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RESEARCH ARTICLE | *Control of Movement*

Contribution of the ventrolateral thalamus to the locomotion-related activity of motor cortex

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Beloozerova IN, Marlinski V. Contribution of the ventrolateral thalamus to the locomotion-related activity of motor cortex. *J Neurophysiol* 124: 1480–1504, 2020. First published August 12, 2020; doi:10.1152/jn.00253.2020.—The activity of motor cortex is necessary for accurate stepping on a complex terrain. How this activity is generated remains unclear. The goal of this study was to clarify the contribution of signals from the ventrolateral thalamus (VL) to formation of locomotion-related activity of motor cortex during vision-independent and vision-dependent locomotion. In two cats, we recorded the activity of neurons in layer V of motor cortex as cats walked on a flat surface and a horizontal ladder. We reversibly inactivated ~10% of the VL unilaterally with the glutamatergic transmission antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and analyzed how this affected the activity of motor cortex neurons. We examined neuronal subpopulations with somatosensory receptive fields on different segments of the forelimb and pyramidal tract projecting neurons (PTNs). We found that the VL contribution to the locomotion-related activity of motor cortex is very powerful and has both excitatory and inhibitory components. The magnitudes of both the excitatory and inhibitory contributions fluctuate over the step cycle and depend on locomotion task. On a flat surface, the VL contributes more excitation to the shoulder- and elbow-related neurons than the wrist/paw-related cells. The VL excites the shoulder-related group the most during the transition from stance to swing phase, while most intensively exciting the elbow-related group during the transition from swing to stance. The VL contributes more excitation for the fast- than slow-conducting PTNs. Upon transition to vision-dependent locomotion on the ladder, the VL contribution increases more for the wrist/paw-related neurons and slow-conducting PTNs.

NEW & NOTEWORTHY How the activity of motor cortex is generated and the roles that different inputs to motor cortex play in formation of response properties of motor cortex neurons during movements remain unclear. This is the first study to characterize the contribution of the input from the ventrolateral thalamus (VL), the main subcortical input to motor cortex, to the activity of motor cortex neurons during vision-independent and vision-dependent locomotion.

cat; CNQX; inactivation; PTN; vision

INTRODUCTION

The activity of motor cortex is profoundly modulated in the rhythm of strides. Individual neurons discharge more in one phase of the stride and less in another phase, and the activity of the entire population is also modulated (Armstrong and Drew 1984a, 1984b; Beloozerova and Sirota 1985, 1993a, 1993b; Bradford et al. 2016; DiGiovanna et al. 2016; Drew 1993; Farrell et al. 2014, 2015; Fitzsimmons et al. 2009; Gwin et al. 2011; Miri et al. 2017; Stout et al. 2015; Stout and Beloozerova 2012, 2013; Xing et al. 2019; Yokoyama et al. in press). The intensity of the modulation depends on the complexity of locomotor behavior. It is typically more pronounced during complex tasks, such as stepping accurately over obstacles or along a horizontal ladder, compared with locomotion on a flat surface. In turn, performance of complex locomotor behaviors that require visuo-motor coordination and accuracy of steps severely suffers when the activity of motor cortex is compromised (Beloozerova and Sirota 1993a; Den Otter et al. 2005; Farr et al. 2006; Friel et al. 2007; Liddell and Phillips 1944; Metz and Whishaw 2002; Trendelenburg 1911). Thus, motor cortex plays an essential role in control of visually guided locomotion. However, how its activity is generated remains unclear. This is in large part because we do not know how signals received by motor cortex during locomotion contribute to the formation of its activity. Understanding how the locomotion-related activity of motor cortex arises is necessary for a better understanding of the role that motor cortex plays in control of locomotion.

Different inputs to motor cortex can convey locomotion-related signals shaping the activity of motor cortical neurons, including somatosensory inputs via the thalamus and cortex, various inputs from parietal and prefrontal cortices, and inputs from the basal ganglia via the thalamus. Signals from a part of the ventrolateral thalamus that connects the cerebellum to motor cortex and is the main source of ascending projections from the thalamus to motor cortex may be particularly important (e.g., Allen and Tsukahara 1974; Asanuma et al. 1974; Hendry et al. 1979; Massion 1976; Massion and Rispal-Padel 1972; Rispal-Padel et al. 1981; Sauerbrei et al. 2020; Shinoda et al. 1985b; Strick 1973). Before considering these signals, we want to clarify the nomenclature for nuclei in the thalamic ventrolateral complex, as it slightly differs between primates and felines. In primates the cerebellum-receiving part of the ventrolateral thalamus is termed VLp, whereas it is called VL in felines. Below we use the term "VL" to refer to the cerebellum-receiving part of the ventrolateral thalamus. This part is largely distinct from the basal ganglia-receiving part,

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which is termed VLa in primates and VA in felines (Asanuma et al. 1983; Ilinsky and Kultas-Ilinsky 1984).

During locomotion, the VL conveys two types of information from the cerebellum to motor cortex. It transmits information about the ongoing locomotion received by the cerebellum from the locomotion-related network of the spinal cord and peripheral somatosensory receptors (reviewed in Arshavsky et al. 1986; Orlovsky et al. 1999). It also conveys visual information about locations of objects in the environment received by the cerebellum via the pons from several areas of the cortical visual "dorsal stream" areas, the "Where?" pathway (Glickstein 2000). However, the VL is more than a simple cerebellum-to-motor cortex relay. During locomotion, motor, somatosensory, and visual information merge in the VL (Marlinski et al. 2012a). This merging occurs in part because different inputs from the cerebellum converge on VL neurons (e.g., Shinoda et al. 1985a; Uno et al. 1970). It also occurs because, in addition to the locomotion-related inputs from the cerebellum, the VL receives direct inputs from the spinal cord (Craig 2008; Mackel et al. 1992; Yen et al. 1991). The information that the VL transmits to motor cortex is important for accurate visually guided movements, as lesions in the VL profoundly impair motor performance. Human patients enduring infarctions in the thalamus that involve the VL have ataxia in the contralateral parts of the body and great difficulty performing accurate arm movements (Garcin 1955; Melo et al. 1992; Solomon et al. 1994). Experimental lesions in the VL in cats compromise accuracy of jumps and locomotion along a horizontal bar, increase latency of reaching movements, and render cats unable to learn a new visual-motor coordination task (Batuev et al. 1983; Cherenkova et al. 1981; Cherenkova and Iunatov 1983; Fabre and Buser 1980; Fabre-Thorpe and Levesque 1991). In a prior study, we also found that for several days after a VL lesion a cat was unable to accurately step over barriers or along a horizontal ladder (Beloozerova and Sirota 1988, 1998).

How the visuo-motor information that the VL transmits to motor cortex contributes to the formation of motor cortex activity during locomotion remains unclear. We have previously suggested that signals from the VL are the main drivers of motor cortex activity during both vision-independent locomotion on a flat surface and visually guided stepping on a complex terrain (Marlinski et al. 2012a). This possibility appeared plausible because the activity of VL neurons during locomotion resembles that of motor cortex neurons in several important aspects. First, during locomotion on a flat surface the activity of the vast majority of VL neurons is modulated in the rhythm of strides and most cells discharge one activity burst per stride, a pattern also typical for motor cortical neurons. Second, upon transition to visually guided locomotion on a horizontal ladder the activity of most VL neurons changes similarly to that of motor cortical neurons, namely, the depth of neurons' stride-related activity modulation typically increases and the discharge becomes more restricted to specific phases of the stride. Although some differences between the activity of neurons in the VL and motor cortex have been seen, the noted similarities allowed us to speculate that the VL may have a decisive role in determining the activity of motor cortical neurons during locomotion. However, this inference was made only by correlation. The goal of this study was to directly determine the contribution of signals from the VL to the formation of the activity of motor cortex neurons during vision-independent and vision-dependent locomotion.

The first question was: How do the monosynaptic excitatory and di-/oligosynaptic inhibitory inputs from the VL influence the activity of motor cortex neurons during locomotion? Cortically projecting neurons of the VL are glutamatergic (Graziano et al. 2008; Salt et al. 1995), and numerous studies have shown that they excite motor cortex neurons (e.g., Baranyi et al. 1993; Baranyi and Fehér 1978; Noda and Yamamoto 1984; Shinoda et al. 1985a). However, an important role of the di-/oligosynaptic inhibitory influence from the VL via cortical inhibitory interneurons was also demonstrated in many studies (e.g., Baranyi et al. 1993; Nashef et al. 2018). The need to further investigate the nature of the VL influence upon layer V motor cortical neurons in ambulating animals arose from the following observation: We found that group activities of VL neurons projecting to motor cortex that have somatosensory receptive fields on a specific segment of the forelimb (shoulder, elbow, or wrist/paw) peak in opposite phases of the stride compared with layer V motor cortical cells with a receptive field on the same segment (Marlinski et al. 2012a; Stout and Beloozerova 2012; reviewed in Beloozerova et al. 2013). For example, the group activity of shoulder-related VL neurons peaks during transition from the swing to stance phase of the stride, whereas that of motor cortex neurons peaks during transition from stance to swing. One possible explanation could be that, in analogy to the somatosensory system, where axons from the ventro-basal thalamus vigorously excite inhibitory interneurons in somatosensory cortex in addition to exciting efferent cells (e.g., Swadlow 2002), neurons in layer V of motor cortex receive their main input from the VL during locomotion not directly but via the cortical inhibitory network (e.g., Baranyi et al. 1993; Beloozerova et al. 2003; Estebanez et al. 2017; Merchant et al. 2008; Nashef et al. 2018). Such a relay via the cortical inhibitory network would provide many functional benefits, as discussed, for example, in Tremblay et al. (2016) and Feldmeyer et al. (2018), and therefore can be expected, particularly during a demanding behavior. Thus, the first aim of this study was to determine the effects of the VL input on the activity of individual neurons and subpopulations of neurons in layer V of motor cortex during locomotion. To do this, we reversibly inactivated a small area of the VL, so that the motor performance was barely affected, while the thalamo-cortical transmission in a part of the pathway was disrupted. We then analyzed changes in the activity of motor cortical neurons during two locomotion tasks: locomotion on a flat floor, a vision-independent task, and locomotion on tops of crosspieces of a horizontal ladder, a complex vision-dependent task (Beloozerova and Sirota 2003). We found that the VL effects on the activity of motor cortex neurons depend on the phase of the stride, the locomotion task, and the subpopulation of neurons.

The second question was whether the VL influence on individual cortical neurons and subpopulations of neurons, be it excitatory or inhibitory, is steady over the step cycle or fluctuates. Although we have previously found that the activity of the overwhelming majority of VL neurons projecting to motor cortex fluctuates in the rhythm of strides (Marlinski et al. 2012a), numerous thalamo-cortical axons converge on individual neurons in motor cortex and their contribution may potentially equalize across the step cycle. Thus, it remained to be

determined whether the net contribution of the VL to the activity of motor cortical neurons fluctuates over the step cycle or is rather tonic. If the VL contribution was found to fluctuate over the step cycle, a question would arise as to what the preferred phases of the stride are for the VL contribution and how these phases are related to the preferred phases of activity of motor cortical neurons. We assessed the contribution of the VL to the activity of motor cortical neurons in two ways: *1*) as the effect on the discharge rate in spikes per second and *2*) as the percentage of VL-driven activity in the total discharge rate. We found that the VL contribution nearly always fluctuates over the step cycle in both aspects. We found that the phase of the VL contribution typically coincides with the preferred activity phase(s) of the recipient cortical neurons. Moreover, we found that the contribution of ~10% of the VL may account for up to 100% of the activity of individual neurons during some periods of the step cycle and for 25–30% of the average activity of the entire layer V population. Thus, we concluded that the VL contribution is very powerful, is step phase dependent, and plays a substantive role in the formation of preferred phases of activity of layer V motor cortex neurons.

The third question was how different the VL contribution to the activity of motor cortical neurons is between vision-independent and vision-dependent locomotion. How different is the contribution when the VL signals are supposedly based on motor information received from the spinal cord (directly or via the cerebellum) compared with when signals from the cerebellum carrying visual information are thought to also contribute? How does the VL contribute to the activity of motor cortex that is specifically related to processing of visual information? One of the major findings about the activity of motor cortex during locomotion is that, when visual control of steps is required on a complex terrain, the depth of the stride-related modulation of the neurons' activity changes, typically increases, and the activity becomes more restricted to specific phases of the stride (Beloozerova et al. 2010; Beloozerova and Sirota 1993a; Drew 1993; Xing et al. 2019). Are these changes produced by the corresponding changes in signals from the VL as we suggested in our earlier study (Marlinski et al. 2012a)?

To answer this question, we compared VL contribution to the activity of motor cortical neurons during vision-independent locomotion on a flat surface and visually guided stepping along a horizontal ladder. We previously showed that while locomotion on the flat surface can be accomplished without vision, locomotion along the horizontal ladder, even well practiced, cannot (Beloozerova and Sirota 2003). At the same time, we found that the mechanics of well-practiced locomotion on the horizontal ladder with wide flat crosspieces positioned at a convenient distance of a typical length of the stride are very similar to those of locomotion on a flat surface (Beloozerova et al. 2010). This similarity allowed us to interpret the changes in the activity of VL and cortical neurons that occur upon the transition from locomotion on the flat surface to the ladder as a reflection of integration of visual information with ongoing locomotion (Beloozerova et al. 2010; Marlinski et al. 2012a). In this study, we compared the contribution of the VL to the activity of individual neurons and subpopulations of neurons in motor cortex during locomotion on the flat surface and the convenient horizontal ladder and interpreted the observed differences as the vision-related contribution of the VL. We found that nearly all tested neurons in layer V of motor cortex received some visual information-related input from the VL. In many cases, the visual information-related contribution of the VL had two maxima during the step cycle, which coincided with the phases of gaze fixations. We concluded that the VL substantially shapes the activity of motor cortex neurons during vision-dependent locomotion.

The fourth question was whether the contribution of the VL differs in respect to cortical circuits controlling different segments of the limb. There is mounting evidence that different segments of the limb are controlled differently during locomotion, which we briefly reviewed in Beloozerova et al. (2013). In addition, in our recent studies we found that different limb segments participate differently in the adaption of limb movement during visually guided locomotion (Stout et al. 2015) and that their movement is controlled actively during different phases of the stride (Zubair et al. 2018). We hypothesized that differences in the control of different segments of the limb during locomotion arise, at least partly, from differences in signals that motor cortical circuits related to different segments of the limb receive from the VL. The average activity of VL subpopulations related to different segments of the forelimb peaks during different phases of the stride (Beloozerova et al. 2013; Marlinski et al. 2012a). VL neurons with somatosensory receptive fields on the shoulder, as a group, are preferentially active during the transition from the swing to stance phase, those with fields on the elbow are most active during the transition from the stance to swing phase, opposite in phase to that of the shoulder-related neurons, and neurons with somatosensory fields on the wrist and paw are most active during the stance phase. If it is assumed that the thalamo-cortical projection is primarily excitatory and influences cortical neurons with similar somatosensory receptive fields, it can be expected that the VL excites the shoulder-related neurons during the transition from the swing to stance phase, activates the elbow-related cells during the transition from the stance to swing phase, and excites the wrist/paw-related neurons during the stance phase. We found that the VL influences cortical neurons with somatosensory receptive fields on different segments of the forelimb differently. However, the phases of the influence were not the ones that could have been expected based on the above assumptions. In fact, for the shoulder- and elbow-related groups, they were opposite. In addition, the efficacy of the VL contribution to the activity of different subpopulations of motor cortical neurons varied greatly, which could not be expected based on VL activity data.

Finally, the fifth question was whether the contribution of the VL to the activity of fast- and slow-conducting pyramidal tract projecting neurons (PTNs) is different. It was shown that during locomotion information conveyed from motor cortex along different efferent pathways is different (e.g., Beloozerova et al. 2003; Sirota et al. 2005). The major difference between the activity of fast- and slow-conducting PTNs is that the slow-conducting PTNs are less active (Armstrong and Drew 1984a; Stout and Beloozerova 2013). However, we found that slow-conducting PTNs respond to the demand for visuo-motor coordination and accuracy of stepping during locomotion more vigorously than fast-conducting PTNs (Stout and Beloozerova 2013). Thus, we wondered whether these dissimilarities in the activity of fast- and slow-conducting PTNs, particularly their dissimilar responses to the demand for

visuo-motor coordination during locomotion, are due to different contributions they receive from the VL. Data briefly reviewed above indicate that the VL conveys to motor cortex information needed for accurate visually guided locomotion on a complex surface. Therefore, we hypothesized that the VL may have a larger contribution to the activity of the slow- than fast-conducting PTNs. Our results have supported this hypothesis, as a larger increase in the VL contribution to the activity of the slow- than fast-conducting PTNs was indeed found upon the transition from vision-independent locomotion on the flat surface to accurate stepping along the ladder, a task for which visual information was needed at the cortex.

Brief accounts of parts of this study have been published in abstract form (Beloozerova 2019; Marlinski et al. 2011).

METHODS

Experimental Strategy

We performed experiments in two freely ambulating domestic cats (*Felis catus*): a female (*cat A*, weight 2.7 kg) and a male (*cat B*, weight 4.0 kg). Cats were purchased from a certified commercial class B dealer. We have used cats because they easily engage in consistent locomotion, which is necessary for collection of statistically sound data sets. We also utilized cats' amenability to petting to accurately examine somatosensory receptive fields of neurons. This allowed us to jointly evaluate neurons with receptive fields on the same segment of the forelimb and characterize their group responses. In addition, the activity of motor cortex during locomotion has been described in great detail in the cat (e.g., Armer et al. 2013; Armstrong and Drew 1984a, 1984b; Beloozerova et al. 2010; Beloozerova and Sirota 1985, 1993a, 1993b; Drew 1993; Farrell et al. 2014, 2015; Stout et al. 2015; Stout and Beloozerova 2012, 2013), which provided a necessary reference for this study. Importantly, motor cortex of both cats used in this study was explored, and results are presented as parts of three prior reports: Armer et al. (2013) and Stout and Beloozerova (2013) for both cats and Beloozerova et al. (2010) for *cat A*. Finally, it was essential for this study that the activity of the VL during locomotion in these two cats was also studied before the initiation of experiments described here, and this is reported in Marlinski et al. (2012a).

We obtained extracellular recordings of the activity of single motor cortex neurons in these two cats as they ambulated around an experimental chamber. We analyzed how the locomotion-related discharges of motor cortex neurons were affected by a short-lasting decrease of the activity in a part of the VL. We decreased the activity in a small part of the VL (~10% of the VL) so that the motor performance was barely affected and analyzed the effect on the activity of motor cortex neurons. Not affecting the motor performance substantially was important because a disturbed movement could have affected the activity of the neurons and thus obscure the specific effect of the reduction in thalamo-cortical signaling. Many sources that provide an input to the thalamus, including cerebellum (Nieoullon et al. 1984), sensorimotor cortex (Kerkerian et al. 1982), and spinal cord (Broman and Ottersen 1992; Ericson et al. 1995) use glutamate as a neurotransmitter and excite thalamic neurons. Thus, to reduce the activity of VL neurons, we blocked excitatory inputs to them, using microinjection of a competitive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate-type glutamate receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Honoré et al. 1988; Lee et al. 2010). The effect of CNQX is reversible, lasting 2–4 h. A unilateral blockade was performed on the same side of the brain as recorded motor cortex. The activity of motor cortical neurons before and after the blockade was recorded and compared for two locomotion tasks: vision-independent locomotion on a flat surface and vision-dependent accurate stepping on crosspieces of a horizontal ladder.

We focused on the areas in motor cortex and VL that are related to the forelimb. We determined the somatosensory receptive fields of cortical neurons and grouped the neurons for analysis according to the segment of the forelimb on which the receptive field was located. All recordings were obtained from cortical layer V, which was identified by the presence of neurons with axons descending within the pyramidal tract. We identified axonal projections of the neurons, calculated the axonal conduction velocity, and grouped the neurons for the analysis according to conduction velocity of the axon. We then analyzed the effects of an inactivation of a part of the VL on the locomotion-related activity of the neurons, and on the activity of subpopulations of neurons with somatosensory receptive fields on different segments of the forelimb, and PTNs with axons conducting with different velocities.

Methods of surgical preparation and recording techniques have been previously described (Marlinski et al. 2012a; Prilutsky et al. 2005) and are only briefly reported here. The experimental protocol was in compliance with National Institutes of Health guidelines for the care and use of animals in research and was approved by the Barrow Neurological Institute Animal Care and Use Committee.

Locomotion Tasks

Positive reinforcement (food) was used to adapt cats to the experimental situation and to engage them in locomotion (Pryor 1975; Skinner 1938). Two locomotion tasks were used: locomotion on a flat surface, and locomotion on crosspieces of a horizontal ladder (Fig. 1*A*). It has been demonstrated in several studies that locomotion on a

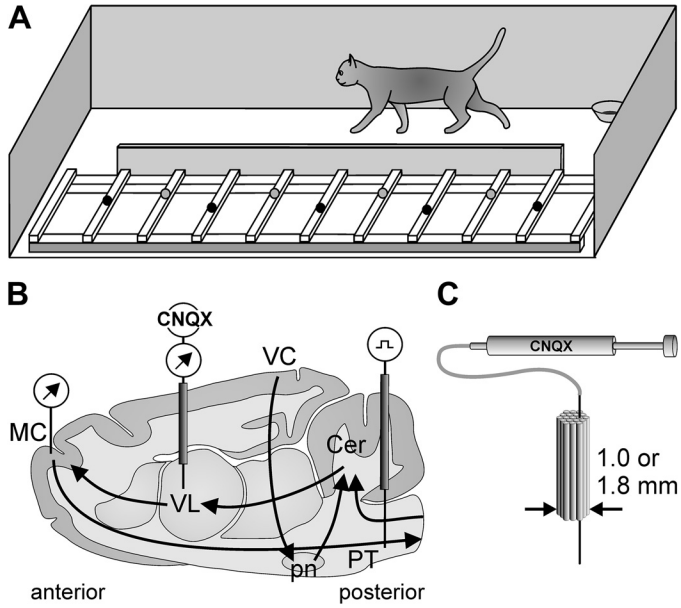


Fig. 1. Experimental paradigm. *A*: locomotion tasks. The experimental chamber was divided into 2 corridors. In one corridor the floor was flat, and the other corridor contained a horizontal ladder. Black circles on the crosspieces of the ladder schematically show placements of the right forelimb paw, and gray circles show those of the left forepaw. *B*: sites of recording (circles with arrows), injections (a circle with letters), and electrical stimulation (a circle with a square shape) are shown on a schematic drawing of a parasagittal section of the cat brain. Cer, cerebellum; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione, a competitive α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate glutamate receptor antagonist; MC, motor cortex; pn, pontine nuclei; PT, pyramidal tract; VC, visual cortex; VL, ventrolateral nucleus of the thalamus. Curved arrows schematically show flow of signals. *C*: an array consisting of 7 (1.0-mm outer diameter) or 19 (1.8-mm outer diameter) stainless steel guide tubes of 28-gauge size was chronically implanted above the VL and allowed insertion of recording electrodes and an injection needle into the VL.

flat surface does not require vision and can be accomplished without the forebrain, whereas accurate stepping on a ladder relies on vision and participation of the thalamo-cortical network (Beloozerova and Sirota 1993a, 2003; Chambers and Liu 1957; Farr et al. 2006; Friel at al. 2007; Liddell and Phillips 1944; Metz and Whishaw 2002; Trendelenburg 1911).

Cats were trained to walk in an experimental chamber, which was a rectangular enclosure with two connected parallel corridors 2.5 × 0.3 m each (Fig. 1*A*). In one corridor the walking surface was flat, and the other corridor contained a horizontal ladder. The centers of the ladder crosspieces were spaced 25 cm apart, equal to one-half of a cat's average stride length during locomotion in the chamber with a flat floor (Beloozerova et al. 2010; Beloozerova and Sirota 1993a). The crosspieces had flat tops and were 5 cm wide, which was slightly greater than the 3-cm diameter support area of the cat paw. The ladder was elevated above the chamber's floor by 6 cm. While ambulating around the chamber, cats passed through the two 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 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 paw for recording paw contact with the floor.

Recording and Analyzing Locomotor Behavior

The aim of recording and analyzing locomotor behavior in this study was to ensure that strides selected for each locomotion task and treatment condition were performed in a stereotypical fashion. The detailed analysis of the effects of inactivation in the VL on biomechanics of locomotion was not the goal of this study. Only general assessments sufficient to characterize the overall performance were conducted.

The cat's passage through the beginning and end of each corridor was recorded with infrared photodiodes mounted in the walls of the chamber. The time to complete the passage was noted. The durations of the swing and stance phases of the stride were determined with the step-mark signal. To obtain this signal, the floor in the chamber and the crosspieces of the ladder were covered with an electrically conductive rubberized material connected to the common ground, and the cat was trained to wear an electro-isolative instrumented sock made of thin rubber. An ~0.1-mm thin metal plate 10 mm in diameter was placed under the paw on the outside of the sock (e.g., Beloozerova et al. 2010; Beloozerova and Sirota 1993a; Prilutsky et al. 2005). A voltage of 2–5 mV was applied to the plate, and the fall of the voltage resulting from contact of the paw with the floor was recorded. The duration of the stance and swing phases and the stride's duty factor, which is the relative duration of the stance phase within the stride and characterizes the temporal structure of the stride, were calculated. We refer to the full movement cycle of one limb (from beginning of swing to beginning of next swing of the same limb) as a step cycle or stride and use these terms interchangeably. The effects of the locomotor task (flat surface vs. ladder) and testing condition (unperturbed vs. CNQX in the VL) on the parameters of strides were tested with the Student's two-tailed *t* test.

In all experiments, the overall motor behavior of the animal was visually assessed and evaluated for any abnormalities. In selected experiments, locomotor performance of the cat in the experimental chamber was videotaped. These video recordings were visually examined and evaluated by two independent investigators who classified each trial as obtained before or after CNQX injection in the VL while being blind to the experimental condition.

Surgical Procedures

Surgery was performed under isoflurane anesthesia using aseptic procedures. 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 fixation of electrical connectors, electrode microdrives, and preamplifiers and to rigidly hold the cat's head while searching for neurons and injecting CNQX. Arrangements of 7 (*cat A*) or 19 (*cat B*) 28-gauge hypodermic guide tubes fitted with stainless steel wires were implanted bilaterally above the VL (Fig. 1, *B* and *C*). The tip of an arrangement was lowered to the vertical Horsley and Clarke coordinate V +7.0, which is 2–5 mm above the top of the VL. On the left side of the head, the dorsal surface of the anterior and lateral sigmoid gyri and the rostral part of the posterior sigmoid gyrus were exposed by removal of ~0.6 cm^2 of bone and dura mater. The region of left motor cortex was visually identified on the basis of surface features and photographed (Fig. 2, *A* and *B*). The exposure was covered with a 1-mm-thick acrylic plate. The plate was preperforated with holes of 0.36-mm diameter spaced by 0.5 mm; the holes were filled with a bone wax and Vaseline mixture. The plate was fixed to the surrounding bone by orthodontic resin (Dentsply Caulk, Milford, DE). For identification of axonal projections of motor cortical neurons to the pyramidal tract, two 26-gauge hypodermic guide tubes fitted with stainless steel wires were implanted 7 mm above the left medullary pyramidal tract (Fig. 1*B*), placed 1 mm apart in the rostro-caudal direction. Later in the awake cat, a 200-μm platinum-iridium in Teflon insulation wire was inserted into the medullar pyramid under physiological guidance (Prilutsky et al. 2005) to serve as a stimulation electrode for identification of axonal projections of motor cortical neurons. Immediately after the surgery and 12 h thereafter, the analgesic buprenorphine was administered intramuscularly.

Single-Unit Recording and Identification of Neurons in Motor Cortex

Several days after the surgery, the cat was placed on a table equipped with a comforting pad and encouraged to take a "sphinx" posture. After the cat rested in this posture for several minutes, the base attached to the skull during surgery was fastened to an external frame so that the resting position of the head was approximated. This procedure minimizes stress on the neck while the head is immobilized. After a few training sessions, both cats sat quietly with their head restrained. They did not seem to be disturbed by the restraint, as they frequently fell asleep while sitting.

Extracellular recordings of single-neuron activity were obtained from the motor cortex's forelimb representation area in the anterior and lateral sigmoid gyri (Fig. 2, *A* and *B*). This area is considered to be the forelimb-related motor cortex on the basis of a considerable body of data obtained by means of inactivation, stimulation, and recording techniques (Armstrong and Drew 1984c, 1985; Beloozerova et al. 2010; Beloozerova and Sirota 1993a; Martin and Ghez 1993; Nieoullon and Rispal-Padel 1976; Phillips and Porter 1977; Vicario et al. 1983). Since the exact location of the area slightly varies among subjects, its position in each cat was identified by multiple-unit mapping procedures before recording experiments were initiated. In addition, the locomotion-related activity of the area identified in each cat was analyzed, and results are presented as parts of three previous reports: Armer et al. (2013) and Stout and Beloozerova (2013) for both cats and Beloozerova et al. (2010) for *cat A*.

Recordings were obtained with tungsten varnish-insulated microelectrodes (120-μm OD; FHC Inc., Bowdoin, ME). The impedance of electrodes was 1–3 MΩ at 1,000 Hz. A custom-made lightweight (2.5 g) manual single-axis micromanipulator chronically mounted on the cat's head was used to advance the microelectrode. Signals from the microelectrode were preamplified with a miniature, custom-made preamplifier positioned on the cat's head and then further amplified and filtered (0.3–10 kHz band pass) with the CyberAmp 380 (Axon Instruments). After amplification, signals were digitized with a sam-

pling frequency of 30 kHz and recorded with a computerized data acquisition and analysis package (Power1401/Spike2 System; Cambridge Electronic Design, Cambridge, UK). The Power1401/Spike2 waveform-matching algorithm was used to initially identify and isolate the spikes of single neurons. Only well-isolated neurons with stable spike shape were used for further analyses (see Fig. 3*A* and Fig. 5, *A*, *C*, *E*, *F*, *H*, *J*, *K*, *M*, and *O*).

The somatosensory receptive fields of the neurons were examined in animals resting with their head restrained. Stimulation was produced by lightly stroking fur, palpation of the muscle bellies and tendons, and passive movements around limb joints. An example response of a neuron to passive flexion of the right elbow is shown in Fig. 3*A*. The size of the receptive field was determined by measuring the entire area from which action potentials could be elicited.

Neurons with axons descending within the pyramidal tract, pyramidal tract projection neurons (PTNs), were identified on the basis of their antidromic responses to electrical stimulation of the tract at the medulla level using the test for collision of spontaneous and evoked spikes (Bishop et al. 1962; Fuller and Schlag 1976; see also, e.g., Stout and Beloozerova 2013). The pyramidal tract was electrically stimulated by individual rectangular pulses of 0.5-mA current with a duration of 0.2 ms (Fig. 1*B*; see Fig. 5, *T* and *U*). The distance between the stimulation electrode in the medullary pyramidal tract and recording sites in the pericruciate cortex was estimated at 51.5 mm, which included the curvature of the pathway. Neurons were classified as fast or slow conducting based on the criteria of Takahashi (1965), i.e., neurons with axonal conduction velocity of 21 m/s or higher were considered to be fast conducting, and those with conduction velocities below this were considered to be slow conducting. Distribution of latencies of PTN responses to stimulation of the pyramidal tract and axonal conduction velocities of PTNs included in

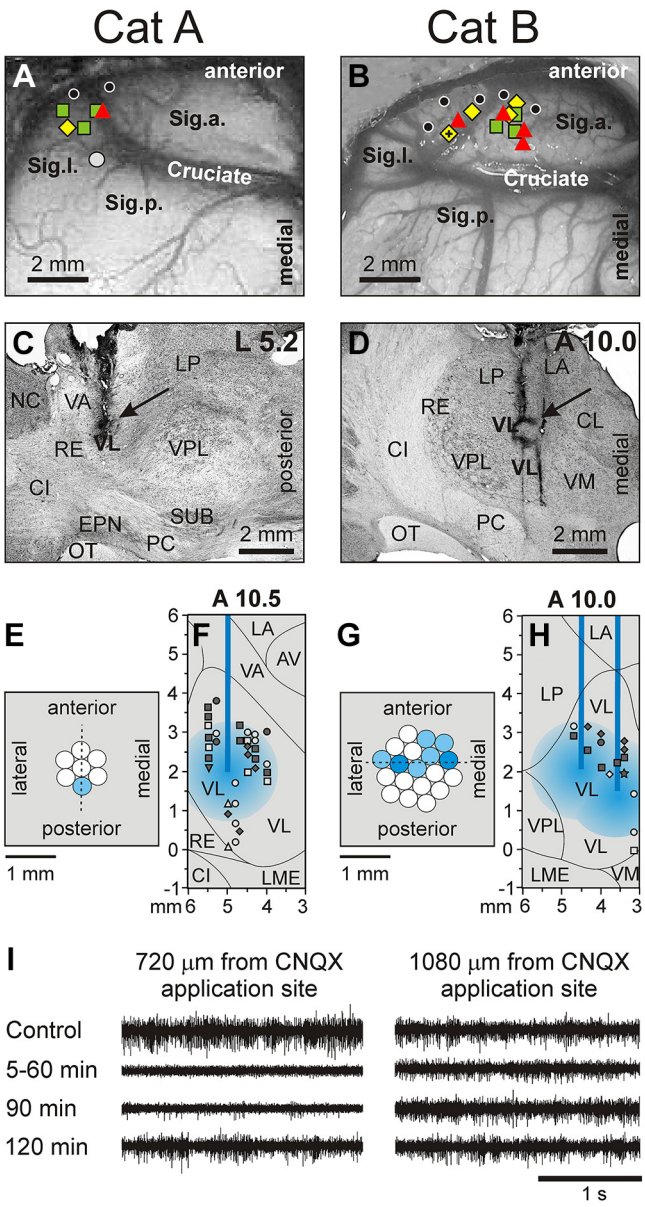


Fig. 2. Reconstructions of recording sites in motor cortex and injection sites in the nucleus ventralis lateralis (VL) and the effect of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the VL. *A* and *B*: sites of recording in the forelimb representation of left motor cortex of *cat A* (*A*) and *cat B* (*B*). Microelectrode entry points into the cortex are shown as symbols of different shapes. Green squares show penetrations where the majority of neurons had receptive fields on the shoulder, arm, and/or forearm but not on the wrist or paw. Yellow diamonds indicate penetrations where most neurons had receptive fields on the arm and/or forearm and also responded to stimulation of the wrist and/or paw. Red triangles show penetrations where most neurons had receptive fields on the wrist and/or paw. Overlapping symbols designate penetrations where different neurons had receptive fields on different segments of the forelimb. A gray circle in *A* shows a penetration where only neurons without a somatosensory receptive field were recorded (2 neurons). In *B*, the microelectrode track where a pyramidal tract projecting neuron (PTN), whose activity is shown in Fig. 5, was recorded is indicated by a black cross. Entrance points of stimulation electrodes into the cortex are schematically shown by black circles with white rims. These electrodes were used for antidromic identification of VL neurons projecting to motor cortex. *C–H*: sites of recording and injection in the VL. *C* and *D*: parasagittal (*C*) and frontal (*D*) sections of the thalamus of *cats A* and *B*, respectively. Cresyl violet stain. Arrows point to electrolytic lesion marks. *E* and *G*: cross sections of guide tube arrangements implanted in the left hemisphere of *cat A* (*E*) and *cat B* (*G*). Blue color indicates guide tubes used for injection of CNQX; in *G*, the 2 tubes marked with dark blue color were used for injection on multiple days. Vertical (in *E*) and horizontal (in *G*) dashed lines indicate position and orientation of the brain sections shown in *C* and *D*, respectively, in relation to the implanted guide tube arrangement. *F* and *H*: reconstruction of areas of VL inactivation in *cat A* (*F*) and *cat B* (*H*). These are modified fragments from Fig. 2D of Marlinski et al. (2012a). Vertical blue bars schematically represent CNQX injection needles. Blue circles with graded color intensity schematically highlight approximate areas that were affected by CNQX. Symbols indicate estimated positions of VL neurons whose activity was described in Marlinski et al. (2012a). Squares represent neurons with somatosensory receptive fields on the shoulder; diamonds show cells that were activated by movements in the elbow; up-facing triangles indicate neurons with receptive fields on the wrist or paw; a down-facing triangle shows a neuron whose receptive field encompassed the entire forelimb; circles represent neurons without somatosensory receptive fields and those whose receptive fields were not identified. Filled symbols indicate neurons with axon projecting to motor cortex; open symbols indicate neurons whose axonal projections were not identified. *I*: inhibition of multiunit activity in the VL after injection of CNQX. Data from an experiment with *cat A* are shown, in which 1.5 μL of 4.5 mM CNQX was injected in the VL. The activity in the VL was recorded at different distances from the injection site: one and two guide tubes away, which was 720 μm and 1,080 μm away, respectively, and at different times after the injection: 5–60 min, 90 min, and 120 min after. Note that the inactivation of the VL was localized and reversible. Approximately 5 min after initiation of the injection, a complete cessation of the activity occurred 720 μm away from it, which lasted for ~1 h (2nd trace from *top*). Ninety minutes later, spikes of a small amplitude, which suggests remoteness of their source, reappeared (3rd trace from *top*). The activity returned close to normal over the course of next 2 h (*bottom* trace). The activity 1,080 μm away from the injection site was only minimally affected. AV, nucleus anterio-ventralis thalami; CI, capsula interna; CL, nucleus centralis lateralis; Cruciate, sulcus cruciatus; EPN, nucleus entopeduncularis; LA, nucleus lateralis anterior; LME, lamina medullaris externa thalami; LP, nucleus lateralis posterior; NC, nucleus caudatus; OT, optic tract, PC, pedunculus cerebri; RE, nucleus reticularis thalami; Sig.a., gyrus sigmoideus anterior; Sig.l., gyrus sigmoideus lateral; Sig.p., gyrus sigmoideus posterior; SUB, nucleus subthalamicus; VA, nucleus ventralis anterior; VL, nucleus ventralis lateralis; VM, nucleus medialis; VPL, nucleus ventralis postero-lateralis.

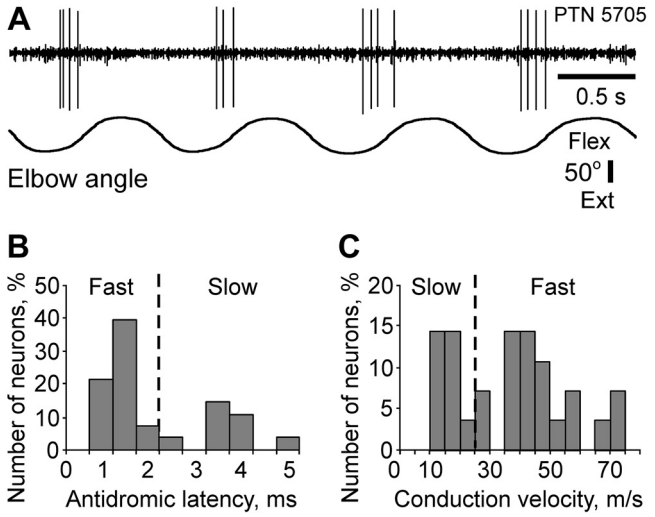


Fig. 3. Identification of neurons by their responses to somatosensory stimulation and responses to electrical stimulation of the pyramidal tract at the medulla level. *A*: responses of a motor cortex neuron [pyramidal tract projecting neuron (*PTN) 5705*] to passive flexion of the right elbow. *B*: distribution of latencies of PTN responses to stimulation of the pyramidal tract. The dashed line denotes the division between fast- and slow-conducting PTNs. *C*: axonal conduction velocities of PTNs. The dashed line denotes the division (21 m/s) between fast- and slow-conducting PTNs.

this report are shown in Fig. 3, *B* and *C*. Each recorded neuron was tested for antidromic activation before, during, and after every locomotion test. Stimulating pulses typically did not evoke any visible motor responses and never produced any signs of discomfort or distress in the cats.

Thus, individual neurons were identified according to *1*) location in the cortex, *2*) somatosensory receptive field, *3*) projection to the pyramidal tract, and, for the projecting neurons, *4*) the axonal conduction velocity.

Identification of the Forelimb Area in the VL

The VL was identified by stereotaxic coordinates, antidromic responses of neurons to electrical stimulation of motor cortex, responses of neurons to somatosensory stimulation, and their activity during locomotion as described in Marlinski et al. (2012a). We recognized divisions of the thalamic ventrolateral nuclear complex in accordance with nuclear delineation of the cat brain atlas of Reinoso-Suarez (1961). We denoted the posterior division of the complex as the ventral lateral nucleus (VL) and the anterior division of the complex as the ventral anterior nucleus (VA). These divisions in the cat are analogous to the posterior and anterior divisions of the primate ventral lateral nucleus, termed the VLp and VLa, respectively.

The location of the VL was verified by postmortem histological examination (Fig. 2, *C* and *D*). It was found that in *cat A* the implanted guide tube arrangement targeted the anterior portion of the VL, whereas in *cat B* the arrangement was aimed at the central portion of the VL (Marlinski et al. 2012a). All sites of CNQX injection were confined within the VL. Their stereotaxic coordinates in each subject are given in *Injection of CNQX* below.

The identity of the injected VL area as the cerebellum-receiving subdivision of the ventrolateral thalamus was confirmed by tracing afferent connections of the area, which was done as a part of the previous study (Marlinski et al. 2012a). As detailed in Marlinski et al. (2012a), in *cat A*, in which horseradish peroxidase-conjugated wheat germ agglutinin (WGA-HRP) was used for the tracing, numerous retrogradely labeled neurons were found in the anterior half of the lateral (dentate) nucleus of the right (contralateral) cerebellum and in the anterior interposed nucleus of the right cerebellum (Fig. 3*A* in Marlinski et al. 2012a). Ipsilaterally, labeled cells were found in the lateral half of the entopeduncular nucleus, which is the feline analog of the primate globus pallidus pars interna (GPi). In *cat B*, in which fluorescent beads were used for the tracing, retrogradely labeled cells were found contralaterally throughout the rostro-caudal extent of the dentate and posterior interposed nuclei of the cerebellum, and a number of cells were found in the anterior interposed and fastigial nuclei of the cerebellum (Figs. 3 and 4 in Marlinski et al. 2012a). Ipsilaterally, numerous labeled cells were found in the frontal cortex, throughout layer VI of the anterior and posterior sigmoid gyri, and across the lateral ansate sulcus into the suprasylvian gyrus. The entopeduncular nucleus was free from label in this cat. Thus, in both cats deep cerebellar nuclei, which form the main output of the cerebellum, were intensively labeled after a retrogradely transporting tracer was injected in the area of the VL that in this study was reversibly silenced with CNQX.

Projection of this VL area to motor cortex was demonstrated in the same previous study by demonstrating that neurons in the area respond antidromically to electrical stimulation of motor cortex. Sites of stimulation used to evoke antidromic responses of VL neurons are marked with black circles on photographs of the cortex shown in Fig. 2, *A* and *B*. Approximately half of tested VL neurons, randomly distributed across the area, responded to the stimulation (Marlinski et al. 2012a). On the reconstructions of areas of CNQX inactivation in the VL shown in Fig. 2, *F* and *H*, VL neurons projecting to motor cortex are depicted by filled symbols.

The fact that the area of the VL that we reversibly inactivated was related to the forelimb was determined in the same previous study by examining somatosensory receptive fields of its neurons. As described in Marlinski et al. (2012a), out of 168 neurons tested 75% (125/168) had somatosensory receptive fields on the right forelimb or neck and only 6 neurons (3.6%) had receptive fields on the right hindlimb. In Fig. 2, *F* and *H*, VL neurons with somatosensory receptive fields on different segments of the forelimb are shown by symbols of different shapes. We have previously reported that we did not see any somatotopy within the forelimb-related VL area, as neurons responding to stimulation of different segments of the forelimb were distributed randomly in this area without any clear clusters of shoulder-, elbow-, or wrist/paw-related cells.

The activity of VL neurons during flat surface and ladder locomotion recorded in these cats during a preceding separate set of experiments is also reported in Marlinski et al. (2012a).

Blockade of Glutamatergic Transmission in a part of the VL with CNQX

Recording protocol. On each experimental day, one to three motor cortical neurons were identified by their somatosensory receptive field, projection of the axon, and the axon's conduction velocity. The neurons' activity during flat surface and ladder locomotion was first recorded in normal conditions. Two or three data sets were collected 1 h apart, each consisting of ~30 rounds of passing around the experimental chamber. It took the cat 10–15 min to complete these rounds. Between the recordings, the cat sat for 45–50 min on a comforting pad with its head restrained. If the activity of at least one neuron in two consecutive data sets was similar (as determined by the analyses of the rasters and histograms of the neurons' stride-related discharges, an example is shown in Supplemental Figure S1; all Supplemental Material is available at https://doi.org/10.6084/m9.figshare.12252086), and the quality of the recording remained good, CNQX was injected into the left VL (see below). Immediately after the injection and 40–60 min, 2 h, 3 h, and 4 h thereafter, neurons' activity was recorded during ~30 rounds of locomotion around the chamber. Similarly to the pre-CNQX data collection, recordings after the injection were separated by 45- to 50-min intervals while the cat sat with the head restrained.

Injection of CNQX. A unilateral reversible blockade of glutamatergic synaptic transmission in the VL was accomplished with the competitive AMPA/kainate-type glutamate receptor antagonist CNQX (Sigma, CAS no. 115066-14-3) injected by pressure (Fig. 2, *E–H*). In each experiment, 1.0–2.5 μL of 4.5 mM (*cat A*) or 1.5–2 μL of 7.4 mM (*cat B*) CNQX dissolved in 0.9% NaCl solution was injected through the 35-gauge needle of a 10-μL Hamilton microsyringe. The needle was connected to the syringe via a calibrated silicone tube; it was manually lowered into the VL through an implanted guide tube (Fig. 1, *B* and *C*; Fig. 2, *E–H*). CNQX was delivered in portions of 0.1 μL over a period of 10–15 min. An additional 5 min was allowed for diffusion of the substance before the needle was slowly withdrawn. The guide tube was then sealed with a fitting wire.

In *cat A*, CNQX was injected in a single site of the left VL with coordinates A 10.5, L 5.0, and V +2.0 (Fig. 2, *C*, *E*, and *F*), whereas in *cat B* CNQX was injected in various sites of the left VL within coordinates in the range of A 10.0–10.5, L 3.5–4.5, and V +1.5 to +2 (Fig. 2, *D*, *G*, and *H*); the two sites marked with dark blue color in Fig. 2*G* were injected on multiple days. Typically only one injection per day was made (see details below).

Verification of the effect of CNQX in the VL. To verify the effect of CNQX on the activity of VL neurons and estimate the size of the affected area, in selected experiments recordings were made from VL sites located one and two guide tubes away from the injection site, which, with the 28-gauge tubes 360 μm in outer diameter, was 720 μm and 1,080 μm away (Fig. 2*I*). These recordings were taken during and immediately after the CNQX injection as well at all later times at which the activity in motor cortex during locomotion was recorded. They showed that, with the volumes and concentrations of CNQX solutions used, a VL area ~2 mm^3 in diameter was inactivated for a period of 2–4 h, as neurons ~1 mm away in all directions from the injection site were mostly silent during this period (Fig. 2*I*). This estimate of the spread of CNQX is consistent with the estimates of drug diffusion in the brain obtained in the study of Myers (1966). Two cubic millimeters represents ~10% of the VL total volume, which in the cat is ~20 mm^3.

Data sample. In *cat A*, seven injections were made in a span of 14 days, one per day. In *cat B*, 18 injections were made in seven sites of the VL over a period of 2 mo, one or two per day. In *cat A* injections were spaced 1–6 days apart, and in *cat B* they were spaced 1–15 days apart. Injections always resulted in a dramatic reduction of the activity in neighboring VL areas and changes in the activity of motor cortex neurons (see below). The effectiveness of multiple subsequent CNQX injections suggests that damage to the thalamic tissue resulting from the injections was small.

Processing of Neuronal Activity

From the four or five strides that cats took along each corridor (Fig. 1*A*), two strides in the middle of a corridor were selected for the analyses. The strides were further selected so that their average duration during flat surface and ladder locomotion in each session differed by no more than 10%. It was previously shown that the activity of only a minority of neurons in motor cortex reflects the speed of locomotion (Armstrong and Drew 1984a; Beloozerova and Sirota 1993b); nevertheless, selecting strides of a similar duration minimized potential differences in the activity of neurons due to difference in the speed of locomotion during the two tasks. Each group of selected strides contained at least 15 strides.

During data analysis, the onset of swing of the right forelimb was taken as the beginning of the step cycle. The step cycles were time normalized, and raster plots were created to visualize the discharge of the neuron over all selected cycles of a locomotor task (see, e.g., Fig. 5, *B* and *D*). The duration of each step cycle was divided into 20 equal bins, and a phase histogram of the discharge rate of a neuron during the cycle was generated and averaged over all selected cycles (see, e.g., Fig. 5, *C* and *E*). The phase histogram was smoothed by recalculating the value of each bin as follows: $F_n^{'} = 0.25F_{n-1} + 0.5F_n + 0.25F_{n+1}$, where F_n is the original value of a bin. The "depth" of modulation, dM, was calculated as dM (%) = $(N_{max} - N_{min})/N \times 100$, where N_{max} and N_{min} are the number of spikes in the maximal and minimal histogram bins and N is the total number of spikes in the histogram. The activity with dM > 4% was judged to be stride related. This was based on an analysis of fluctuations of the activity in the resting animal (Efron and Tibshirani 1993; Stout and Beloozerova 2013). 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 a "period of elevated firing" (PEF; see Fig. 5, *C* and *E*). PEFs were smoothed by removing all one-bin peaks and troughs. In neurons with a single PEF per cycle, the "preferred phase" (PrPh) of the activity was assessed with circular statistics (Batschelet 1981; Beloozerova et al. 2003; Drew and Doucet 1991; Fisher 1993).

The following activity parameters were calculated for each neuron: the mean discharge frequency, dM, number of PEFs, duration of PEF(s), and, for neurons with a single PEF, the PrPh. For individual neurons, the difference in each parameter between locomotion in normal conditions and each data set after the CNQX injection in the VL was determined. For the comparison of the discharge frequency in different conditions 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 established based on the results of a bootstrapping analysis that compared differences in the parameters between various reshufflings of strides of the same locomotion condition (Efron and Tibshirani 1993; Stout and Beloozerova 2013).

For populations of neurons, the following activity parameters were calculated and compared between normal and each test condition: the percentage of neurons at their PEF during different phases of the step cycle, the distribution of the average population discharge frequency over the step cycle, the range of coefficients of modulation, and the average widths of PEFs. The difference of each mean parameter of population activity between conditions was tested with the Student's two-tailed t test. Unless noted otherwise, for all mean values the standard deviation (SD) is given. When data were categorical, a nonparametric Mann–Whitney (U) test, Fisher's two-tailed test, or Z test for proportions was performed. The significance level for all tests was set at 0.05.

The analysis of neuronal activity was not blind to the experimental condition.

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 of 40-μm thickness were cut in the regions of recording and stimulating electrodes and the sites of CNQX injections. The tissue was stained for Nissl substance with cresyl violet or thionin. The positions of recording tracks in the cortex and injection sites in the VL were estimated in relation to the reference lesions. The positions of stimulation electrodes in the medullar pyramids were verified. Further details of histological procedures can be found in our previous publications (e.g., Beloozerova et al. 2010; Marlinski et al. 2012a).

RESULTS

Blockade of Glutamatergic Transmission in ~10% of the VL Had a Very Minor Effect on Motor Behavior

Although CNQX always silenced the injected VL area (Fig. 2*I*) and caused changes in the activity of motor cortical neurons

as described below, only very minor changes in motor behavior were observed, and only in *cat A*. In this cat, the first three injections resulted in the cat missing a ladder crosspiece a couple times shortly after the injection. On each of those experimental days, however, the cat completed between 35 and 65 rounds around the chamber within an hour of the injection, making between 150 and 250 ladder strides. Thus, missing a crosspiece on a couple of strides (there were exactly 2 misses on each of the 3 days) can be considered a very minor deficit. The placement of the paw was normal, and otherwise the cat behaved and moved normally. No movement abnormalities were seen in *cat B*.

During trials immediately preceding an injection of CNQX in the VL, cats walked 12–40 times around the chamber. From these trials, between 20 and 120 strides were selected for the analyses of each locomotion task according to criteria outlined in METHODS. Across cats and testing days, the duration of the strides ranged between 550 and 850 ms, which corresponded to locomotion speed of 0.6–0.9 m/s. Stride characteristics of each cat in normal conditions are shown in Fig. 4 with black bars, and aggregated data are given in Table 1.

Blocks of postinjection trials were selected for the maximal effect of CNQX in the VL on the locomotion-related activity of each motor cortical neuron (see below). These trials were typically those immediately following the injection (n = 13/15 for simple/ladder locomotion, respectively) or recorded 30–45 min after the injection (n = 16/18 for simple/ladder locomotion, respectively) or, in some cases, 60 min after the injection (n = 12/9 for simple/ladder tasks, respectively). During these blocks of trials cats walked 11–38 times around the chamber. From these trials, between 15 and 107 strides were selected per data set for each locomotion task according to criteria detailed in METHODS. Across cats and testing days, the duration of the strides ranged between 660 and 730 ms, which corresponded to locomotion speed of 0.7–0.8 m/s. Stride characteristics of each cat in the test condition are shown in Fig. 4 with gray bars, and aggregated data are given in Table 1.

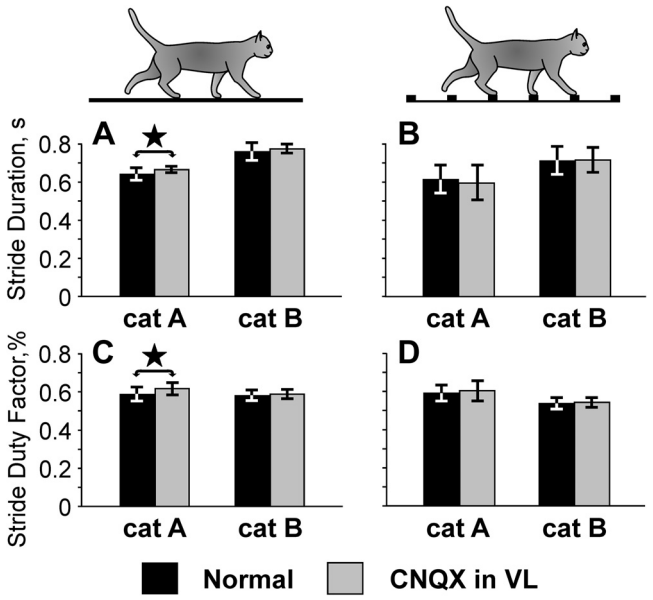


Fig. 4. Characteristics of flat surface (*left*) and ladder (*right*) locomotion in normal conditions and after an injection of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the ventrolateral thalamus (VL). Silhouettes of cats indicate locomotor task. *A* and *B*: the average duration of the step cycle during flat surface (*A*) and ladder (*B*) locomotion. *C* and *D*: the stride duty factor (ratio of stance duration to step cycle duration). Black bars show normal condition data, and gray bars show data obtained after injection of CNQX in the VL. Error bars show SDs. Stars indicate statistically significant difference between normal and CNQX in the VL conditions, *t* test.

Table 1. *Characteristics of locomotion behavior*

		CNQX in the VL	
	Parameters of Strides	Before	After
Flat surface locomotion	Number of passages	30 ± 7	31 ± 5
	Number of strides	78 ± 24	81 ± 17
	Duration of stride, ms	706 ± 72	722 ± 59
	Duration of swing, ms	294 ± 34	289 ± 39
	Duration of stance, ms	412 ± 51	433 ± 32*
	Stride duty factor, %	58.4 ± 3.2	60.1 ± 2.9*
Ladder locomotion	Number of passages	31 ± 6	32 ± 4
	Number of strides	57 ± 27	62 ± 29
	Duration of stride, ms	666 ± 87	658 ± 99†
	Duration of swing, ms	291 ± 46	282 ± 55
	Duration of stance, ms	375 ± 58†	377 ± 62†
	Stride duty factor, %	56.2 ± 4.0†	57.0 ± 4.9†

Values are means ± SD. CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; VL ventrolateral thalamus. *Values that are statistically significantly different between normal and CNQX inactivation in VL conditions (Student's unpaired *t* test for averages). †Values that are statistically significantly different between locomotion on the flat surface and on the ladder.

After injection of CNQX, *cat A* walked on the flat surface a little slower (by 3.4%; $P < 0.05$, *t* test) but walked with a similar pace on the ladder (Fig. 4, *A* and *B*). *Cat B* walked slightly slower than *cat A* overall during both tasks; however, it walked with a similar pace before and after a CNQX injection in the VL during both tasks (Fig. 4, *A* and *B*). The stride's duty factor, which characterizes the structure of the stride, in both cats was similar before and after the injection for ladder locomotion and in *cat B* was similar for flat surface locomotion as well (Fig. 4, *C* and *D*).

Neuronal Sample

The activity of 35 neurons from layer V of motor cortex was recorded during locomotion before and after a blockade of glutamatergic transmission in the VL with CNQX. Eighteen neurons were recorded from five tracks throughout the anterior aspect of the lateral sigmoid gyrus in *cat A* (Fig. 2*A*), and 17 neurons were collected from eight tracks in the lateral aspect of the anterior sigmoid gyrus in *cat B* (Fig. 2*B*). Neurons had diverse somatosensory receptive fields. Ten neurons responded to movement in the shoulder joint, nine reacted to movement in the elbow joint, and eight responded to movement of the wrist or pressure on the paw. Receptive fields of eight neurons were not identified.

Twenty-four neurons were identified as PTNs. Their axonal conduction velocities ranged between 10 and 74 m/s, and according to the criteria of Takahashi (1965), 15 PTNs with axonal conduction velocities of 29–74 m/s were considered to be fast conducting and 9 PTNs with axonal conduction velocities of 10–21 m/s were considered to be slow conducting. The distribution of latencies of PTN responses to stimulation of the pyramidal tract and distribution of axonal conduction velocities of PTNs are shown in Fig. 3, *B* and *C*.

On each experimental day, responses of one to three neurons to an application of CNQX in the VL were tested. For five

neurons recorded in *cat B*, the effects of two consecutive CNQX injections in different, medial and lateral, VL areas were tested. Altogether, the effect of CNQX was examined 40 times.

Neuronal Activity in Normal Conditions

An example activity of a typical neuron is shown in Fig. 5, *A–E*. This neuron (*PTN 5705*) recorded in *cat B* was located laterally in the anterior sigmoid gyrus (Fig. 2*B*; the track where the neuron was encountered is indicated by a cross). In the resting cat the neuron was activated by flexion of the elbow (Fig. 3*A*). The activity of the neuron during standing was 2.6 ± 1.4 spikes/s. During locomotion on the flat surface its activity was strongly modulated in the rhythm of strides: it peaked at ~25 spikes/s around the transition from swing to stance and was near zero at the end of stance and beginning of swing (Fig. 5*A*, left side of bottom trace). The raster in Fig. 5*B* shows that the activity of the neuron was very consistent across many strides (68 strides). The activity is summed in Fig. 5*C*, which shows the distribution of the discharge frequency across the step cycle of locomotion on the flat surface. The period of elevated firing (PEF) is indicated by a black horizontal bar, and the preferred phase of the activity (PrPh) is depicted with a white circle and stated at Fig. 5*C*, *top right*, panel along with the value of the depth of modulation (dM). A rainbow bar below the graph shows the average discharge frequency in each 1/20th portion of the step cycle, color-coded according to the scale at Fig. 5, *bottom left*. During ladder locomotion, the discharge of the neuron throughout the step cycle was higher, particularly during the transition from swing to stance phase, when it peaked at 40 spikes/s (Fig. 5, *A*, *D*, and *E*). The PrPh differed by only 1/20th of the cycle, which was considered insignificant (see METHODS), while the dM was lower.

The activity of the entire group of 35 neurons during locomotion on the flat surface and horizontal ladder in normal conditions and subpopulations of these neurons grouped according to neurons' somatosensory receptive field and axonal conduction velocity is detailed in the APPENDIX and illustrated in Supplemental Figs. S2, S3, and S4.

This activity was consistent with previously reported data (Beloozerova et al. 2010; Beloozerova and Sirota 1993a; Drew 1993; Farrell et al. 2014, 2015; Stout and Beloozerova 2012, 2013), thus suggesting that the 35 neurons whose responses to application of CNQX in the VL are reported here are a representative group.

Blockade of Glutamatergic Transmission in a Part of the VL Differently Affects the Activity of Motor Cortex Neurons During Different Phases of the Stride

A typical response of a motor cortical neuron (*PTN 5705*) to CNQX application in the VL is shown in Fig. 5, *F–J*. This is the same cell whose activity during locomotion before the application (in normal conditions) is shown in Fig. 5, *A–E*. Forty minutes after 2 μL of 7.4 mM CNQX was injected in a lateral VL area (Fig. 2, *G* and *H*), the activity of the neuron during standing significantly decreased (to 0.1 ± 0.1 spikes/s; $P < 0.05$, *t* test). Its activity during locomotion was still sharply modulated in the rhythm of strides and consistent across many step cycles (Fig. 5, *F–J*). However, the peak discharge on the flat surface was now only 10 spikes/s, less than half of that in normal conditions, and the peak discharge on the ladder decreased by a quarter to 30 spikes/s. The PrPh did not change, but the duration of the PEF during flat surface locomotion became 37% shorter, while staying unchanged during the ladder task. The dM became greater during both tasks. Figure 5, *P* and *Q*, show the activities of the neuron during flat surface (Fig. 5*P*) and ladder (Fig. 5*Q*) locomotion superimposed over its activities in normal conditions. Black area histograms in Fig. 5, *R* and *S*, show the difference between the activities during flat surface (Fig. 5*R*) and ladder (Fig. 5*S*) locomotion before and after CNQX injection. These difference histograms highlight the components of the PTN activity that in normal conditions were contributed by the inactivated VL area. Rainbow bars below the histograms show the difference in each 1/20th portion of the step cycle of flat surface (Fig. 5*R*) and ladder (Fig. 5*S*) locomotion, color-coded according to the scale at Fig. 5, *bottom right*.

Over the next few hours, the activity of the neuron gradually returned to normal levels. A test conducted 2 h after the injection and illustrated in Fig. 5, *K–O*, showed that, by this time, the neuron's activity during ladder locomotion was similar to that in normal conditions, whereas its activity during locomotion on the flat surface was still low. However, the flat surface locomotion-related activity also showed signs of recovery and recovered in an additional 2 h. The shape of the PTN's spike did not change over the period of observation (insets in Fig. 5, *C* and *E*, *H* and *J*, and *M* and *O*). The ability of the neuron to respond to electrical stimulation of the pyramidal tract at a fixed latency of 0.9 ms (Fig. 5*T*) and to satisfy the collision test (Fig. 5*U*) also did not change.

Blockade of glutamatergic transmission in ~10% of the VL (the estimated volume of the inactivation, see above) led to a reduction of the discharge rate during standing of 74% of neurons, by 54 ± 23% (range 18–100%; $P < 0.05$, *t* test); the activity of only four cells increased. The average discharge rate of the whole population decreased by 4.1 spikes/s ($P = 0.02$, *t* test). During locomotion on the flat surface, the activity of half of the neurons (51%) was also lower than normal, by 57 ± 19% (range 22–92%; $P < 0.05$, *t* test) (Fig. 6*A*). The activity of the whole population was lower by 4.8 spikes/s ($P = 0.02$, *t* test). Similarly, during ladder locomotion the activity of 66% of cells was lower by 47 ± 18% (range 25–85%; $P < 0.05$, *t* test) (Fig. 6*B*), and the activity of the population was lower by 3.9 spikes/s ($P = 0.04$, *t* test).

For the majority of neurons, changes in the discharge rate after application of CNQX in the VL were not uniform across the step cycle. For example, the flat surface locomotion-related activity of the neuron shown in Fig. 5 was affected the strongest during the swing phase of stride, when its activity was almost entirely suppressed, whereas during the stance phase some activity persisted after the CNQX application (Fig. 5*P*). During ladder locomotion, the discharge rate of this neuron during different phases of the stride decreased to a different degree as well (Fig. 5*Q*). In addition, in many neurons the effect of the VL inactivation was positive during some phases of the stride, meaning that the activity of a neuron increased rather than decreased after the inactivation, showing that in normal conditions the VL was suppressing the activity of the neuron during these phases. Profiles of the effects of the VL contribution to the activity of individual neurons and the whole population of neurons during the step cycle of

flat surface and ladder locomotion are summarized in Fig. 7. Each horizontal bar of a colored plot (plots numbered *1* and *3*) shows the difference in the discharge frequency of a neuron before and after an application of CNQX in the VL and is analogous to the rainbow bars for PTN 5705 in Fig. 5, *R* and *S*. This difference represents the effect of the VL contribution to the activity of the neuron in spikes per second.

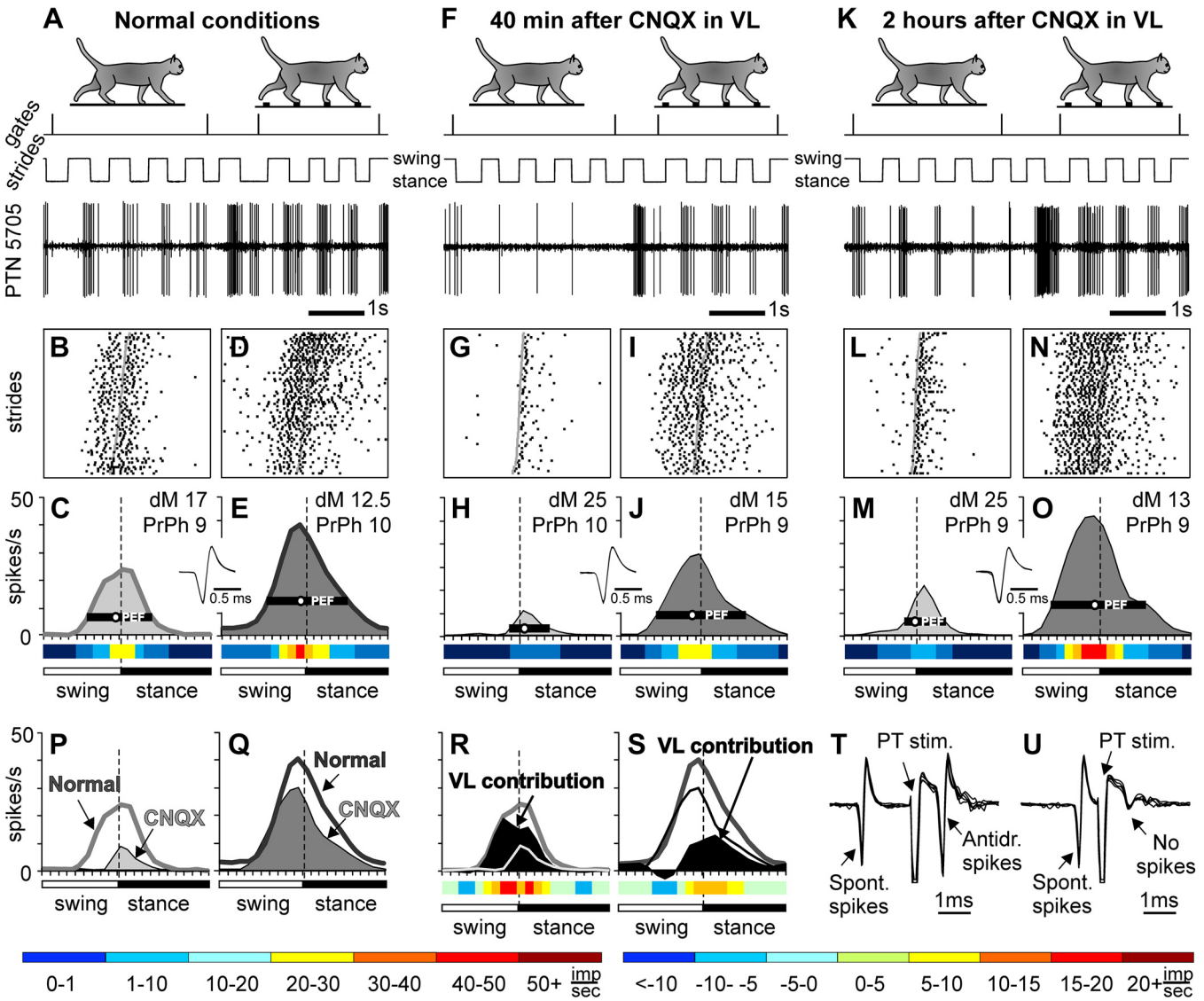


Fig. 5. Effect of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) application in the ventrolateral thalamus (VL) on the locomotion-related activity of an example motor cortical neuron [pyramidal tract projecting neuron *(PTN) 5705*]. Two microliters of 7.4 mM CNQX were injected in the left VL of *cat B* (a lateral track), and the neuron was recorded in the lateral aspect of the left anterior sigmoid gyrus. Representative recordings of the activity of the neuron during flat surface and ladder locomotion before (*A–E*) and 40 min (*F–J*) and 2 h (*K–O*) after the CNQX injection are shown. For each group of panels, silhouettes of cats at *top* indicate locomotion on the flat surface (*left*) and the ladder (*right*). In *A*, *F*, and *K*, periods of flat surface and ladder locomotion are marked with vertical ticks on the "gates" trace, the "strides" trace indicates the swing and stance phases of the stride of the right forelimb, and the "*PTN 5705*" trace shows a raw recording from the neuron. *B*, *D*, *G*, *I*, *L*, and *N*: rasters show the occurrence of spikes during 68 strides on the flat surface (*B*, *G*, and *L*) and the ladder (*D*, *I*, and N). The duration of step cycles is normalized to 100%, and the strides are rank ordered according to the duration of the swing phase. In the rasters, the end of swing and the beginning of stance in each stride is indicated by a gray square. *C*, *E*, *H*, *J*, *M*, and *O*: area histograms show the average discharge rate of the neuron across the step cycle on the flat surface (light gray) and the ladder (dark gray) before (*C* and *E*) and 40 min (*H* and *J*) and 2 h (*M* and *O*) after CNQX injection in the VL. Vertical dashed lines denote the end of swing and beginning of stance phase. *Insets* show 10 superimposed spikes for each condition. Rainbow bars below the histograms show the average discharge frequency of the neuron in each 1/20th portion of the step cycle, color-coded according to the scale at *bottom left*. dM, depth of modulation; PrPh, preferred phase. *P* and *Q*: the average discharge rates across the step cycle on the flat surface (*P*) and the ladder (*Q*) before (line graphs) and 40 min after (area graphs) application of CNQX are superimposed. *R* and *S*: the difference is shown as a black area histogram for flat surface (*R*) and ladder (*S*) locomotion. Rainbow bars below these histograms show the difference in each 1/20th portion of the step cycle of the flat surface (*R*) and ladder (*S*) locomotion, color-coded according to the scale at *bottom right*. These difference histograms highlight the components of the PTN activity that in normal conditions were contributed by the inactivated VL area. *T* and *U*: collision test determines that the neuron's response to stimulation of the pyramidal tract (PT) was antidromic. *T*: the PTN spontaneously discharges, and the PT is stimulated 1.75 ms later. The PTN responds with a latency of 0.9 ms. Five repetitions are superimposed. *U*: the PTN spontaneously discharges, and the PT is stimulated 0.8 ms later. PTN does not respond because in 0.8 ms its spontaneous spike was still en route to the site of stimulation in the PT, and thus collision/nullification of spontaneous and evoked spikes occurred. Five repetitions are superimposed. The neuron consistently satisfied the collision test during all periods of the observation.

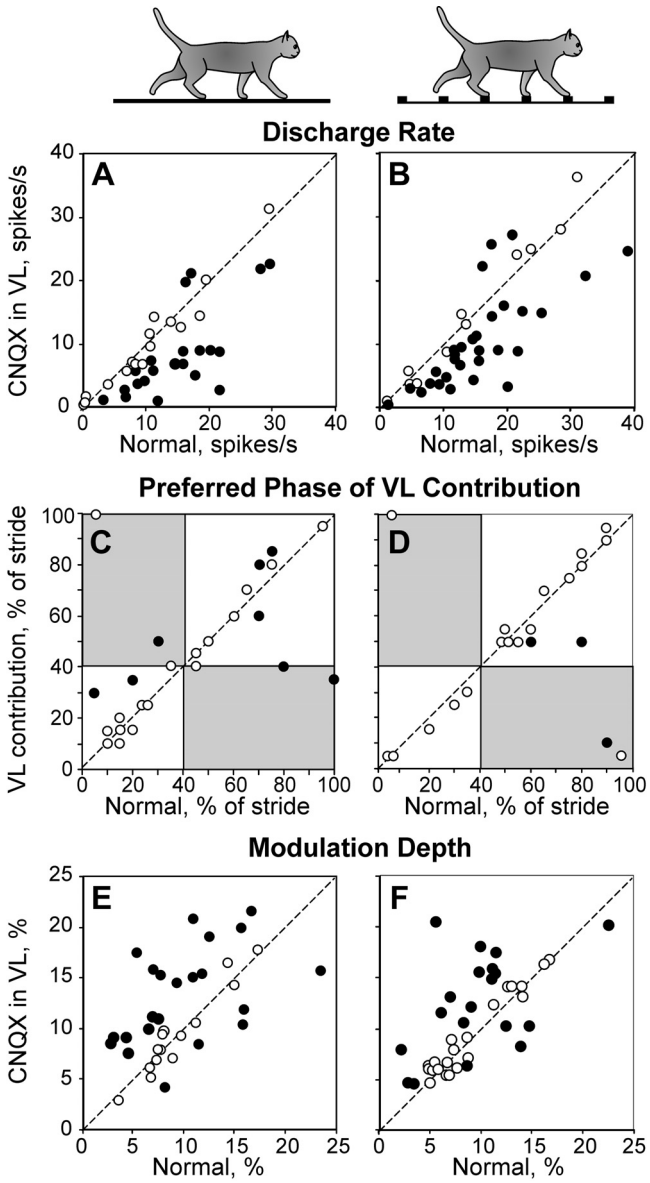


Fig. 6. Effect of blockade of glutamatergic transmission in a part of the ventrolateral thalamus (VL) on the activity characteristics of individual neurons in motor cortex during flat surface (*left*) and ladder (*right*) locomotion. The *x*-axis and *y*-axis of each point show the values observed during normal and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in VL conditions, respectively. Neurons whose characteristics were statistically significantly different between the two conditions (see METHODS) are shown by filled circles; the others are shown by open circles. *A* and *B*: mean discharge rate averaged over the step cycle. *C* and *D*: preferred phase of the VL contribution, determined based on the difference in the discharge rate between normal and CNQX in VL conditions (see text for details). Areas that correspond to the swing phase during one condition but stance phase during the other condition are shaded. *E* and *F*: depth of frequency modulation (dM).

For 88% (35/40) of the cases examined during flat surface locomotion and for almost every case (95%, 38/40) tested during ladder locomotion, the VL contribution was changing over the course of the step cycle (Fig. 7, *A1* and *A3*). In 33% (13/40) of cases tested during flat surface locomotion and 30% (12/40) of cases tested during ladder locomotion neurons received excitation only (green, yellow, and red colors in Fig. 7, *A1* and *A3*). The excitation could change by 4–20 spikes/s over the course of the step cycle of flat surface locomotion and by 4–34 spikes/s over the step cycle of ladder locomotion. The majority of neurons, however, were excited during some phases of the cycle and inhibited during other phases: in 53% (21/40) of cases during flat surface locomotion and in 63% (25/40) of cases during ladder locomotion. In these cases, the excitation could result in an increase of firing by as much as 30 spikes/s and the inhibition could lead to a decrease of firing by as much as 17 spikes/s.

Figure 8 shows profiles of the effects of VL contribution to the activity of motor cortex neurons grouped into six groups according to the phase of the stride when the contribution peaked during the step cycle of flat surface and ladder locomotion. In each panel, the profiles of cases belonging to a particular group are shown superimposed in a single plot (for clarity, only those exceeding ±5 spikes/s are presented). The overwhelming majority of the profiles had one peak during the step cycle: 95% (38/40) during flat surface locomotion and 80% (32/40) during ladder locomotion. *Group 1* includes profiles in which the VL contribution peaked during transition from the stance to swing phase. *Group 2* includes those in which the contribution peaked during the swing phase. In cases of *group 3*, the contribution was maximal during transition from the swing to stance phase. *Group 4* includes profiles in which it peaked during the stance phase of the stride. There were more *group 4* profiles during ladder than flat surface locomotion ($P = 0.014$, Z test for proportions). *Group 5* includes profiles in which the contribution's inhibitory component dominated; the inhibition often peaked during the transition phase from swing to stance. Finally, *group 6* includes profiles in which the contribution was bimodal, peaking twice during the step cycle; there were more such profiles during ladder than flat surface locomotion ($P = 0.044$, Z test for proportions).

Despite the fact that many neurons received inhibition during some phases of the step cycle, the average effect of the VL contribution to the activity of motor cortex layer V population was excitatory and fluctuated around 4 spikes/s during either locomotion task (Fig. 7, *B1* and *B3*).

With respect to how much the input from the estimated 10% of the VL contributed to the activity of neurons in motor cortex in relation to other inputs such as inputs from the other 90% of the VL, other areas of the thalamus, and beyond, we found that during a 1/20th period of the step cycle the activity of this portion of the VL could evoke anywhere from below 1% to 100% of a neuron's discharge rate. The inhibition could also range widely from below 1% to 90%. Profiles of the effects of the VL contribution during the step cycle of flat surface and ladder locomotion expressed as percentages of the VL-driven activity in the total activity of individual neurons and the population of neurons, the "relative" VL contribution, are shown in Fig. 7 plots numbered *2* and *4*. Each horizontal colored bar in these plots shows percentages of the activity that the ~10% of the VL evoked in the neuron during different phases of the stride (Fig. 7, *A2* and *A4*), and area histograms show population averages (Fig. 7, *B2* and *B4*).

The relative contribution of the VL always fluctuated over the step cycle (Fig. 7, *A2* and *A4*). In 38% (15/40) of cases tested during flat surface locomotion and 28% (11/40) of cases tested during ladder locomotion the contribution was positive throughout the cycle, ranging between 10% and 100% during flat surface locomotion and between 4% and 100% during ladder

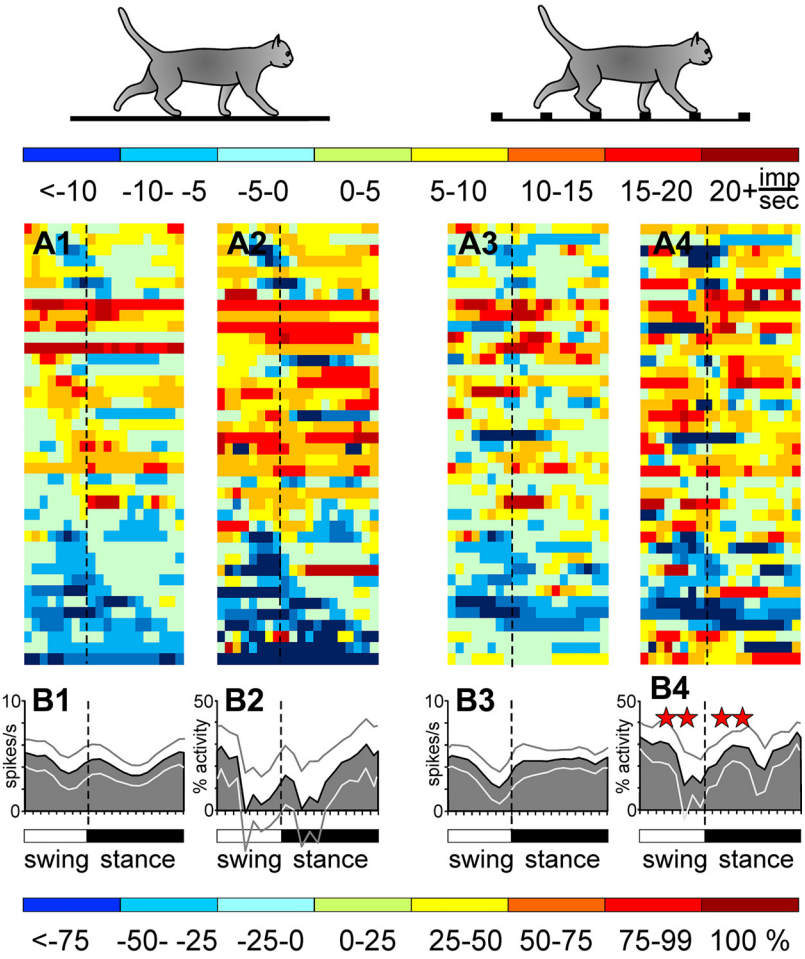


Fig. 7. Stride phase distributions of the ventrolateral thalamus (VL) contribution to the activity of individual neurons and the population of neurons of motor cortex. *Left*: data for flat surface locomotion. *Right*: data for ladder locomotion. *A1* and *A3*: stride phase distributions of the effects of the VL contribution to the activity of individual neurons in spikes per second. For each neuron, the average effect of the contribution in each 1/20th portion of the step cycle is color-coded according to the scale shown above *A1–A4* and is similar to rainbow bars in Fig. 5, *R* and *S*. Neurons are rank-ordered so that those with the excitatory VL contribution earlier in the step cycle are plotted at *top* of graph. *A2* and *A4*: corresponding stride phase distributions of the VL contribution to the activity of individual neurons expressed as the % of the VL-driven activity in the total activity of the neuron. For each neuron, the % of the discharge rate that resulted from the VL contribution during each 1/20th portion of the step cycle is color-coded according to the scale at *bottom*. Negative values denote VL contribution to the inhibition. Neurons are rank-ordered identically as in *A1* and *A3*. *B1–B4*: the mean effect of the VL contribution on the activity of motor cortex neurons in spikes per second (*B1* and *B3*) and as % of the motor cortex population's total activity (*B2* and *B4*). Thin lines show SE. Red stars indicate periods of the stride when the VL contribution during ladder locomotion was significantly greater than during flat surface locomotion ($P < 0.05$, *U* test).

locomotion. In the majority of cases the relative contribution alternated between positive and negative values over the course of the step cycle, negative values indicating contribution to the inhibition: in 60% (24/40) of cases during flat surface locomotion and in 70% (28/40) of cases during ladder locomotion. In these cases, the excitation from the VL could be responsible for as much as 100% of a neuron's discharge rate during some phases of the cycle, whereas the inhibition could suppress it by as much as 95% during other phases.

The average relative contribution of the VL to the activity of motor cortex layer V population was excitatory. During flat surface locomotion it evoked ~25% of the population's activity during the first half of the swing phase and the second half of stance (Fig. 7*B2*). The VL contribution evoked ~10% of the activity during transition from swing to stance and was small during the second half of the swing phase and the first half of stance (Fig. 7*B2*). Upon transition to the ladder, the relative contribution of the VL increased: during the first half of the swing phase and the second half of stance it evoked ~30% of the activity, ~25% of the activity during the first half of stance, and ~7% of it during the second half of swing (Fig. 7*B4*).

Although there were neurons for which the VL contribution peaked twice during the step cycle (*group 6* in Fig. 8) and many neurons for which it had different signs during different phases of the cycle, in 63% (25/40) of cases the VL's excitatory contribution had a single peak and a preferred phase. In the great majority of such cases, the preferred phase of the contribution during both flat surface and ladder locomotion was identical or close to the PrPh of the neuron's activity during the task in normal conditions (open circles in Fig. 6, *C* and *D*), indicating that an important, PrPh-forming, part of the neuron's activity during the task was evoked by the input from the VL.

The nonuniform changes in the discharge rate of neurons across the step cycle after application of CNQX in the VL led to changes in the depth of modulation (dM) of neuronal activity after the application. The dM often increased. During flat surface locomotion the dM increased in 51% of neurons by 81 ± 57% (Fig. 6*E*), and it rose in 37% of cells during ladder locomotion by 96 ± 80% (Fig. 6*F*). Also as a result of the nonuniform changes in the discharge rate of neurons over the step cycle, the pattern of activity of a number of neurons changed. Most notably, 11 of 13 neurons that during flat surface locomotion in normal conditions had two PEFs per stride discharged only one PEF after CNQX was applied in the VL. Similarly, five of nine neurons that had two PEFs per stride during ladder locomotion in normal conditions had only one PEF per stride after CNQX application in the VL. Changes of the modulation pattern in the opposite direction were significantly less frequent ($P = 0.04$, Fisher's test). This shows that in addition to often contributing to formation of the preferred phase of the neurons' activity during locomotion, the input from the VL may enrich their discharge pattern by adding peaks to it.

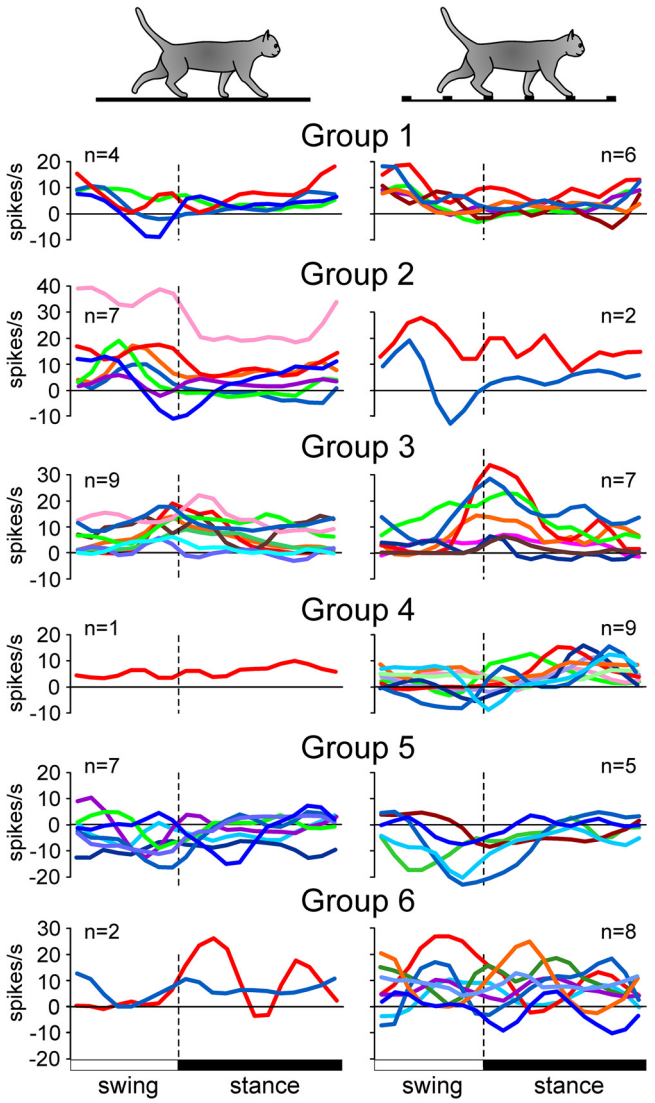


Fig. 8. Six groups of stride-related patterns of the ventrolateral thalamus (VL) contribution to the activity of motor cortex neurons during flat surface and ladder locomotion. Each plot shows superimposed profiles of the effects of the VL contribution of all studied cases belonging to a particular group. Only those for which the effect was step cycle modulated and exceeded ±5 spikes/s in any 1/20th portion of the stride are shown.

Compared with the very common changes in the average discharge rate, the depth, and the pattern of activity modulation after blockade of glutamatergic transmission in a part of the VL, changes in the duration of the PEF and the preferred phase of the activity were significantly less frequent during either locomotor task ($P = 0.004$, Fisher's test). The duration of PEF changed in only six and seven neurons during flat surface and ladder locomotion, respectively; all these changes except for one were reductions in the PEF duration. Among 24 cells that had one PEF in both normal and CNQX in the VL conditions, the preferred phase of the activity was altered in only six cases during flat surface locomotion and in only four cases during ladder locomotion.

All tested neurons, except one, responded to blockade of glutamatergic transmission in a part of the VL by changes in at least one parameter of their activity. During flat surface locomotion, only in five cases (12%) did a cell not respond to the blockade, and in only six cases (15%) did a cell not respond during ladder locomotion. In all but four cases responses of individual neurons during two locomotion tasks were different.

Blockade of Glutamatergic Transmission in a Part of the VL Differentially Affects Flat Surface and Ladder Locomotion-Related Activity of Neurons in Motor Cortex

Responses of the example neuron shown in Fig. 5 demonstrated that an application of CNQX in the VL could greatly reduce activity of a neuron during one locomotor task (flat surface locomotion in this example) while reducing it to a lesser degree during another task (ladder locomotion in this example). In addition to the difference in the magnitude, the phases of the VL influence could be different between the tasks. In Fig. 5, the largest effect of CNQX application on the activity of the neuron during flat surface locomotion was in the swing phase of the stride, whereas the largest effect during ladder locomotion was in the stance phase (Fig. 5, *P* and *Q*). These graphs are replotted in Fig. 9, *A1* and *A2*, and Fig. 9*A3* shows the effect of the VL contribution to the activity of this neuron during the two tasks superimposed; the difference is highlighted by an orange curve. It has two components: a negative deflection in the late swing phase, indicating that during locomotion on the ladder the VL contributed less during this phase than during flat surface locomotion, and a positive deflection during the stance phase, indicating that on the ladder the VL contributed more during this phase. As explained in INTRODUCTION, we interpret the difference in the VL contribution to the activity of motor cortex neurons between vision-independent locomotion on the flat surface and vision-dependent locomotion on the convenient ladder as the vision-related portion of the VL contribution (see also DISCUSSION).

In 45% (18/40) of cases, the difference in the VL contribution between flat surface and ladder locomotion, the vision-related portion of the VL contribution, had one peak. We have grouped difference profiles with one peak into three groups (*groups 1–3* in Fig. 9*C*) according to the stride phase of the maximal positive or negative difference. *Group 1* includes profiles in which the maximal difference occurred during transition from the stance to swing phase. *Group 2* includes profiles in which the difference peaked in the middle of the swing phase. *Group 3* includes profiles in which it was maximal during transition from the swing to stance phase. The one-peak profiles were distributed equally among *groups 1–3*.

The majority of difference profiles (55%, 22/40) had two peaks during the step cycle. An example is shown in Fig. 9*B*. During locomotion on the flat surface the activity of *neuron 5684* shown here peaked at ~20 spikes/s in the beginning of the swing phase and fluctuated around 10 spikes/s during late swing and throughout stance (gray line in Fig. 9*B1*). The part of the VL that we inactivated in this experiment evoked ~50% of the neuron's activity during the beginning of the swing phase, suppressed it during the second half of swing by more than half, and had only a small effect during stance (black area histogram in Fig. 9*B1*). During locomotion on the ladder, the activity of the neuron during the first half of swing increased, peaking at ~30 spikes/s, and was largely unchanged during the rest of the cycle (gray line in Fig. 9*B2*). On the ladder, the VL area that we inactivated contributed almost 100% to the activity of this neuron during the beginning of the swing and

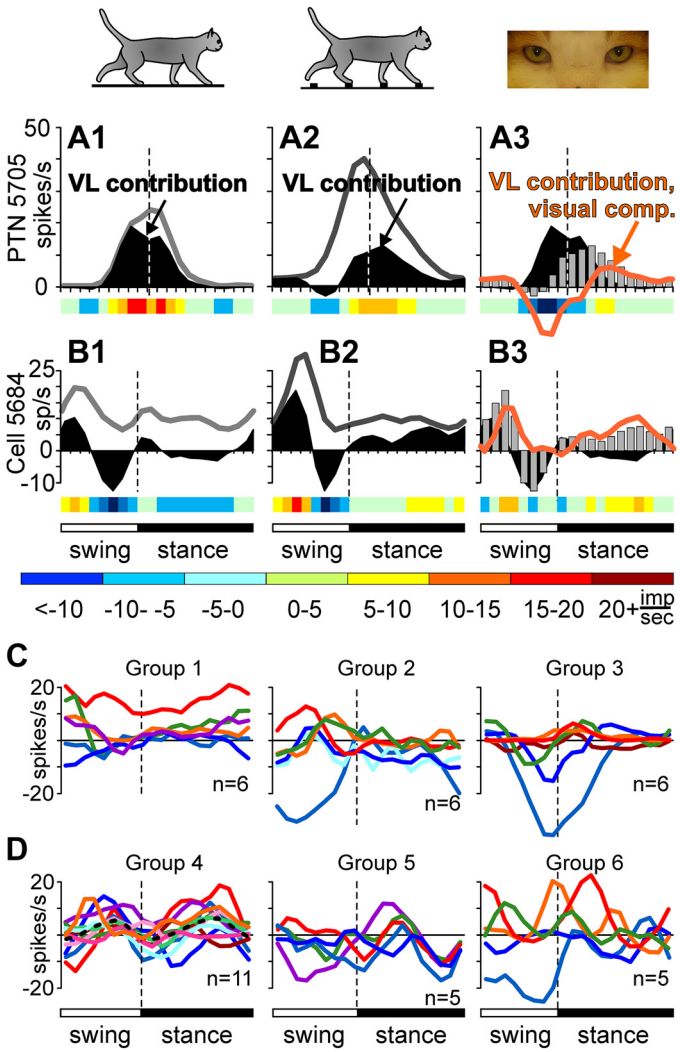


Fig. 9. The difference in the ventrolateral thalamus (VL) contribution to the activity of motor cortical neurons between vision-independent locomotion on the flat surface and vision-dependent locomotion on the convenient horizontal ladder, the vision-related component in the VL contribution. *A*: example neuron, pyramidal tract projecting neuron *(PTN) 5705*. *A1* and *A2*: Fig. 5, *R* and *S*, replotted. Black area histograms show components of the PTN activity that were contributed by the inactivated VL area during flat surface (*A1*) and ladder (*A2*) locomotion. *A3*: the contributions during the two tasks are superimposed (to assist visual comparison, the one for ladder locomotion is shown here as a gray bar histogram), and the difference between the contributions is shown with an orange curve. This difference is interpreted as the vision-related contribution of the VL to the activity of the neuron. See text for details. The vision-related contribution to the activity of *PTN 5705* had 1 negative and 1 positive peak during the step cycle. *B*: effects of the VL contributions on the activity of a different neuron, *cell 5684*. Plots are analogous to those in *A*. For this neuron, the vision-related component of the VL contribution had 2 positive peaks during the step cycle. *C*: 3 groups (*groups 1–3*) of patterns of the VL vision-related contribution to the activity of motor cortex neurons that have 1 peak during the step cycle. *D*: 3 groups (*groups 4–6*) of patterns of the VL vision-related contribution to the activity of motor cortex neurons that have 2 peaks during the step cycle. In *C* and *D*, each plot shows superimposed the profiles of the VL vision-related contribution of all studied cases belonging to a particular group. Other designations are as in Fig. 7 and Fig. 8.

the end of stance phase and inhibited it during the second half of swing by about half (black area histogram in Fig. 9*B2*). This VL contribution was different from that on the flat surface, and the difference is shown by an orange curve in Fig. 9*B3*. It has two peaks: one during the swing and one during the stance phase.

We have grouped the difference profiles with two peaks into three groups (*groups 4–6* in Fig. 9*D*) according to the stride phases of maximal positive and negative differences. *Group 4* includes profiles in which the positive differences first peaked during the swing phase of the stride and then peaked again during stance. *Group 5* includes profiles in which one of the negative peaks occurred at the end of the stance phase. *Group 6* includes the remaining profiles of bimodal difference. The observed two-peak profiles distributed unequally among *groups 4–6*, as twice as many profiles belonged to *group 4* than to either *group 5* or *group 6* ($P = 0.059$, Z test for proportions).

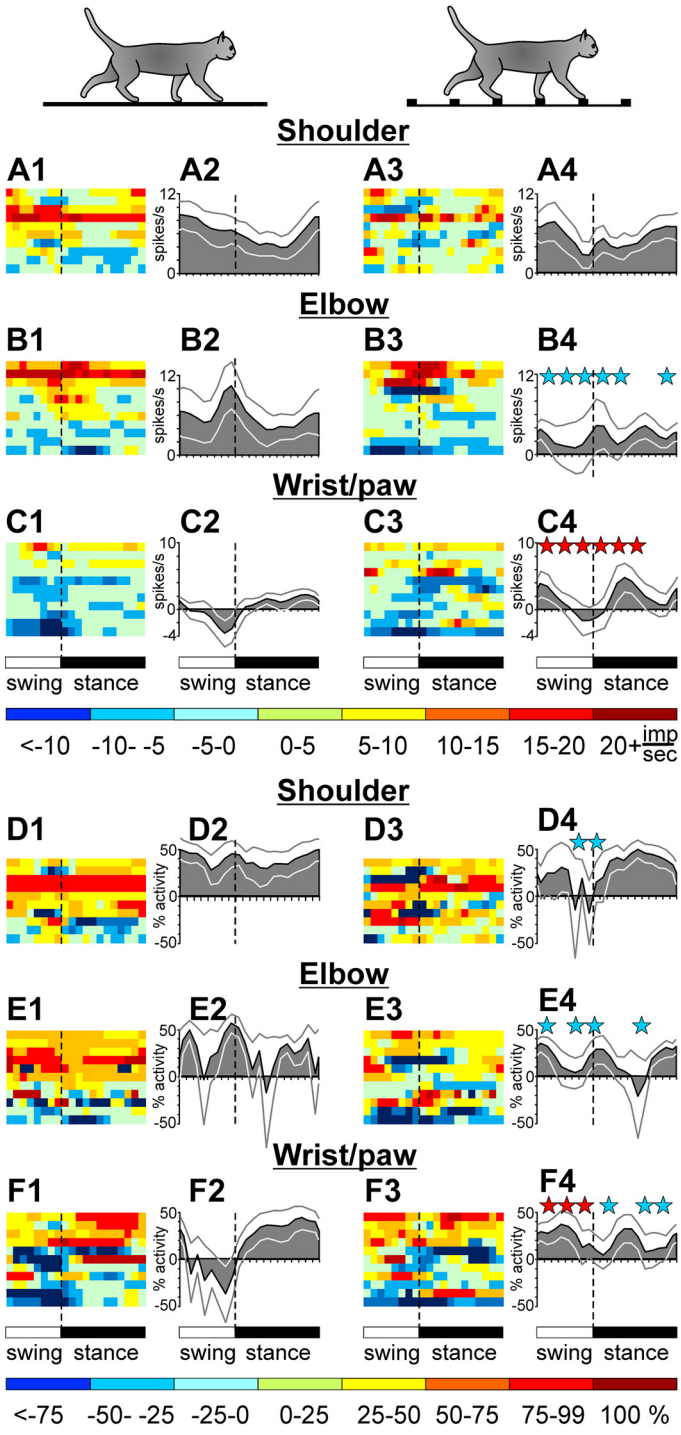
The effect of the vision-related contribution of the VL to the activity of individual neurons could range between 4 and 40 spikes/s and in 88% of the cases (35/40) had an inhibitory component in excess of 1 spike/s.

Blockade of Glutamatergic Transmission in a Part of the VL Differentially Affects Activity of Shoulder-, Elbow-, and Wrist/Paw-Related Neurons in Motor Cortex

The contribution of the VL to the activity of shoulder-, elbow-, and wrist/paw-related neurons in motor cortex was different during either locomotion task. Profiles of the VL contribution during the step cycle of flat surface and ladder locomotion to the activity of single neurons and populations of neurons with somatosensory receptive fields on different segments of the forelimb are summarized in Fig. 10, *A–C*. Each horizontal bar of a colored plot (plots numbered *1* and *3*) shows the difference in the discharge frequency of a neuron before and after an application of CNQX in the VL, similar to Fig. 7, *A1* and *A3*. In the graphs for the shoulder- and elbow-related neurons one can see a lot of yellow and red colors (Fig. 10, *A1* and *A3*, *B1* and *B3*), whereas the graphs for the wrist-related cells are more blue (Fig. 10, *C1* and *C3*). Indeed, the average effect of the VL contribution to the activity of the wrist-related cell group at 0.2 ± 1.7 spikes/s was significantly smaller than that to the activity of either shoulder- or elbow-related group, by ~6 spikes/s ($P = 0.0001$, t test; Fig. 10*C2*). In fact, for many wrist-related neurons the effect was inhibitory rather than excitatory, particularly during the swing phase of the stride (Fig. 10*C1*). The effects of the VL contributions to the activity of the shoulder- and elbow-related groups, although similar in the average magnitude of ~6 spikes/s, differed in the phase. The shoulder-related group received most of the VL influence at the end of the stance and beginning of swing phase (Fig. 10*A2*), whereas the contribution to the activity of the elbow-related group peaked during the transition from the swing to stance phase (Fig. 10*B2*).

With respect to how much the input from an estimated 10% of the VL contributed to the activity of neurons with somatosensory receptive fields on different segments of the forelimb in relation to other inputs to these neurons, we found that neurons of each of the three groups could receive a very substantial excitation, up to 95–100% of their total activity, as well as a significant inhibition, by as much as 80–90% (Fig. 10, *D1*, *E1*, and *F1*). However, the profiles of population averages were different for different groups (Fig. 10, *D2*, *E2*, and *F2*). The relative VL contribution to the activity of the

shoulder-related group was fairly steady over the step cycle, fluctuating between 30% and 50% (Fig. 10*D2*). In contrast, the contribution to the activity of the wrist-related group was profoundly modulated: it was inhibitory during most of the swing phase, suppressing the group's activity by 20–35%, and was excitatory during the stance phase, evoking 30–40% of the group's activity during this phase (Fig. 10*F2*). The VL relative contribution to the activity of the elbow-related group was very variable over the step cycle, peaking at ~50% in the beginning of the swing phase and during transition from swing to stance (Fig. 10*E2*).



Upon transition from locomotion on the flat surface to vision-guided accurate stepping on the ladder, the VL contribution to the activity of neurons with receptive fields on different segments of the forelimb changed, differently for different groups. For the elbow-related group, the VL contribution decreased in both the absolute and relative value, particularly during the swing phase of the stride ($P = 0.018$, *U* test; Fig. 10, *B3* and *B4* vs. *B1* and *B2* and *E3* and *E4* vs. *E1* and *E2*), and there were fewer periods during which the estimated 10% of the VL contributed 99–100% of individual neurons' activity than there were such periods during flat surface locomotion ($P = 0.018$, *Z* test for proportions; Fig. 10, *E3* vs. *E1*). In contrast, for the wrist-related group, the VL contribution increased in its excitatory component, particularly during the swing phase of the stride ($P = 0.019$, *U* test; Fig. 10, *C3* and *C4* vs. *C1* and *C2* and *F3* and *F4* vs. *F1* and *F2*). For individual neurons of this group, there were now fewer periods during which the ~10% of the VL suppressed activity of the neuron by >50% than there were such periods during flat surface locomotion ($P = 0.011$, *Z* test for proportions; Fig. 10, *F3* vs. *F1*). The absolute value of the VL contribution to the activity of the shoulder-related group did not change ($P > 0.05$, *U* test; Fig. 10, *A3* and *A4* vs. *A1* and *A2*), but its relative value during the transition phase from swing to stance diminished ($P = 0.012$, *U* test; Fig. 10, *D3* and *D4* vs. *D1* and *D2*).

Blockade of Glutamatergic Transmission in a Part of the VL Differentially Affects Activity of PTNs with Fast- and Slow-Conducting Axons

The contribution of the VL to the activity of PTNs with different conduction velocities along the axon was different as well. During flat surface locomotion, the contribution was larger for the fast- than slow-conducting PTNs, with 5.1 ± 1.1 vs. 2.2 ± 1.0 spikes/s effect, respectively ($P = 0.0001$, *t* test; Fig. 11, *A1* and *A2* vs. *B1* and *B2*). For fast-conducting PTNs, this constituted ~40% of their activity during the transition from stance to swing phase and ~30% during the stance phase (Fig. 11*D2*). The contribution to the activity of slow-conducting PTNs was more variable in respect both to individual neurons (Fig. 11*E1*) and the phases of the step cycle when it

Fig. 10. Stride phase distributions of the ventrolateral thalamus (VL) contribution to the activity of individual neurons and populations of neurons with somatosensory receptive fields on different segments of the forelimb. *A1* and *A3* through *C1* and *C3*: stride phase distributions of the effects of the VL contribution to the activity of individual neurons in spikes per second. For each neuron, the average effect of the contribution in each 1/20th portion of the step cycle is color-coded according to the scale shown below *C1–C4* and is similar to rainbow bars in Fig. 5, *R* and *S*. *A2* and *A4 through C2* and *C4*: the mean effect of the contribution on the activity of subpopulations of neurons. Thin lines show SE. Blue stars indicate periods of the stride when the VL contribution during ladder locomotion was significantly smaller than during flat surface locomotion, and red stars indicate when it was larger ($P < 0.05$, *U* test). *D1* and *D3* through *F1* and *F3*: stride phase distributions of the effects of the VL contribution to the activity of individual neurons expressed as % of their total activity. For each neuron, the % of the activity evoked by the estimated 10% of the VL during each 1/20th portion of the step cycle is color-coded according to the scale at *bottom*. Neurons are rank-ordered identically as in *A1* and *A3* through *C1* and *C3*. *D2* and *D4* through *F2* and *F4*: the mean % that the estimated 10% of the VL evoked in the activity of subpopulations of neurons. Thin lines show SE. Blue stars indicate periods of the stride when the relative VL contribution during ladder locomotion was significantly smaller than during flat surface locomotion, and red stars indicate when it was larger ($P < 0.05$, *U* test). Other designations as in Fig. 7.

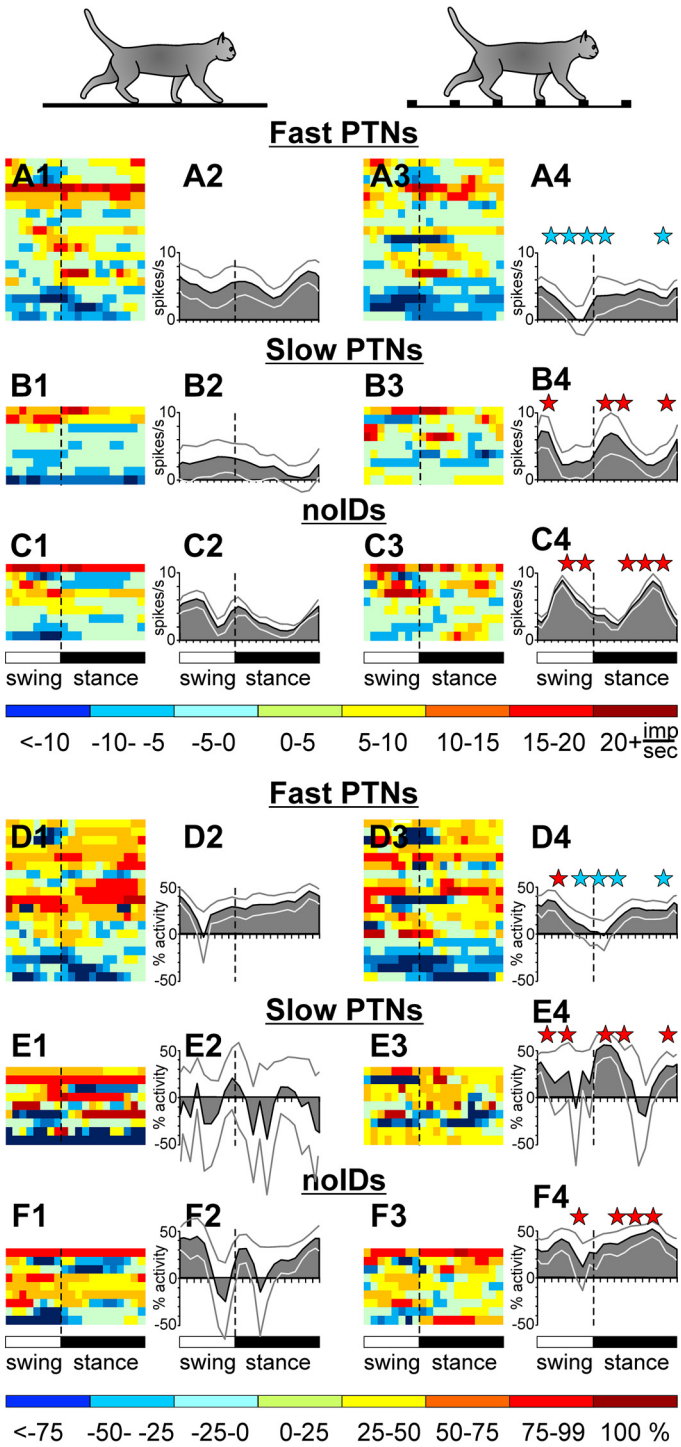


Fig. 11. Stride phase distributions of the ventrolateral thalamus (VL) contribution to the activity of individual pyramidal tract projecting neurons (PTNs) and subpopulations of PTNs with fast- or slow-conducting axons and to the activity of neurons without identified projection of the axon (noIDs). *A1* and *A3* through *C1* and *C3*: stride phase distributions of the effects of the VL contribution to the activity of individual neurons. *A2* and *A4 through C2* and *C4*: the mean effect of the VL contribution on the activity of subpopulations of neurons. *D1* and *D3* through *F1* and *F3*: stride phase distributions of the effects of the VL contribution to the activity of individual neurons expressed as % of their total activity. Neurons are rank-ordered as in *A1* and *A3* through *C1* and *C3*. *D2* and *D4* through *F2* and *F4*: the mean % of the VL-evoked activity in the total activity of subpopulations of neurons. Other designations as in Fig. 7 and Fig. 10.

was delivered for the population (Fig. 11*E2*). Compared with fast-conducting PTNs, there were more periods of the step cycle during which the estimated 10% of the VL suppressed the activity of a slow-conducting PTN by >50% ($P = 0.0001$, Z test for proportions; Fig. 11, *E1* vs. *D1*).

Upon transition to locomotion on the ladder, the VL contribution to the activity of fast-conducting PTNs decreased, resulting now in only 3.2 ± 1.4 spikes/s ($P = 0.001$, t test; Fig. 11, *A3* and *A4* vs. *A1* and *A2*). It represented now only ~25% of these neurons' group activity in the end of stance and was very small in the end of the swing phase and beginning of stance, albeit increasing in the middle of swing (Fig. 11*D4*). At the same time, the contribution increased for slow-conducting PTNs ($P = 0.001$, t test; Fig. 11, *B3* and *B4* vs. *B1* and *B2*). There were now substantially fewer periods of the step cycle during which the ~10% of the VL suppressed the activity of a slow-conducting PTN by >50% ($P = 0.0008$, Z test for proportions; Fig. 11, *E3* vs. *E1*). The absolute effect of the VL contribution at 4.2 ± 1.8 spikes/s was almost twice as large compared with that during flat surface locomotion (Fig. 11, *B4* vs. *B2*), and the contribution evoked >50% of slow-conducting PTN group activity in the beginning of the stance phase and ~30% during transition from the stance to swing phase (Fig. 11*E4*).

The effect of the VL contribution to the activity of the group of neurons without identified projections to the pyramidal tract was 3.6 ± 1.6 spikes/s on average, fluctuating between ~6 spikes/s during the swing phase and 3 spikes/s during stance (Fig. 11*C2*). This contribution evoked ~40% of the activity of these neurons during the swing phase and ~30% of it in the beginning and end of stance (Fig. 11*F2*). Upon transition to the ladder, the effect of the VL contribution increased to 5.3 ± 2.2 spikes/s ($P = 0.008$, t test), and the contribution now evoked ~40% of the activity of this group during the stance phase of the stride (Fig. 11*F4*). During locomotion on the ladder, the VL contributed to the activity of neurons without an axon in the pyramidal tract during largely the opposite phases of the stride compared with its contribution to the activity of PTNs (Fig. 11*C4*).

DISCUSSION

The goal of this study was to clarify the contribution of signals from the VL to formation of activity in motor cortex during locomotion. In two cats we have carefully mapped the areas of the VL that contain neurons with ascending projections to the forelimb representation in motor cortex and described their activity during vision-independent and vision-dependent locomotion (Marlinski et al. 2012a). We also investigated the locomotion-related activity of motor cortex in these cats (Armer et al. 2013; Beloozerova et al. 2010; Stout and Beloozerova 2013). This allowed us to examine effects of local inactivation in the VL on the locomotion-related activity of motor cortex while knowing both characteristics of the activity being removed from the network by VL inactivation and the activity being modified in motor cortex.

We estimated the spread of CNQX and the area of the VL inactivation to be ~2 mm^3, which is ~10% of the VL in the cat. This estimate is based on our recordings of activity in the areas neighboring the site of CNQX injection (Fig. 2*I*) and is consistent with estimates of drug diffusion in the brain obtained by

other researchers (e.g., Myers 1966). It is likely that the inactivated area was not a perfect sphere as schematically depicted in Fig. 2*F* because CNQX likely diffused differently in different directions. However, even though not perfectly spherical and possibly somewhat larger than estimated, the area of inactivation was small in respect to the large nucleus and was largely confined to the VL. By visually assessing locomotor performance of the cats and measuring the duration of the swing and stance phases of the stride before and after inactivation of a part of the VL, we observed only very minor abnormalities, and only in *cat A*. This cat walked 3.4% slower than normal on the flat surface and, on the ladder, albeit walking with a normal pace, missed a crosspiece a couple of times on the first 3 days of experiments. No movement abnormalities were seen in *cat B* (Fig. 4). Having largely normal movement was important for this study because a disturbed movement could by itself affect the activity of motor cortex neurons and thus obscure specific effects of the local reduction in the thalamo-cortical transmission. We acknowledge, however, that the mechanics of locomotion in subjects with a small inactivated area in the VL should be investigated in detail in a future study to determine whether any characteristics of locomotion are in fact affected in such subjects. For this study, we argued that even if there were some small changes in the movement after inactivation in the VL in addition to those we have detected, they were unlikely to cause significant changes in the activity of motor cortex neurons observed. We argued so because it was previously shown that even big changes in the locomotor movement such as those associated with changes in the speed of locomotion are reflected in the activity of only a minority of motor cortex neurons (Armstrong and Drew 1984a; Beloozerova and Sirota 1993b) and that the activity of motor cortex during locomotion is more concerned with the visuomotor coordination and accuracy of stepping than with other aspects of the movement (Armstrong and Marple-Horvat 1996; Beloozerova et al. 2010; Beloozerova and Sirota 1993a; Drew et al. 2008; Friel et al. 2007). Particularly, given the data from Armstrong and Drew (1984a) and Beloozerova and Sirota (1993b) on the rather weak dependence of the activity of motor cortex neurons on the speed of locomotion in the cat, it is safe to assume that the 3.4% change in the pace of locomotion of *cat A* on the flat surface after CNQX application in the VL (Fig. 4, *A* and *C*), which affected aggregated data for flat surface locomotion in Table 1, was not the major cause of the massive changes in the activity of motor cortex neurons after the application.

VL Contributes Both Excitation and Inhibition, Which Fluctuate over the Step Cycle and Substantially Influence Preferred Phases of Activity of Motor Cortex Neurons

The first finding of this study is that the VL substantially contributes to the locomotion-related activity of neurons in motor cortex by supplying them with both excitation and, di-/oligosynaptically, inhibition (Fig. 7, Fig. 8). The excitation dominates overall, as the activity of a small part of the VL can evoke up to 100% of the activity of individual neurons during some phases of the stride and up to 25–30% of the average activity of the population (Fig. 7). The inhibition is as powerful but is less frequently observed across the population (Fig. 7, Fig. 8). These VL contributions are consistent with the fact that the thalamo-cortical projection is glutamatergic (Graziano et al. 2008; Salt et al. 1995) and richly synapses on PTNs and other excitatory cortical neurons, as well as on inhibitory interneurons (Kuramoto et al. 2009; Strick and Sterling 1974). The VL contributions are also consistent with the described effects of VL activity on the activity of motor cortex neurons in anesthetized or awake but sitting animals (e.g., Baranyi et al. 1993; Noda and Yamamoto 1984; Sauerbrei et al. 2020; Shinoda et al. 1985a). This is the first study to characterize the contribution of the VL to the activity of motor cortex neurons during locomotion.

One of the striking findings is that the contribution of a fixed part of the VL to the locomotion-related activity of neurons in motor cortex substantially fluctuates over the step cycle, often going from excitatory to inhibitory. The average population activity of the VL is more steady over the step cycle than are most profiles of the VL contribution to the activity of individual cortical neurons (compare Fig. 8 with Fig. 6, E and K, and Fig. 8, D and I, in Marlinski et al. 2012a). The VL projection to motor cortex is both divergent and convergent, such that a small VL area projects to a wide cortical territory and projection fields of different VL parts overlap in the cortex (Shinoda et al. 1993). Having such a projection and knowing that the VL substantially influences motor cortex activity, one could believe that the effects of VL inputs in the cortex can be fairly well predicted based on the population activity of VL neurons projecting to cortex. However, this is not what we found.

The more variable VL contribution profiles as compared with the VL population activity profile suggest that there is a certain selectivity in the VL-to-motor cortex projection such that only particular neurons from any VL area affect the activity of any given neuron in motor cortex. The frequent presence of inhibitory components in the VL contribution indicates that the effect of the VL input to the cortex is shaped in the cortex by intracortical interactions involving inhibitory interneurons. Influences from inputs other than the VL, such as inputs from other thalamic regions, including those conveying signals from basal ganglia, and inputs from various cortical areas are likely to contribute as well. The very variable shapes of the VL contribution profiles suggest that all these interactions constantly change throughout the step cycle. In addition, it is possible that the size of the VL area influencing each cortical neuron fluctuates over the step cycle, thus also shaping the VL contribution.

We previously found that the activity of 91–95% of VL neurons projecting to motor cortex is modulated in the rhythm of strides (Marlinski et al. 2012a). Among these, 63% discharge one activity burst per stride and 35% discharge two. The VL contribution to the activity of cortical neurons found in this study, however, was more often unimodal than could be expected based on the activity patterns of VL neurons. Namely, it was unimodal in 95% of cases during flat surface locomotion and in 80% of cases during ladder locomotion (Fig. 8). This can be explained if one assumes that the VL neurons discharging one activity burst per cycle have wider or more powerful effects in motor cortex compared with those of their two-burst counterparts. A different explanation would be that one of the bursts of the two-burst VL neurons is blocked at the cortex.

We found that the contribution of the VL to the activity of motor cortex neurons depended on the phase in the stride even during locomotion on the flat surface, a task that does not

require participation of motor cortex or the VL to be successful (Fig. 7, Fig. 8). For neurons receiving a one-peaked excitatory contribution, the excitation most often came during transition from the stance to swing phase of the stride or in the middle of the swing phase (Fig. 8, *groups 1* and *2*), whereas during transition from the swing to stance phase neurons received either excitation or inhibition (Fig. 8, *groups 3* and *5*, respectively). Very often, the VL contribution was the greatest during the preferred phase of the activity of the neuron (Fig. 6, *C* and *D*) and could account for up to 100% of its activity (Fig. 7, *A2* and *A4*). The VL contribution could also account for up to 25–30% of the activity of the entire population during some phases of the stride (Fig. 7, *B2* and *B4*). These findings suggest that the activity of motor cortex neurons during their preferred phases of activity to a large extent results from the contribution from the VL. During vision-independent locomotion this contribution is likely to be based on the information about the endogenous locomotion-related activity of the spinal cord and information from the body somatosensory receptors.

VL Contributes Vision-Related Information During Vision-Dependent Locomotion on a Complex Terrain

One of the main roles of motor cortex in control of locomotion is in the adjustment of locomotor movements to the visually perceived features of the environment (Armstrong and Marple-Horvat 1996; Beloozerova et al. 2010; Beloozerova and Sirota 1993a, 1993b; Drew 1993; Drew et al. 2008; Xing et al. 2019; Yokoyama et al. in press). Does the VL contribute anything to the activity of motor cortex to specifically assist it in its role of adjusting locomotion based on visual information aside from the contribution of the basic locomotion-related information as discussed above? Results of this study show that it does (Fig. 9).

For many years our approach to isolating the parts of neuronal activity that are related to processing of visual information was to compare the activity between vision-independent locomotion on a flat surface and vision-dependent locomotion that has mechanical characteristics close to those of the flat surface locomotion (e.g., Beloozerova et al. 2010; Beloozerova and Sirota 1993a, 2003; Marlinski et al. 2012a). Locomotion along a horizontal ladder with wide crosspieces placed at a distance of a typical length of the stride appeared to be a suitable comparison. We showed that locomotion along such a ladder, even well practiced, requires vision (Beloozerova and Sirota 2003). We also found that although on the ladder cats assume a more bent-forward posture, change some of the joints' moments during selected phases of the stride, and increase activity of some distal muscles, the great majority of mechanical variables out of 229 tested are similar between locomotion on the flat surface and the convenient ladder (Beloozerova et al. 2010). These findings allowed us to interpret the differences in the activities of neurons observed between flat surface and convenient ladder locomotion as a reflection of processing of visual information during ladder locomotion. The probably increased attention during ladder locomotion may contribute too but does not appear to play the leading role in determining neuronal stride-related responses on the ladder because during locomotion along a narrow strip these responses are different (Farrell et al. 2015) despite a likely similar level of required attention.

Thus, in this study we compared VL contributions to the activity of motor cortex neurons during locomotion on the flat surface and the convenient ladder with the goal of revealing the (chiefly) vision-related component of the contribution. We found that the VL contributions during locomotion on the flat surface and the ladder are nearly always different (Fig. 9). Following the above reasoning, this means that during ladder locomotion the VL contributes some vision-related information to the activity of neurons in motor cortex in addition to supplying them with movement-related information. Among individual neurons these contributions were quite diverse, falling into two major categories: with one peak and with two peaks during the step cycle. The most populous is a two-peak group, the members of which have one peak in the middle of the swing phase and another peak in the middle of the stance phase of the stride [27.5% (11/40); *group 4*, Fig. 9*D*]. In a recent study we found that during locomotion cats fixate gaze on the walking surface during the middle of the swing phase of each forelimb (Zubair et al. 2019). Since swing of the other forelimb occurs in the middle of stance of the recorded forelimb, one can hypothesize that *group 4* contribution profiles reflect arrival of visual information about the crosspieces obtained during gaze fixations during swing phase of each forelimb.

Also interesting are the profiles of *group 5* that have a trough at the end of the stance phase. The end of stance is a phase during which both forelimbs are on the ground and the "constant gaze" behavior takes place (Zubair et al. 2019). Constant gaze, also called "travel fixation" in humans, is a gaze behavior during which the subject looks a fixed distance ahead during locomotion. Many studies suggest that images of objects that travel across the retina in a constant pattern during constant gaze form an "optic flow," which provides information about objects in the environment (Gibson 1958). Inhibition that the VL contributes to motor cortex neurons in the end of the stance phase might reflect arrival of visual information about the ladder's crosspieces obtained with constant gaze.

VL Contribution is Limb Segment Specific

An important finding of this study is that the VL contributes differently to the activities of motor cortical neurons with receptive fields on different segments of the limb, which are likely to be related to control of different limb segments. In the motor cortex there is a reasonable correspondence between the location of the source of the afferent somatosensory input and the target of motor output (Asanuma et al. 1968; Murphy et al. 1975; Rosén and Asanuma 1972; Sakata and Miyamoto 1968). In this study, similar to our earlier study (Stout and Beloozerova 2012), we used this correspondence to infer which part of the limb a motor cortex neuron might be controlling based on the somatosensory receptive field it has.

Our previous comparison of population activities of neurons with somatosensory receptive fields on the same segment of the forelimb residing in the VL and layer V of motor cortex showed that they reach their maxima largely in opposite phases of the stride (Marlinski et al. 2012a; Stout and Beloozerova 2012; reviewed in Beloozerova et al. 2013). We suggested three possible explanations, *1*) that the inhibitory network of the cortex "inverts" the VL signal, *2*) that each cortical neuron receives inputs only from those VL neurons that are active

during similar phases of the stride, or *3*) that influences from VL neurons related to other segments of the limb substantially affect motor cortex neurons (Beloozerova et al. 2013). Numerous inhibitory contributions of the VL found in this study indicate that the cortical inhibitory network is certainly involved. Putative inhibitory interneurons with suitable properties were found in motor cortex (Beloozerova et al. 2003, rabbit; Murray and Keller 2011, rat; Beloozerova, unpublished observations, cat), and such a relay via inhibitory neurons would have many benefits (discussed in, e.g., Feldmeyer et al. 2018; Tremblay et al. 2016). It was shown that cortical inhibitory interneurons importantly participate in motor-related responses of PTNs and experimental reduction of cortical $GABA_A$ inhibition enhances PTN activity during voluntary movements (Matsumura et al. 1992) and postural corrections (Tamarova et al. 2007). Whether the activity of cortical inhibitory interneurons inverts the VL signal or modulates it in some other manner remains unclear. Results of this study suggest very significant cortical interactions among the VL inputs and probably between the VL and other inputs to individual cortical neurons during locomotion, which ultimately determine the effect of any particular input from the VL.

In this study we found that the VL contributes a lot of excitation to the activity of shoulder- and elbow-related neurons (Fig. 10, *A* and *B*). For the shoulder-related group, the contribution is greatest during transition from stance to the swing phase, whereas for the elbow-related cells it is largest during the opposite phase, the transition from the swing to stance phase. Unlike the phases of activities of the shoulder- and elbow-related VL neurons, these phases of the VL contribution are exactly the ones when neurons with the respective somatosensory receptive fields in the cortex are most active during locomotion (Supplemental Fig. S3, A1 and B1; also see Stout and Beloozerova, 2012 for data on larger populations). The similarity of the profiles of the VL contributions and motor cortex population activities further supports the suggestion that the VL plays a pivotal role in driving locomotion-related discharges in motor cortex.

In contrast to the substantial excitatory contribution to the activity of neuronal groups related to the proximal limb, during locomotion on the flat surface the VL contributes very little excitation to the activity of the wrist/paw-related group (Fig. 10, *C1* and *C2*). This contribution is also different from the group activity of wrist/paw-related VL neurons, which are quite active during locomotion, discharging at ~30 spikes/s at the peak in the stance phase of the stride (Marlinski et al. 2012a). The small excitatory contribution of the VL to the activity of cortical neurons related to the wrist/paw is, however, consistent with our previous finding that neurons in the reticular nucleus of the thalamus (RE) that have somatosensory receptive fields on the wrist/paw are vigorously active during locomotion, in contrast to the less active RE neurons with receptive fields on the proximal limb (Marlinski et al. 2012b; Marlinski and Beloozerova 2014). The RE primarily consists of inhibitory neurons that receive inputs from both collaterals of thalamo-cortical and cortico-thalamic axons and project to the thalamic nuclei, inhibiting them. We have previously suggested (Marlinski and Beloozerova 2014) that during locomotion the highly active RE neurons related to the wrist/paw powerfully inhibit the wrist/paw-related thalamo-cortical transmission, potentially opening an opportunity for cortical inputs to influence the stride-related activity of the wrist/paw-related population in motor cortex. Results of the present study support this suggestion. In addition, they show that the wrist/paw-related neurons of motor cortex receive only a limited amount of excitation from the VL and are often inhibited by it.

We want to note that the dissimilarity of the effects of the VL inactivation on the activity of motor cortex neurons with somatosensory receptive fields on different segments of the forelimb was not due to a selective inactivation of clusters of neurons with receptive fields on different segments of the forelimb in the VL, as no such clusters were observed in the injected area in the course of the previous study (Marlinski et al. 2012a).

Overall, our findings demonstrate that the effect of the activity of an area providing inputs to another area cannot be predicted based on its activity alone and that the actual contribution has to be measured. This is because each neuron receives inputs from many sources and they interact.

In respect to the influence of somatosensory information on the locomotion-related activity of motor cortex, results of our previous study suggest that this influence differs for different motor cortex subpopulations (Stout and Beloozerova 2012). The locomotion-related activity of shoulder flexion-responsive PTNs appears to be significantly influenced by afferent signals from the shoulder, as they typically discharge in phase with flexion of the shoulder joint during locomotion (Fig. 9 in Stout and Beloozerova 2012). In contrast, the locomotion-related activity of the shoulder extension-responsive PTNs and PTN subpopulations related to other segments of the forelimb appear to be less affected by signals from their somatosensory receptive fields, as neurons of these subpopulations typically have no preference to discharge in or out of phase with stimulation of their receptive field during locomotion or may even preferentially discharge out of phase (Fig. 9 in Stout and Beloozerova 2012). The very dissimilar contribution of the VL to the activity of motor cortical populations related to different segments of the forelimb found in this study must play a role in determining how somatosensory signals transmitted by the VL and other sources shape the locomotion-related responses of these populations. However, additional experiments are needed to better understand this role.

In respect to processing visual information during locomotion on a complex terrain, we found that the VL contributions to responses of motor cortex neurons related to different segments of the forelimb to the demand on visuo-motor coordination were sharply dissimilar. The VL contribution to the activity of the wrist/paw-related group increased upon the transition from flat surface to ladder locomotion (Fig. 10*C*). It decreased for the elbow-related group (Fig. 10*B*) and did not change for the shoulder-related group (Fig. 10*A*). We want to suggest that the increase in the VL contribution to the activity of the wrist/paw-related group during a task requiring visual control of strides indicates that, for such a task to succeed, important information needs to be relayed from the cerebellum to the wrist/paw-related motor cortical group. Conveying visual information for guiding movements appears to be one of the important functions of the cerebellum (reviewed in, e.g., Stein and Glickstein 1992). During walking on the ladder, while the paw is transported in space through body translation and shoulder and elbow rotations (Zubair et al. 2018), it is the movement in the wrist that orients the paw so that it accurately

lands on a crosspiece (Beloozerova et al. 2010). Visual information for such wrist orientation during reaching is believed to be conveyed via the cerebellum (Glickstein et al. 1998, 2005; Sultan and Glickstein 2007).

VL Contribution Differs for Fast- and Slow-Conducting PTNs

The differential contribution of the VL to the activity of motor cortex neurons related to different segments of the forelimb could in itself result in a difference in the contribution to the activity of fast- and slow-conducting PTNs because it is known that fast-conducting PTNs primarily influence spinal networks related to distal segments of the limb whereas slow-conducting PTNs do not have a preference (Brookhart 1952; Wiesendanger 1981). Thus, one could have expected that fast-conducting PTNs receive a VL contribution that is similar to that of the wrist/paw-related cells whereas slow-conducting PTNs receive a contribution more similar to that of the shoulder- and elbow-related groups. However, our data showed differently. We found that fast- and slow-conducting PTNs do receive different VL contributions but not as hypothesized above. During flat surface locomotion, unlike the wrist/paw-related cells, which receive very little excitatory contribution from the VL during this task, fast-conducting PTNs as a group receive a solid contribution that results in an ~5 spikes/s increase in their discharge (Fig. 11*A2*). At the same time, the contribution to the activity of the slow-conducting PTNs is less than half of this, increasing discharge of this population by ~2 spikes/s only (Fig. 11*B2*). Since the VL contribution to the activity of fast-conducting PTNs represents 30–40% of the activity of these neurons during most phases of the stride (Fig. 11*D2*), whereas the contribution to the activity of slow-conducting PTNs is very variable (Fig. 11*E2*), we concluded that the observed difference in the average discharge rate between fast- and slow-conducting PTNs on the flat surface is to a large extent due to a much larger contribution of the VL to the activity of fast-conducting PTNs.

The VL also contributes differently to responses of fast- and slow-conducting PTN groups during visually guided locomotion on the ladder. Upon transition from the flat surface to the ladder, the contribution increases for slow-conducting PTNs and decreases for fast-conducting PTNs (Fig. 11, *A*, *B*, *D*, and *E*). On the ladder, the VL contribution accounts for over 50% of the activity of slow-conducting PTNs, while contributing less than one-third to the activity of fast-conducting PTNs (Fig. 11, *D* and *E*). As noted above, we have previously found that upon transition from flat surface to ladder locomotion the slow-conducting PTNs of motor cortex change their activity more than fast-conducting PTNs (Stout and Beloozerova 2013). Results of this study suggest that to a large extent this is the input from the VL that drives them.

Overall, the uniqueness of responses of the fast- and slow-conducting PTNs compared with groups of neurons with somatosensory receptive fields on distal versus proximal segments of the limb suggests that these two PTN groups have distinct functions during locomotion that are related to dissimilarities in their properties other than the preference in the target limb segment.

The fact that neurons without identified projection of the axon, noIDs, received the VL contribution during ladder locomotion largely in opposite phases of the stride compared with those of the PTNs (Fig. 11, *C4* vs. *A4* and *B4*) suggests that some of those noIDs might be inhibitory interneurons that invert the VL signal for the PTNs.

Concluding Remarks

When discussing results of this study, some of my colleagues asked how it could be that the activity of motor cortex changes substantially during inactivation in the VL while locomotor behavior shows only very minor changes. This is a very important question, but it is outside the scope of this study. Our goal was to elucidate the contribution of the VL to the activity of motor cortical neurons, and investigation of motor effects of cortical output during VL inactivation should be a topic for separate study. There is at least one other situation when the activity of motor cortex changes without a motor effect, which is during preparation for a reaching movement, and the study by Kaufman and colleagues (2014) offers a possible explanation for this latter phenomenon.

Limitations of this study are mainly related to a relatively small number of neurons tested ($n = 35$). This neuronal sample allowed us to reveal the major features of the VL contribution to the activity of motor cortex during locomotion such as the complex excitatory/inhibitory nature of the contribution, its fluctuation over the step cycle, dissimilarity during vision-independent and vision-dependent locomotion, and the specificity in respect to cortical networks related to different segments of the limb and cortico-spinal efferent systems. A larger sample, however, would allow a more accurate profiling of the contribution to the specific subpopulations of neurons (Fig. 10 and Fig. 11).

This is the first study to characterize the stride phase-, limb segment-, and cortico-spinal projection-specific contribution of inputs from the VL to the activity of motor cortex neurons during vision-independent and vision-dependent locomotion. These data allow for a better understanding of how the activity of motor cortex that is critical for guiding strides on a complex terrain is formed.

APPENDIX: ACTIVITY OF MOTOR CORTEX NEURONS IN NORMAL CONDITIONS

When the cat was standing, all neurons were active. The activity varied between 0.9 and 37 spikes/s and was 12.3 ± 8.2 spikes/s on average. It was similar among groups of neurons with receptive fields on different segments of the forelimb ($P > 0.05$, *t* test) but tended to be higher in the fast- than the slow-conducting PTNs ($P = 0.07$, *t* test).

During locomotion on the flat surface, the average activity of neurons was 13.7 ± 10.1 spikes/s, similar to that during standing ($P > 0.05$, *t* test). The activity of 89% (32/36) of neurons was modulated in the rhythm of strides. The majority of neurons (59%, 19/32) had a single PEF, and 41% (13/32) had two PEFs. The dM was 9.0 ± 4.2, similar between one- and two-PEF populations. In both groups, PEFs were distributed evenly across the step cycle (Supplemental Fig. S2A1). The average duration of the PEF was 60 ± 15% of the cycle. Because of a relatively long duration of the PEFs and their wide distribution across the step cycle, PEFs of different neurons overlapped, and between 55% and 75% of cells were simultaneously active in most phases of the cycle (Supplemental Fig. S2B1). However, neurons were slightly more active during the swing than stance phase (Supplemental Fig. S2A2), and therefore the entire population

was slightly more active during the swing than stance phase ($P = 0.007$, U test; Supplemental Fig. S2B2).

Groups of neurons with receptive fields on different segments of the forelimb were preferentially active during different phases of the stride (Supplemental Fig. S3, A1 and A2 through C1 and C2). The shoulder- and elbow-related groups were most active during the end of stance and beginning of the swing phase (Supplemental Fig. S3, A1 and A2 and B1 and B2), whereas the wrist/paw-related neurons were most active at the end of the swing phase (Supplemental Fig. S3, C1 and C2). The average activities of these groups were similar at 13.0 ± 3.9, 16.0 ± 17.8, and 10.2 ± 6.0 spikes/s, respectively ($P > 0.05$, U test). The activity of the fast-conducting PTNs, however, was more than double of that of the slow-conducting PTNs (18.0 ± 12.1 vs. 6.9 ± 7.1; $P = 0.009$, U test; Supplemental Fig. S4, A1 and A2 and B1 and B2). It was also more modulated than the activity of either the slow-conducting PTNs or neurons without identified projections of the axon (noIDs; Supplemental Fig. S4, A1 and A2 vs. B1 and B2 and C1 and C2).

Upon transition from locomotion on the flat surface to accurate stepping on the horizontal ladder, 89% (31/35) of neurons changed their activity, typically in two or three aspects. The mean discharge rate of 60% (21/35) of neurons changed, increasing in 13 cells and decreasing in 8 cells ($P < 0.05$, t test). The dM changed in 54% (19/35) of neurons, increasing in 8 cells and decreasing in 11. The number of PEFs changed in nine cells: six neurons that had two PEFs per stride during flat surface locomotion had only one PEF during ladder locomotion, whereas the pattern of the modulation of three cells changed in the opposite direction. Duration of the PEF changed in eight neurons, typically by shortening for ladder locomotion. Changes in the preferred phase of the activity were seen least frequently, as of 15 cells that had one PEF during both locomotor tasks, and thus could be evaluated for the PrPh, 10 had the same PrPh during both tasks. Despite significant differences in the activity between flat surface and ladder locomotion displayed by the great majority of neurons, for the entire population the average discharge rate at 15.4 ± 8.6 spikes/s, the dM at 9.7 ± 4.2, and the duration of PEF at 59 ± 20% were similar between the tasks ($P > 0.05$, U test). This similarity was due to a roughly equal number of neurons changing activity in opposing ways between the tasks. The average discharge frequency during the stance phase of the ladder task was, however, slightly higher, by 3 spikes/s ($P < 0.0001$, U test; Supplemental Fig. S2, A and B).

Groups of neurons with receptive fields on different segments of the forelimb reacted differently to the accuracy demand of the ladder. Shoulder-related cells showed only a modest response, slightly decreasing their activity during the late swing phase and increasing it during late stance phase (Supplemental Fig. S3, A1–A4). The activity of the elbow-related group changed substantially by developing a peak during the transition from the swing to stance phase (Supplemental Fig. S3, B1–B4). At variance, the activity of the wrist/paw-related group increased in the beginning of the swing and throughout the stance phases, becoming more evenly distributed over the cycle (Supplemental Fig. S3, C1–C4). The average discharge rates of neuronal groups did not change, however, staying at 13.0 ± 5.4, 19.0 ± 12.8, and 14.1 ± 4.4 spikes/s for the shoulder-, elbow-, and wrist/paw-related groups, respectively ($P > 0.05$, U test). The average discharge rate of fast-conducting PTNs also did not change, and, similarly to that of wrist/paw-related neurons, their group activity became more evenly distributed over the step cycle (Supplemental Fig. S4, A1–A4). In contrast, the activity of the slow-conducting PTN group tended to increase (to 10.1 ± 6.3 spikes/s) and became step cycle modulated (Supplemental Fig. S4, B1–B4). The average activity of the group of noID neurons did not change but became more modulated, showing two peaks: one during the swing and one during the stance phase (Supplemental Fig. S4, C1–C4).

These population activity characteristics are consistent with previously reported data (Beloozerova et al. 2010; Beloozerova and Sirota 1993a; Farrell et al. 2014, 2015; Stout and Beloozerova 2012, 2013), thus suggesting that the 35 neurons whose responses to application of CNQX in the VL are reported here are a representative group.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

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