least the ones that were studied so far. Thus, the

function of the phase change of the activity of areas 6aa and 6ac neurons upon flat-to-ladder transition remains unclear.

How do changes in the firing activity of areas 6aa and 6ac neurons help lift limbs higher during stepping over barriers? Between locomotion on the ladder and with barriers, perhaps the most striking difference is that the population activity of neurons with a 2-PEF discharge pattern during locomotion with barriers particularly increases in the middle of the swing and middle of the stance phases of the contralateral forelimb, the latter is also the swing phase of the ipsilateral forelimb (Fig. 8D4 vs. Fig. 5D4). This suggests, first, that the firing of this neuronal group conveys a signal that helps activate muscles for lifting the limbs, and second, that it may contribute to the coordination between the forelimbs. Nakajima and colleagues (12) also noted that the activity of some cells in subdivisions of the premotor cortex that they studied is consistent with a view that these cells contribute to interlimb coordination for stepping over an obstacle. In addition, it was shown that electrical microstimulation within areas 6aa and 6ac activates limb muscles on both sides with nearly equal efficacy (79).

It is also possible that the activity of neurons in areas 6aa and 6ac contributes to control of the movement of the head during stepping over barriers. The head of the cat is held actively even during locomotion on the flat surface with reflexes playing only a partial role in determining head movement and vision further diminishes their role (51). Our preliminary data show that the movement of the head during locomotion with barriers is quite similar to that on the flat surface (53). This suggests that the head movement during stepping over barriers is also controlled actively, being maintained within a specific range. The firing activity of neuronal populations of areas 6aa and 6ac is well suited for providing such control because electrical microstimulation within these areas readily activates neck muscles and evokes head movements (69, 72, 79). During locomotion with barriers, the dM of the activity of neurons that are associated with more than just the contralateral (right) forelimb (those with RFs also including the right hindlimb, body, or head) is higher compared with that of cells associated with the right forelimb only, and their PEFs are shorter. No such difference was observed during locomotion on the ladder. This indicates that stepping over barriers requires a tighter coordination between the limbs and the limbs and the head than stepping on the ladder. Neurons of areas 6aa and 6ac with widely branching descending axons are perfectly posed for contributing to the interlimb and limb-head coordination. The contribution of different subdivisions of area 6 to interlimb coordination during stepping over an obstacle was recently discussed by Dr. Drew and colleagues (12, 79).

What are the routes for the activity of areas 6aa and 6ac neurons to affect the musculature during locomotion? A better understanding of how signals from areas 6aa and 6ac influence the activity of downstream motor centers is required to answer this question. At present, we can only point out that it does not seem likely that the main route of the influence of areas 6aa and 6ac on the peripheral apparatus during locomotion goes via motor cortex. One important difference in the activity of these areas argues against this route. The stride phase of the discharge of the majority of areas 6aa and 6ac neurons changes on the transition from locomotion on the flat surface to accurate stepping on the ladder or over barriers. In contrast, the characteristic feature of the activity of neurons in motor cortex during the flat-to-ladder and flat-to-barriers transitions is the rather stable phase distribution of the firing (17, 22–24, 36). It seems unlikely that a stable phase distribution of firing is constructed out of a flexible one. It seems more likely that areas 6aa and 6ac act independently of motor cortex imposing their direct influence on the downstream motor centers and the spinal cord. Projections from areas 6aa and 6ac to both the cervical and lumbar spinal cord as well as rich projections to the pontomedullary reticular formation were documented by a number of studies (69, 76, 77, 79, 86, 87).

When investigating the strategies for obstacle avoidance during walking in the cat we have previously found that while cats prefer to alter muscle activity to avoid an obstacle without altering the locomotor rhythm, when such a strategy is not sufficient, they also adjust the rhythm of the stride and/or the swing-to-stance ratio (57). These strategies are not exclusive but complement each other. This suggests that during locomotion on the complex surface distinct motor commands concurrently adjust different aspects of the stride. In that earlier study, we hypothesized that this flexibility in using different strategies for modifying the stride on a complex terrain is achieved by the way of concurrent processing of parallel motor commands that originate in distinct brain areas and target different groups of spinal neurons (57). The comparison of the activity of several cortical areas during visually guided stepping on the complex surface presented above shows that these different areas, all of which house neurons that send axons down to the pyramidal tract, contribute simultaneously but differently to the task of making the strides accurate. The existence of both parallel and serial cortical processing for planning and executing a step over an obstacle was also suggested by Drew and colleagues based on the analysis of discharges of cortical neurons during locomotion on the treadmill (12, 29, 79).

Limitations of the Study

The major limitation of this study is that only a portion of area 6aa and a small portion of area 6ac were explored. Analyzing the activity of larger extents of these areas will provide a more accurate assessment of their involvement in visually guided locomotion.

DATA AVAILABILITY

The data supporting the findings of this study are available from the corresponding author (I.N.B.; ibeloozerova3@gatech.edu) upon request.

SUPPLEMENTAL DATA

Supplemental Figs. S1–S3: https://doi.org/10.6084/m9.figshare.22270321.

ACKNOWLEDGMENTS

We are indebted to Peter Wettenstein for exceptional engineering assistance.

GRANTS

This research was supported by National Science Foundation (grant 1912557) to I.N.B., and in part, by the National Institute of

Neurological Disorders and Stroke (grant R01 NS-058659) to I.N.B and the National Eye Institute (grant R21 EY-033071) to I.N.B.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

I.N.B. conceived and designed research; G.V.D.P., V.M., and I.N.B. performed experiments; G.V.D.P., V.M., and I.N.B. analyzed data; G.V.D.P., V.M., and I.N.B. interpreted results of experiments; G.V.D.P. and I.N.B. prepared figures; G.V.D.P. and I.N.B. drafted manuscript; G.V.D.P., V.M., and I.N.B. edited and revised manuscript; G.V.D.P., V.M., and I.N.B. approved final version of manuscript.

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