# Cortical and Thalamic Contribution to Visual and Somatosensory Control of Locomotion in the Cat

by

Wijitha Udayalal Nilaweera

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved March 2016 by the
Graduate Supervisory Committee:

Irina Beloozerova, Co-Chair
Brian Smith, Co-Chair
Natalia Dounskaia
Eric Vu

ARIZONA STATE UNIVERSITY

May 2016

ABSTRACT

Navigation through natural environments requires continuous sensory guidance. In addition to coordinated muscle contractions of the limbs that are controlled by spinal cord, equilibrium, body weight bearing and transfer, and avoidance of obstacles all have to happen while locomotion is in progress and these are controlled by the supraspinal centers.

For successful locomotion, animals require visual and somatosensory information. Even though a number of supraspinal centers receive both in varying degrees, processing this information at different levels of the central nervous system, especially their contribution to visuo-motor and sensory-motor integration during locomotion is poorly understood.

This dissertation investigates the patterns of neuronal activity in three areas of the forebrain in the cat performing different locomotor tasks to elucidate involvement of these areas in processing of visual and somatosensory information related to locomotion. In three studies, animals performed two contrasting locomotor tasks in each and the neuronal activities were analyzed.

In the first study, cats walked in either complete darkness or in an illuminated room while the neuronal activity of the motor cortex was recorded. This study revealed that the neuronal discharge patterns in the motor cortex were significantly different between the two illumination conditions. The mean discharge rates, modulation, and other variables were significantly different in 49% of the neurons. This suggests a contextual correlation between the motor cortical activity and being able to see.

In two other studies, the activities of neurons of either the somatosensory cortex (SI) or ventrolateral thalamus (VL) were recorded while cats walked on a flat surface (simple locomotion) or along a horizontal ladder where continuous visual and somatosensory feedback was required (complex locomotion).

We found that the activity of all but one SI cells with receptive fields on the sole peaked before the foot touched the ground: predictably. Other cells showed various patterns of modulation, which differed between simple and complex locomotion. We discuss the predictive and reflective functionality of the SI in cyclical sensory-motor events such as locomotion.

We found that neuronal discharges in the VL were modulated to the stride cycle resembling patterns observed in the cortex that receives direct inputs from the VL. The modulation was stronger during walking on the ladder revealing VL's contribution to locomotion-related activity of the cortex during precision stepping.

ACKNOWLEDGMENTS

Throughout the years I had many mentors, advisors, and guiding counselors, who helped me to progress through my education and career. Because of their vision and understanding I was able to reach my goals and realize my dreams. I was fortunate to join Dr. Irina Beloozerova's laboratory at Barrow Neurological Institute (BNI) in Phoenix, Arizona to conduct research in neurophysiology of locomotion. Before long it became clear to me how exciting the work in the lab was. Dr. Beloozerova helped me, guided me, and taught me to become a successful researcher. She also encouraged me to enroll in the neuroscience graduate program jointly offered by BNI and Arizona State University. She became not only my supervisor at work but also a co-chair of my dissertation committee. First and foremost I am thankful to Dr. Beloozerova for her kindness, persistent support, and guidance.

I am deeply grateful to Dr. Eric Vu at BNI for his continuous support during my academic years. I am also very thankful to Dr. Brian Smith for agreeing to serve as a co-chair of my dissertation committee, without whom this neuroscience graduate program at ASU may have not been possible. Dr. Natalia Dounskaia, I am so grateful to you for your help during my graduate years and being a member of my dissertation committee. I cannot forget at this time the enormous support and guidance provided by Dr. Alan Gibson, the Chair of Division of Neurobiology at BNI during years of my employment at the institute.

I also would like to acknowledge Dr. Mikhail Sirota at BNI for his guidance during my tenure in the laboratory. I also would like to thank Mr. Peter Wettenstein, Dr. Vladimir Marlinski, Ms. Madison Armer, Dr. Trevor Rivers, Dr. Eric Stout, at BNI and Dr. Oleg Favorov at University of North Carolina, all with whom I worked closely during

my years at BNI and without whom this work may have not become a reality. I deeply appreciate the current members as well as the veterans of Dr. Beloozerova's lab for their help and encouragement. In addition, I will never forget the support of Wendi Simonson, Beverly McBride, and Yuan Delgado at the School of Life Sciences at ASU.

Thank you my dear wife, Sandhya Kanthi Yaparatna, for helping me at each step of not only in my academic endeavors, but also in my entire life. My family, especially my sister, who never stopped believing in me being able to do great things, needs to be named here as well. My deepest appreciation must also go to the learning and memory laboratory at Iowa State University, especially to Dr. Vlastislav Bracha, who believed in me and accepted me to his lab as a researcher. I learned there systematic experimentation as a novice to neuroscience research.

Thank you my friends in Arizona and Iowa for your encouragements and support throughout my academic years.

I am thankful to BNI foundation for granting me a fellowship toward this graduate program during my first year. The work of this dissertation was partly supported by NIH grant R01 NS-058659 to Dr. Irina Beloozerova.

Specific acknowledgments are given in respective Chapters. The specific contributions of the author of this dissertation include: shared responsibility for planning the dissertation studies with co-chair Dr. Irina Beloozerova; primary responsibility for behavioral training of the animals; shared responsibility for animal surgeries, neuronal and behavioral data collection, and preparation of tissues for histological analysis; primary responsibility for data analysis for Chapters 3 and 4; shared responsibility for data analysis for Chapter 5; and shared responsibility for drafting the publications.

TABLE OF CONTENTS

CHAPTER Page

CHAPTER Page

LIST OF FIGURES

# 1. INTRODUCTION

## Locomotion as a Fundamental Necessity of Animals

Locomotion is one of the most significant features of the animal world and a fundamental necessity for the vast majority of species including humans (Dickinson et al., 2000; Grillner 1975; Orlovsky et al., 1999). For many types of animals, locomotion is essential for their mere survival. The main reasons for animals to move from one location to another are to find food and water, avoid predators and hostile weather, and find mates (Biewener, 2003). Animals use different means of locomotion. From single-cell organisms to the most complex and largest mammals on Earth such as whales and elephants, animals use movements of their body parts for locomotion. Fish and other water-dwelling beings swim, terrestrial animals use their legs and/or bodies for locomotion, and airborne animals fly. Even though it is a common behavior, locomotion is a complex motor act. The goal of this review is to discuss locomotion of terrestrial animals, especially visually guided locomotion and its control by networks of cortex and thalamus.

Any movement is produced by muscle contraction. The signals for these muscle contractions are generated within the central nervous system (CNS) and sent via the axons of motoneurons to the target muscles. Even though the signals to the muscles are sent from motoneurons located in the spinal cord or brain stem, there are several other CNS structures that contribute in varying degrees to the efficient function of muscles. Modulation of the activity during different movements, including locomotion, was observed in a number of brain areas; and lesions in many of them impair movements (Hultborn, 2006; Stuart and Hultborn, 2008; Kuypers, 1964). Not only do these brain

structures control the “lower level” neuronal networks in the spinal cord, they also receive signals from the peripheral receptors about the status of movements and body’s position relative to the environment.

The simplest movements are the somatic reflexes, which are generally quick, protective movements of the body parts. They are triggered by sensory stimuli: when a strong enough sensory stimulus specific to a particular reflex is applied to the receptor (skin, muscle spindle, tendon, retina, etc.) it triggers contraction of a specific muscle group as a response (rev. in e.g., Hultborn, 2006; Sherrington and Laslett, 1903). For example, when one touches a very hot surface inadvertently, a withdrawal reflex is activated, and the hand is quickly removed from the hot surface to prevent further damage. Similarly, when one goes out to a bright area from a darker place, the pupils of the eyes shrink to protect the retina from damage by the bright light. These reflexes perform necessary adaptations without direct involvement of the higher level structures of CNS. The reflexes are involuntary in nature and are controlled by hardwired neuronal “chain reactions” called reflex arcs. Withdrawal reflexes and deep tendon reflexes have the shortest neuronal arcs and are exhibited by contracting one group of muscles. For example, the knee jerk reflex is elicited by stretching the patellar tendon that in turn excites the quadriceps muscle by the motor neurons in the spinal cord (Renshaw, 1940; Sherrington and Laslett, 1903). The quadriceps muscle contraction and reciprocal inhibition of hamstring muscles cause the limb to straighten. This reflex arc is completed between receptor → spinal cord → and the effector muscles without direct involvement of supraspinal motor structures such as motor cortex. However, it should be noted that the information about the stimulus and triggered reflex activity is sent to the higher levels of CNS. Reflexes are critical to any organism because they are normally protective. They are stereotypic, non-repetitive, and are not rhythmic in nature. The neuronal

communication of the reflexes occurs in the spinal cord or brainstem and involvement of the forebrain is not required for the response.

The other group of simple movements is fixed actions such as sneezing and coughing. These are also stereotypic, unidirectional, typically non-repetitive, but are more complex than reflexes. In contrast, rhythmic motor patterns such as breathing, scratching, and locomotion are stereotyped and repetitive. They are under substantial voluntary control. Directed movements such as reaching to grasp or throwing are more complex than any other movements mentioned above but neither are stereotyped nor strictly repetitive. It has been shown that many types of movements including locomotion can be maintained by the neuronal networks residing in the lower-levels of the CNS such as the spinal cord or brainstem. (Duysens and. Van de Crommert, 1998; Frosberg et al., 1980 a, b; Grillner, S. (2011); Hultborn, and Nielsen, 2007; Shik and Orlovsky, 1976)

## Spinal Cord

Locomotion is a voluntary, rhythmic act of the animal. Even though it is still not clear how exactly locomotion is triggered, it is clear that the spinal cord networks are the ultimate targets of the descending commands arising from the supraspinal centers. It is also clear that to produce locomotion the spinal cord motor neurons propagate well-modulated neuronal signals to the appropriate muscles. It had been shown that locomotion-like rhythmic movements can be maintained by the spinal cord (Frosberg et. al, 1980a, b; Grillner, 1973, 2011; Grillner and Zangger 1979; Shik and Orlovsky, 1976). In other words, locomotor pattern does not require descending supraspinal signals for rhythmic locomotor patterns to occur and the spinal cord alone can maintain the locomotor patterns when the right conditions are met (see below). The spinal cord

networks that enable locomotor rhythmic patterns are called central pattern generators (CPGs). During locomotion, the CPGs operate in such a harmony so that each muscle group contracts at a precisely right moment while the opposite muscles relax for the body to move smoothly.

The spinal cord alone cannot carry out locomotion successfully in a natural terrain, however, because it requires much more than simply making rhythmic limb movements. During natural locomotion maintaining equilibrium, bearing body weight, transferring body weight from one side to the other, assessing the postural relationship with the surrounding environment, and evaluating and avoiding obstacles must happen in parallel while locomotion is in progression. Several supraspinal locomotor centers acquire, analyze, and integrate the signals from the surroundings to provide necessary information to the spinal cord for smooth locomotion in natural environments.

It was evident as early as at the dawn of the 20$^{th}$ century that mammals (the cat was the most popular test subject) could walk on a moving treadmill belt after the forebrain is separated from the spinal cord, hence after removing the inputs from the cerebral cortex and other supraspinal structures to the spinal cord (Sherrington, 1910a, b). Even after the spinal cord of the cat is transected at the mid-thoracic level, the cat's hind limbs can maintain locomotion-like rhythmic movements on a running treadmill (Grillner & Rossignol, 1978; Grillner and Zangger, 1984). These animals also adjust the walking speed to the speed of the treadmill. If high spinal transection is performed (at upper or mid-cervical levels), all hind and fore limbs would move rhythmically and synchronously (Miller & Van der Meche, 1976). However, to initiate these movements by the spinal cord, appropriate conditions should be met: the spinal cord needs to be stimulated, physically, electrically or pharmacologically (Forssberg et al. 1980a, b; Grillner, 1973; Wallen & Williams, 1984).The first report that showed the ability to walk

after the spinal cord was transected at the brain stem in the cat and dog was the work of C. S. Sherrington in as early as 1910 (Sherrington, 1910a, b). Sherrington thought that the spinal cord-dependent locomotion was due the functions of a chain of reflexes triggered by the sensory inputs. Graham Brown (Brown, 1911) being agreed with Sherrington about independent functional capability of the spinal cord disagreed with the notion about reflex chain. He showed that even after removing the sensory afferents from the limb muscles to the spinal cord (deafferentation) and transecting the spinal cord at T12 level, the animals were still able to produce locomotion-like rhythmic movements with their hind limbs. Since then it has been shown many times, in many animals that the spinal cord CPGs can function without afferent inputs from either supraspinal centers or peripheral sensory receptors (Forssberg et al. 1980a, b; Grillner, 1975; Grillner & Zangger, 1979; Miller et al., 1976). Surprisingly, it appears that CPGs' function across different species is similar (Duysens et. al., 1998; Pearson, 1993) regardless of the vast differences in animals' locomotor patterns that range from swimming to hopping, to walking, to flying. Moreover, the spinal cord that is completely isolated from all inputs, i.e. the supraspinal inputs, inputs from the peripheral sensory appendages, is capable of producing rhythmic patterns by its motor neurons (Chrachri and Clarac, 1990; Dubuc et al., 2008; Sirota et al., 2000). This is called the fictive locomotion. However, whether it is in vivo or in vitro, for the isolated spinal cord to produce locomotion-like movements, the CPG areas of the spinal cord need to be manipulated (stimulated) with an appropriate electrical current or pharmacological agent or agitate the limbs manually, without which the system would not sustain the rhythmicity or may even completely fail to function.

Despite these capabilities of the spinal cord, it cannot navigate the body in the natural environment because the spinal cord does not receive distant information and

therefore cannot avoid obstacles in the natural environment. Analysis and integration of sensory information to produce appropriate locomotor signals to the spinal cord is the function of supraspinal centers. What are the supraspinal locomotor centers that could analyze and/or integrate this information? In this review I will briefly discuss some of the supraspinal structures that are known to be involved in control of locomotion. I will focus on the motor and somatosensory cortex, thalamus, and cerebellum.

This dissertation contains 3 studies that I have conducted in the cat to better understand the activities of three critical areas of the forebrain that are involved in locomotion: the motor cortex, somatosensory cortex, and motor thalamus- the ventrolateral thalamus.

I would like to note that parts of these studies were presented in abstract form at international neuroscience meetings (Armer et al., 2011; Favorov et al., 2010; cerebellar poster) and also are published in peer reviewed journals (Armer et al., 2013; Favorov et al., 2015; Marlinski et al., 2012). I present the journal articles mostly unchanged (the text is changed minimally but the figures have been repositioned to suit the format of this dissertation.)

## Motor Cortex

The motor cortex and related areas of the cerebral cortex are closely involved in voluntary movements including locomotion (Porter and Lemon, 1993; Armstrong and Drew 1984; Beloozerova and Sirota 1993a, b). Electrophysiological studies have revealed that regions of the motor cortex are active during performance of motor tasks. It is well known that people with injuries to specific areas of the motor cortex due to cerebrovascular accidents or other causes such as mechanical injury suffer from different motor deficiencies. Some of more pronounced deficiencies are dragging the leg along the

ground, changes of limb trajectories, and interlimb discoordination (Mayer et al., 1997). In experimental animals, removal of or lesions to the motor cortex or lesions to the pyramidal tract lead to motor deficiencies (Adkins et al., 1971; Chambers and Liu, 1957, Cambers et al., 1966; Courtine et al., 2005, Drew et al., 2002; Eidelberg and Yu 1981; Eidelberg et al., 1981). It had been shown that cats are unable to walk on a wire mesh or elevated horizontal bar after cooling of the motor cortex or lesions to it, or to the pyramidal tract (Trendelenburg, 1911). Cats were unable to walk on a horizontal ladder after bilateral section of the pyramidal track (Liddell and Phillips, 1944): cats overstepped the rungs instead of stepping on tops because they lost the ability of precise placement of the limbs. A similar study conducted by Beloozerova and Sirota (1993a) reported while bilateral motor cortical lesions or its inactivation by cooling or microinjections of tetrodotoxin, a potent sodium channel blocker, did not affect cats locomotion on a flat surface, uphill walking, or even with weights on both forelimbs. However, it did abolish their ability to walk on a horizontal ladder or accurately overstep obstacles. Similar results have been reported for kittens when they were continuously injected with muscimol, a GABA receptor agonist, in the motor cortex during a critical period of their development (Friel et al., 2007; Martin et al., 2007).

Electrophysiological recordings in cats and monkeys revealed that the activity of motor cortical neurons is step-modulated: meaning that neurons are more active during one phase of the stride cycle and less active during another phase (Armstrong and Drew 1984a, b; Beloozerova and Sirota 1985a, b; 1993a, b; Widajewicz et al. 1994; Drew 1988, 1993; Fitzsimmons et al., 2009). An important study conducted by Beloozerova and Sirota (1993a) showed increasing modulation of the activity in the motor cortex in the cat when requirements for precision limb placement increased. Cats were trained to overstep barriers and the motor cortex activity was recorded. When the gap between the

barriers became smaller (from 25 to 12 to 6 cm), thus forcing the animals to make more precise foot placements, the modulation of the cortical activity became stronger. Amos, Armstrong, & Marple-Horvat (1990) also have shown substantially increasing neuronal activity in motor cortex when cats walked on a horizontal ladder. Motor cortical cell activity also has increased in the cats when unexpected perturbations occurred while walking on a treadmill (Armstrong and Drew 1984a, b; Drew 1993; Widajewicz et. al. 1994, Marple-Horvat et al., 1993).

The motor cortex conveys motor related information to the spinal cord via the pyramidal tract. When the medullary pyramid was electrically stimulated in decerebrate cats within specific parameters of the electrical current and impulse frequency, the rhythmic stepping patterns could be initiated (Orlovsky, 1972). This indicates that the descending corticospinal inputs have a major influence on the function of spinal cord CPGs. Electrical stimulation of the motor cortex also produced responses in the limb muscles demonstrating that the motor cortex actually influences the muscle activity in the limbs (Armstrong and Drew, 1985 a, b) via spinal cord.

Most investigations studied the functional relationship of the motor cortex with movements, but not the visual information processing. There are direct and indirect anatomical connections between the visual and motor areas of the cerebral cortex, potentially conducting visual information from visual areas to the pre-motor, supplementary, and motor cortical areas (Andujar and Drew, 2007; Babb et al., 1984; Symonds and Rosenquist, 1984). Indeed, several studies had found that the motor cortex responds to visual information, suggesting the motor cortex may play a role in integrating visual information for performance of movements (Garcia-Rill and Durovsky, 1974; Mushiake et al., 1991; Weyand et al., 1999).

We wanted to understand how neurons in the motor cortex react to the level of illumination during locomotion. We recorded the activity of motor cortical neurons in cats walking on a flat surface in two different illumination conditions: in a fully illuminated room and in a completely dark room. We found that the neurons fired differently in these illumination conditions. This study was published in Behavioural Brain Research Journal and forms chapter 3 of this dissertation (Armer et al, 2013).

## Somatosensory Cortex

Effective locomotion is impossible without supraspinal contribution. Acquisition and analysis of environmental cues as well as planning and execution of the locomotion-related signal are major functions of these centers. In their natural environments animals adjust their limb placements according to visual information and characteristics of the walking terrain. The somatosensory cortex allows animals to acquire information about the outside world as touch, vibration, pain, temperature, body movement and so forth (Kaas and Pons 1988, Kaas 1990, 1993).The parietal cortex consists of primary somatosensory area (SI), which includes Brodmann's areas 3a, 3b, 1, and 2, and posterior parietal cortex (areas 5 and 7). These are in the center of my discussion. The other major subdivisions are parietal operculum (area 43), inferior parietal lobule including supramarginal gyrus (area 40), and the angular gyrus (area 39). The parietal cortex receives all modalities of sensory information such as somatic, visual, and auditory, except olfaction.

The primary somatosensory cortex (SI) receives projections from several different parts of the central nervous system. Somatosensory information from the skin, muscles, and joints are conveyed via thalamic nuclei, the ventroposterior nucleus being

the largest (Kaas et al. 1984). The ventroposterior nucleus projects to areas 3b and 1 while the ventroposterior superior nucleus projects to areas 3a and 2. Other thalamic nuclei project to different areas of the SI and secondary somatosensory cortex. Afferentations to subdivisions of SI are organized in a hierarchical manner (Felleman and Van Essen, 1991; Iwamura, 1998). Lesions in areas 3a and 3b "inactivate" areas 1 and 2. Area 3b projects to area 1 and it projects to area 2 (Garraghty et al. 1990a, b). With this transition, the size of the receptive fields becomes larger in the higher levels in the hierarchical system. More complex properties such as center-surround effects and direction-selectivity are evident in the higher levels of the hierarchy (Iwamura et al. 1993). Primary somatosensory cortex selectively projects to different areas of the CNS including the motor cortex, with areas 3a and 2 being the largest contributors, pre-motor and supplementary motor areas, posterior parietal areas 5 and 7, brainstem, spinal cord, and contralateral somatosensory cortex (rev. in Kaas, 1993). These connections are reciprocal.

Similar to the motor cortex and primary somatosensory cortex, the posterior parietal cortex (PPC) is critically involved in locomotion and also involved in motor planning and sensory motor integration. Neuronal activity in this cortical area, especially area 5 is increased by the somatosensory stimuli (Pons and Kaas, 1985; Pons et al., 1985) and modulated to the gait cycle. This modulation dramatically changes when it requires precision foot placement involving visual attention (Andujar et al. 2010; Beloozerova and Sirota, 2003; Drew and Marigold, 2015). In an overstepping task in cats, peak activity of some area 5 neurons were observed 2-3 steps before the animal actually overstepped, and continued to maintain high level of activity until the hind limbs cleared the obstacle (Marigold et al., 2011). It is hypothesized that the effect is due to planning to avoid the obstacle rather than the stimulation by afferent visual information about the ongoing

locomotion. The cats were able to see the obstacle several steps, not 2-3 steps, before taking real action. Also lesions to this area caused animals to misstep. When the visual input was interrupted for a short period of time, it did not affect the activity patterns (Marigold and Drew, 2011). It has been postulated that the area 5 process the information concerning the temporal relation between the animal and obstacle. However results of one of our studies indicate that area 5 in cats does not convey this information to the motor cortex despite the fact that the two areas are closely interconnected (Beloozerova et al., 2011). Further studies need to be conducted to better understand the function of the posterior parietal cortex during locomotion.

## Thalamus

The thalamus is a complex, paired organ located in the center of the brain. The thalamus is comprised of many nuclei, most of which receive sensory information and convey it to the sensory-motor cortex and other cortical and subcortical targets. One of the major afferents to the thalamus is the cerebellum. Ventrobasal and ventrolateral thalamic nuclei that receive inputs from cerebellar nuclei function as intermediary to the sensory and motor information.

The thalamic ventrolateral and ventroanterior nuclear complex (VL-VA), defined functionally as the motor thalamus, is the main source of subcortical afferent pathways to the sensory-motor cortex (Strick, 1973; Larsen and Asanuma, 1979; Fang et al., 2006). According to cytoarchitectonic features and afferent connections, this complex is divided into anterior and posterior compartments (Asanuma et al 1983; Ilinsky and Kultas-Ilinsky 1984). The anterior part (the VA) receives its primary input from the basal ganglia, whereas the major projections to the posterior part (the VL) originate in the interposed and dentate nuclei of the cerebellum (Evrard and Craig, 2008; Ilinsky and

Kultas-Ilinsky, 1984; Na et al., 1997; Nakano et al., 1980; Rinvik and Grofová 1974; Steriade, 1995). It also receives inputs form the spinal cord (Craig 2008; Mackel et al., 1992; Yen et al., 1991). Lesions in the VL cause ataxia and deficits in purposeful motor performances (Fabre-Thorpe and Levesque, 1991; Solomon et al., 1994). VL neurons change their activity in parallel with the activity of cerebellar cortex prior to conditioned or self-generated voluntary movements (Evarts 1971; Strick 1976; Neafsey et al., 1978; Schmied et al., 1979; van Donkelaar et al., 1999; Kurata, 2005).

As noted above, it has been shown in decerebrate animals that the brainstem nuclei-vestibular nucleus, red nucleus, and reticular formation-projecting directly to the spinal cord could maintain step-related modulation only if the cerebellum was intact (Orlovski 1970, 1972a, b). The motor cortex does not receive direct inputs from the cerebellum, however, the cerebellum is a major afferent to the VL thalamus which conveys information directly to the motor cortex (Na et al., 1997; Strick, 1973). The VL thalamus is essential for normal performance of locomotor behaviors that require an accurate foot placement. After the VL thalamus was lesioned, cats were able to maintain walking on flat surface with even different loadings and gait variations but could not accomplish any locomotion task that required precise positioning of paws on the surface (Beloozerova and Sirota, 1988, 1998). Moreover, locomotion-related activity of motor cortical cells in these animals became sparse or changed dramatically so that most neurons lost their step-related modulation. It was concluded that the VL contributes to integration of sensory (including visual) and motor information during locomotion, or, at the very least, transmits integrated sensory-motor information that is necessary for an appropriate gait modifications from cerebellum to sensory-motor cortex. Previous lesion studies also have demonstrated that important information is conveyed to the motor

cortex via the VL thalamus during locomotion (Fabre-Thorpe and Levesque, 1991; Solomon et al., 1994).

However, until recently there was no data on the activity of neurons in the VL thalamus during locomotion. Therefore, it was unknown what type of information is conveyed. From Dr. Beloozerova's preliminary studies, it was only known that during locomotion the activity of VL is modulated in the step cycle and depends on whether or not visuo-motor coordination of locomotion is required (Beloozerova and Sirota 2002). In this dissertation, neuronal mechanism of integration of motor and visual information in the VL was investigated in detail and is presented in chapter 5.

## Cerebellum

The cerebellum provides the main input to the ventrolateral thalamus. There is an unambiguous consensus that the cerebellum is vital for motor control, including visuo-motor and sensory-motor coordination during locomotion. Removal of the cerebellum or its partial lesions results in motor deficits with a "discoordination" being a most vivid symptom. Signals from cerebellar outputs reach all motor centers including spinal cord, cerebral cortex via motor thalamus, red nucleus, vestibular nuclei, and reticular formation. The cerebellum receives signals from all motor centers and also signals from limb proprioceptors, and the spinal locomotor generators. The cerebellum also receives visual information via several structures in the brain stem (Brodal, 1979; Glickstein et al. 1994). Stimulation of the cerebellum evokes motor responses and the activities of neurons in cerebellum correlate with movements. Therefore one of the best candidates of the subcortical structures that receive sufficient visual information and also related to locomotor activities is the cerebellum (Bloedel, 1992., Brooks, 1995., Stein and Glickstein 1992, Glickstein et al., 1998, Thach et al., 1992).

Nearly 200 years ago (in 1824) Florence reported that after removal of cerebellum animals were "grossly dis-coordinated" (in Fulton & Dow, 1937), however, they still could walk. Therefore Florence concluded that the cerebellum is not necessary for producing locomotor movements but plays a role in coordination. Later Babinski (1901) (in Stein and Glickstein, 1992) confirmed this by concluding that the deficits in patients with cerebellar lesions are, in fact, due to lack of coordination. Sherrington (1906) emphasized the importance of cerebellum in movements by suggesting that it was the "head ganglion" of the proprioceptive system. A few years later Holms (1917, 1939) discovered that disturbance in visual processing also contributes to the loss of coordination in movements by showing that cerebellar lesions affect visually-guided movements. This probably was the first report that suggested the importance of the cerebellum in visuo-motor coordination of movements. In 1955, William Chambers and James Sprague (1955a, b) published two articles describing series of investigations on the effects of cerebellar lesions in the cat. They performed systematic lesions in the vermis, paravermal region, or lateral cerebellar hemispheres with or without the corresponding deep nuclei, and recorded changes in behaviors short-term (few days) or long-term (for up to 2 years). They concluded that the cerebellum plays a critical role in visually guided locomotion.

In the 1970s a group of scientists from Moscow, Russia (Shik, Arshavsky, Orlovsky, Gelfand and others), reported important findings about the cerebellum and also the brain stem nuclei related to locomotion. Orlovsky (1972b) reported that thalamic cats, that is, cats decerebrate at the level of the thalamus, which were capable of producing rhythmic locomotor movements, had stride-related rhythmic activity in the reticulo-spinal neurons only if their cerebellum was intact. Furthermore, Orlovsky (1972a, b) demonstrated that the activities of two other major nuclei in the brainstem; the

vestibulo-spinal nucleus and the red nucleus are well-modulated to the step cycle only in the presence of the cerebellum. Therefore, it was concluded that the locomotion-related spike activities of the brainstem nuclei, that in turn influence the spinal cord neurons, are under control of the cerebellum. All these nuclei receive direct inputs from the cerebellum. Both reticular nucleus and the vestibular nucleus receive either direct Pukinje cell input or input from the fastigial nucleus of the cerebellum. The red nucleus receives monosynaptic excitatory input from the interposed nucleus of the cerebellum.

When neuronal activities of the deep cerebellar nuclei were recorded in the intact animals during locomotion, it was found that the neuronal discharges were stride-cycle modulated (Armstrong and Edgly, 1984; Marple-Horvat and Criado, 1999), and the activity patterns were different when the precision stepping was required (Armstrong and Marple-Horvat, 1996). In our preliminary studies, we found that when cats walked on a flat surface and a horizontal ladder, the discharge patterns of both the interposed and dentate nuclei were stride-modulated. However, the characteristics of the activities recorded from the two nuclei were different (Nilaweera and Beloozerova, 2009). Namely, upon transition from flat surface walking to accurate stepping on horizontal ladder, the activity of the interposed nucleus increased mainly in the average rate. In contrast, the lateral nuclear activity changed mainly in the depth of its stride-related modulation, which became stronger during accurate stepping on the ladder.

# 2. MATERIALS AND METHODS

The cat was used as a subject for the studies (male and female adult animals) because it is a classic animal for research in locomotion. All experiments were conducted in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals and upon approval by the Barrow Neurological Institute's Animal Care and Use Committee. All experiments were conducted in Dr. Irina Beloozerova's laboratory at the Barrow Neurological Institute.

## Animal Training

Positive reinforcement (food) was used to habituate cats to the experimental situation and engage them in locomotor behavior (Pryor, 1975; Skinner, 1938). At the beginning of training, an animal was food restricted to 50% of normal ration for two days. It was then taken to a quiet empty room where small amounts of food were given simultaneously with a buzzer sound. Three 15 min training sessions per day were conducted for three days. During training period and throughout the time of experiments, the cat's weight was monitored and maintained within 80-120% of its original value.

When the cat showed a clear association between the sound and food, the sound was used to train the cat to walk around the chamber. Training was started with the cat moving gradually further away from the food dish, and continued until the cat walked to the end of one corridor and came back along the other side. At this time, a five second pause was introduced between cat's arrival to the food dish and dispersal of food. The cat had to stand on all four limbs to receive the food reward. During recording experiments, the neuronal activity during one second in the middle of this period was used to assess the activity of neurons during "standing". When the animal walked confidently on the

flat surface, it was trained to walk on tops of crosspiece of a horizontal ladder. We first used very wide (18 cm) crosspieces, and then gradually decreased their width. Crosspieces of all ladders were 25 cm apart, which was empirically established to be a half of an adult cat's stride length. The final width of the crosspieces was 5 cm, which was 2 cm larger than the size of the cat's paw.

Once the cats were well-trained to walk on the horizontal ladder they were trained to wear a "sock" on one or more of the limbs. This sock was used for recording of footsteps. Cats were also trained to carry a communication cable that during experiments connected the electronics on the cat with data acquisition system.

Cat Walking Chamber

Experiments were conducted in a rectangular walking chamber that was 2.5 m long, 0.6 m wide and 0.4 m deep. The chamber was placed about 0.8 m above the ground. It was divided into two corridors by a divider that runs in the middle of the chamber (2.1). During experiments, cats walked away from the food dish along one corridor and came back along the other one. To monitor cats entering and exiting the sections of the corridors, infrared detectors were mounted within the chamber. The chamber's floor was covered with an electroconductive material and so were the stepping surfaces of the ladder's crosspieces. During walking, the foot contact with the floor was recorded by means of an elecromechanical sensor attached to the sock on the foot.

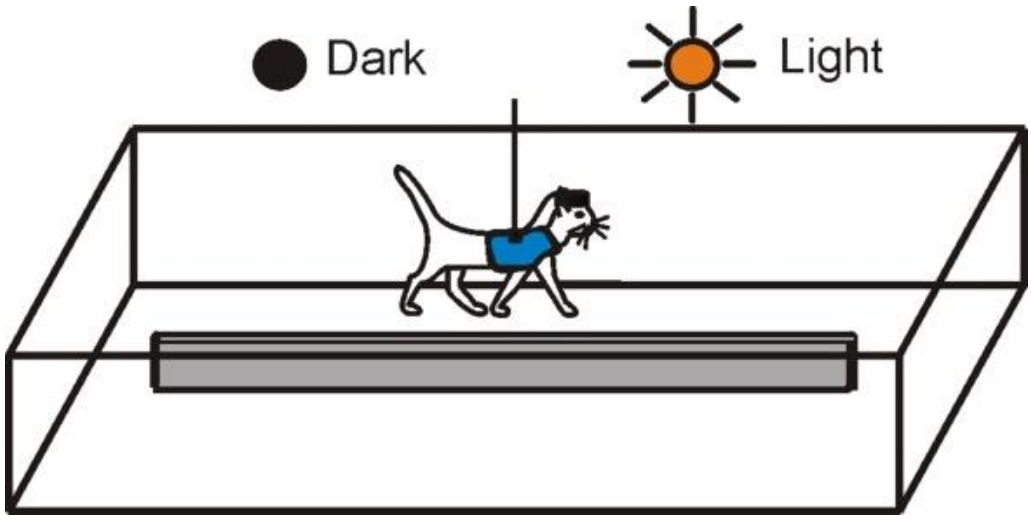


**Fig. 2.1:** Cat walking chamber. Cats walked continuously in the chamber along the two pathways during selected experiments a ladder was positioned in one of the corridors. In the light/dark study (chapter 3) the lights were turned off as the cats walked along the straight portion of the path.

Surgical Procedures

When the cat was well accustomed to the experimental environment and consistently walked in the experimental chamber, a surgery was performed to prepare the animal for extracellular recording experiments. The surgery was conducted under aseptic conditions and isoflurane was used as the method of anesthesia. The surgical procedures followed previously described methods used by Dr. Beloozerova's laboratory (Beloozerova and Sirota, 1993a; Prilutsky et al. 2005).

Once the animal was under anesthesia, its head was fixed in a stereotaxic device, and the skin over the skull was thoroughly cleaned with Betadine followed by 70% ethyl alcohol. Eyes of the animal were protected from drying with a lubricant eye ointment. Body temperature, pulse rate, and the oxygen saturation were monitored throughout the surgery. Normal body temperature was maintained by keeping the animal on a heating

pad during the surgery. An incision was made on top the head. The skin and fascia were moved to the sides and held with retractors. Then the skull was cleaned with normal saline solution and dried with sterile gauze. Ten small perforations were made around the circumference of the skull and ten anchoring screws were implanted (fig. 2.2). A pre-made plastic mold was placed covering the heads of the screws to build a polymer ring-like shaped base. This base later allowed immobilizing the cat's head during experiments with the awake animal. This was needed for insertion of recording electrodes and identification of neurons. The base was also used to affix miniature drives, preamplifiers, and connectors. The gaps between the screws below this plastic base were covered with a silicone material (Silicone Elastomer, Factor II Inc. AZ). At the end of the surgery, the base was capped with a plastic cap for mechanical protection, and electrical shielding during recordings.

Several limb muscles were typically implanted with electrodes for electromyography (EMG) following standard procedures (Beloozerova et al. 2010; Prilutsky et al. 2005; Armer et al., 2013). Following the surgery immediately and 12 hours later, an analgesic buprenorphine was administered.

After the cat has recovered from the surgery, it was trained to sit quietly with its head restrained by an external apparatus. During this training, the cat was placed in this apparatus for increasing periods of time ranging initially from 10 sec to ~30 minutes by the end of the training. After a few sessions, the animal calmly sat in the apparatus with their head restrained. They did not appear to be in discomfort because they often fell asleep in the apparatus.

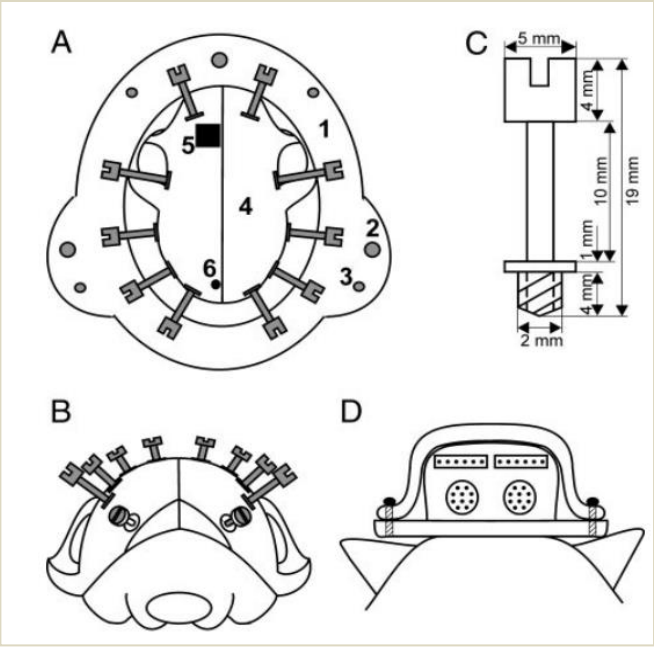


**Fig. 2.2:** Assembly of the surgically implanted head stage. A: Surgically implanted ten anchoring screws around the circumference of the head at approximately perpendicular angles to the surface (top view). B: The implanted anchoring screws, view from behind. C: Dimensions of a typical anchoring screw. D: Appearance of the final assembly on the cat's head viewed from behind. The head stage, protective cap and electronic connectors are seen. 1. Rigid plastic ring (head stage), 2. Screw holes for head restraining apparatus,

3. Screw holes for the protective cap, 4. The exposed skull; this area is completely covered with a thin layer of orthodontic resin. 5. An example of opening of the skull for superficial cortical recording. This opening is covered with a plastic disc with multiple perforations (see the text in chapter 3 and fig. 3.1). 6. An example of a much smaller, circular opening for guide tube implantation for either recording electrodes or stimulating electrodes. (Adapted from Prilutsky et al., 2005 with permission)

Identification of Neurons and Neuronal data Acquisition

All neuronal recording experiments started by inserting a recording electrode in the brain and connecting it to a moving arm of a micromanipulator, which in turn, connected to a preamplifier on the head of the animal. Either quartz-insulated platinum-tungsten electrodes (40 μm outer diameter) that were pulled to a fine tip and then mechanically sharpened (Reitboeck, 1983) or commercially available varnish-insulated tungsten electrodes (FHC Inc. Bowdoin, ME) were used. All electrodes had 2-4 MΩ impedance at 1 kHz. Once an electrode was inserted, the arm of the microdrive was slowly lowered (1 turn was 250 μm) while the activity of the brain was monitored. The signals from the electrode were initially amplified by a preamplifier of the head of the

animal and then further amplified using stationary amplifier CyberAmp 380 (Axon Instruments). Amplified signals were filtered (0.3 – 10 KHz band pass), digitalized (30 kHz sampling frequency) and recorded to a computer hard disk by means of a data acquisition hardware and software package, Power-1401/Spike-2 system (Cambridge Electronic Design, Cambridge, UK).

EMG signals were amplified with individual miniature preamplifiers carried by the animal, then further amplified with a stationary amplifier, filtered (30-1,500 Hz band pass), digitalized with 3 kHz sampling frequency, and stored on a computer hard disk. Both neuronal and EMG signals were displayed in real-time on a computer screen. When a stable neuronal signal with a good signal to noise ratio was found, responses of the neuron to somatosensory stimulation were tested.

Somatosensory stimulation was produced manually while the cat was sitting with its head restrained. Palpation of the parts of the body and limbs, palpation of muscle bellies, touching the skin and fur, and passive movements of the joints were used. The responsive neurons were further tested for directional preference.

### Analysis of Neuronal Activity

During each recording session, animals completed two tasks. They either walked in the light or darkness on a flat surface (chapter 3) or walked on a flat surface and along a horizontally placed ladder (chapters 4 and 5). The lift of the right forelimb was considered to be the beginning of the stride cycle. One stride cycle consisted of the swing and stance periods. For the analysis, each cycle was divided into 20 equal bins. Phase histograms were produced from the neuronal data after averaging over all selected strides (e.g., Beloozerova et al. 2010).

In order to determine the difference between neuronal activity during different tasks, data were analyzed by comparing average discharge rates ($s^{-1}$), the coefficients of stride-related frequency modulation (M%), the depths of modulation (dM%), periods of elevated firing (PEF), the number of PEFs during one stride cycle, and the preferred phases of the activity (PP).

The coefficient of stride-related frequency modulation (M) was calculated using the phase histogram as follows: $M\% = (1-F_{min} \div F_{max}) \times 100$; where $F_{min}$ was the minimum discharge frequency in the histogram, and $F_{max}$ was the maximum frequency in the histogram. Neural activity with M > 50% was considered stride-cycle modulated. This estimate was empirically established using bootstrapping technique (Efron & Tibshirani, 1993).

The depth of modulation (dM) was calculated as follows: $dM\% = (N_{max} - N_{min}) \div (N \times 100)$, where $N_{max}$ was the number of spikes in the highest bin of the histogram, $N_{min}$ was the number of spikes in the lowest bin of the histogram, and N was the total number of spikes in the histogram. Activity with dM >4% was considered to be stride-related, which was also determined using bootstrapping analyses.

The bins of the phase histogram where the value of the activity exceeded a set threshold were considered to be the period of elevated firing (PEF). The preferred phase (PP) of neuronal activity was calculated using circular statistics (Batshelet 1981; Drew and Doucet 1991; Fisher 1993; see also Beloozerova et al. 2003; Sirota et al. 2005). For this purpose, the stride cycle was treated as a circle and each action potential occurring within the stride was given a unit value and an angle to the corresponding time stamp. Then the final vector was calculated over all the strides in the experiment.

The statistical methods used for comparing the neuronal activities, and the details of these tests are provided in corresponding chapters.

## Histological Procedures

After completion of recordings, the cats were deeply anesthetized with pentobarbital sodium. Reference marks at the recording and stimulating sites were made by passing electrical current. In selected experiments, fluorescence microbeads were injected in the brain several weeks prior to the termination of the animal in order to trace the neuronal connections of the structures. The positioning of EMG electrodes was verified by testing limb movements in response to electrical stimulation and by visual inspection. The animals were given an overdose of pentobarbital sodium and perfused with phosphate buffered saline followed by 10% formaldehyde. The extracted brains were sectioned at 50 μm in the coronal or sagittal plane. The sections were mounted on gelatin coated microscope slides, stained, with cresyl violet for Nissl substance and covered with coverslips for preservation.

# 3. EFFECT OF LIGHT ON THE ACTIVITY OF MOTOR CORTEX NEURONS DURING LOCOMOTION

Published in Behavioural Brain Research (Armer et al. 2013)

## Abstract

The motor cortex plays a critical role in accurate visually guided movements such as reaching and target stepping. However, the manner in which vision influences the movement-related activity of neurons in the motor cortex is not well understood. In this study we have investigated how the locomotion-related activity of neurons in the motor cortex is modified when subjects switch between walking in the darkness and in light.

Three adult cats were trained to walk through corridors of an experimental chamber for a food reward. On randomly selected trials, lights were extinguished for approximately four seconds when the cat was in a straight portion of the chamber's corridor. Discharges of 146 neurons from layer V of the motor cortex, including 51 pyramidal tract cells (PTNs), were recorded and compared between light and dark conditions. It was found that cats' movements during locomotion in light and darkness were similar (as judged from the analysis of three-dimensional limb kinematics and the activity of limb muscles), while the firing behavior of 49% (71/146) of neurons was different between the two walking conditions. This included differences in the mean discharge rate (19%, 28/146 of neurons), depth of stride-related frequency modulation (24%, 32/131), duration of the period of elevated firing ((PEF), 19%, 25/131), and number of PEFs among stride-related neurons (26%, 34/131). 20% of responding neurons exhibited more than one type of change.

We conclude that visual input plays a very significant role in determining neuronal activity in the motor cortex during locomotion by altering one, or occasionally multiple, parameters of locomotion-related discharges of its neurons.

**Acknowledgements:** The idea for this study was suggested by Dr. Alan R. Gibson. In addition, authors are indebted to Mr. Peter Wettenstein for his exceptional engineering assistance and Mr. Neet Shah and Mr. Dan Griffiths for assistance with analyses. The research was supported by NIH Grant R01 NS-058659 to INB.

## Introduction

The role of the motor cortex in accurate visually guided movements such as reaching and target stepping is important. It was shown that neuronal discharges in the motor cortex are timed to phases of reaching and target stepping (Armstrong and Drew, 1984a; Beloozerova and Sirota, 1985a, b; 1993a, b; Beloozerova et al., 2010; Drew, 1993; Georgopoulos et al., 1982; Kalaska, 2009; Scott, 2008; Yakovenko et al., 2011) and that lesions or inactivation of the motor cortex impair these movements (Bastian et al., 2000; Beer et al., 2000; Beloozerova and Sirota, 1993; Liddell and Phillips, 1944; Martin and Ghez, 1993; Mihaltchev et al., 2005; Trendelenberg, 1911). The focus of nearly all of previous studies, however, has been on determining only the motor parameter(s) of reaching and target stepping that are controlled by the motor cortex. The inflow of visual information about the location of the target and the manner by which this information influences the movement-related activity of neurons in the motor cortex has received much less attention.

Anatomical studies show that the motor cortex receives input from several higher order visual areas. In the cortex, parietal area 5 intensely projects to the motor cortex (Andujar and Drew, 2007; Babb et al., 1984; Ghosh, 1997). Area 5 receives inputs from a

host of cortical and subcortical visual centers, including the pulvinar and a number of visual cortical areas such as 18, 19, 20, 21, lateral suprasylvian and splenial visual areas (Avendano et al., 1985, 1988; Hyvarinen, 1982; Niimi et al., 1983; Symonds et al., 1981). Rostral subdivisions of cortical parietal area 7 also project to the motor cortex (Andujar and Drew, 2007; Babb et al., 1984; Ghosh, 1997). Area 7 receives inputs from visual cortical areas 19, 20, 21, 22, lateral suprasylvian areas as well as from the pulvinar and rostral intralaminar complex (Divac et al., 1977; Hyvarinen, 1982; olson and Lawler, 1987; Robertson, 1977; Symonds and Rosenquist, 1984). The main thalamic input to the motor cortex arrives from the ventrolateral thalamus. Although the ventrolateral thalamus contains very few visually responsive neurons, it receives intensive afferentation from the lateral cerebellum, which is known to have a substantial visual input (Cerminara et al., 2005; Stein and Glickstein, 1992). In our recent publication (Marlinski et al., 2012) it was argued that the stream of visual information that travels via the cerebellum and ventrolateral thalamus significantly contributes to the transmission of vision-based signals to the motor cortex during motor behavior.

In agreement with anatomical data, physiological experiments show that, in both anesthetized and awake animals, neurons in the motor cortex respond to visual stimuli (Garcia-Rill and Dubrovskey, 1974; Weyand et al., 1999; Martin and Ghez, 1985). In the study by Weyand and colleagues (1999), when receptive fields in paralyzed cats were tested using stationary or moving spots or bars of light, 40% of sites within the motor cortex area 4γ gave moderately reliable visual responses, with most other sites showing some responsiveness, albeit weaker. Receptive fields were found to be large, occasionally encompassing most of the contralateral hemifield, and always included the area centralis. Sites with good responses were intermingled with nearly non-responsive areas. In chronically implanted behaving cats, Martin & Ghez (1985) found that in many motor

cortical neurons the onset of the forearm flexion/extension-related activity, although well-timed to the movement, was even better time-locked to the visual stimulus. For 56% of these cells, however, the specificity of the visual stimulus was unimportant as they were well tuned to any stimulus signaling a certain movement (e.g., elbow flexion), and their visual responses were contingent upon the movement.

In previous studies from Dr. Beloozerova laboratory it was found that when visual information is required for accurate target stepping, the activity of the cat motor cortex differs dramatically from the activity observed during simple locomotion (Beloozerova and Sirota, 1993a, b; Beloozerova et al., 2010). Taking into account that the inactivation of the motor cortex impairs visually guided stepping, this altered activity was interpreted as a control signal for accurate feet placement. However, the manner by which visual information contributes to the formation of a control signal in the motor cortex remained unclear. In this study we have investigated the effect of light on locomotion-related activity of neurons in the motor cortex. We trained cats to walk along a straight path with a flat surface under normal laboratory illumination and when the lights were turned off (complete darkness) for approximately 3-4 seconds. The analysis of three-dimensional kinematics and EMGs of limb muscles showed that cats walked very similarly in the light and darkness. The locomotion-related discharges of 49% (71/146) of neurons, however, were different between the two conditions. We conclude that the motor cortex responds to visual stimulation during locomotion, and describe how the light, and thus the ability to see, shapes its locomotion-related activity.

## Methods

Recordings were obtained from three adult cats prepared for chronic experiments, of which two were female and one was male. Methods of surgical preparation and

recording techniques have been described in detail in the methods section of this dissertation and in previous publications from Dr. Beloozerova laboratory (Beloozerova and Sirota, 1993; Prilutsky et al., 2005). Therefore only the specific methods used in this study will be described here while the general methods will be only briefly reported.

Locomotion Tasks

Positive reinforcement (food) was used to habituate cats to the experimental situation and engage them in locomotion behavior (Pryor, 1975; Skinner, 1938). Cats walked in a rectangular chamber that had two corridors, each 2.5 m long and 0.3 m wide (Fig. 2.1). The cat's passage through the beginning and the end of each corridor was monitored using infrared photodiodes (emission wavelengths of 850-900 nm, which is outside of the visible spectral range of the cat (Guenther and Zenner, 1993).

Cats walked in two experimental conditions: under normal laboratory illumination of approximately 500 lux (the spectral sensitivity of cats is very similar to that of humans (Guenther and Zenner, 1993) and in full darkness. The experimental room had no windows, its door was light-proofed, and all lights from electronics were masked. Computer monitors were located in a neighboring room. The darkness condition was randomly presented in approximately 50% of the rounds. It started upon the cat entering a straight portion of the walkway and ended when it reached a photodiode at the end of the corridor. Typically, it took cats between 2.5 and 4 seconds to walk through a corridor. Cats were trained to walk around the chamber in this experimental setting daily for about 2-3 weeks until they were confident in doing so.

Cats were accustomed to wearing a cotton jacket, a light backpack with connectors, and a sock with a small metal plate on the sole of the foot for recording foot contact with the floor. On selected trials, cat movements were recorded using the

Visualeyez System (3D Real Time Motion Capture and Analysis System, Phoenix Technologies Inc., Canada). This system detects positions of light-emitting photodiodes (LEDs) in three-dimensional space and calculates various kinematical parameters. Wide-angle, six-chip infrared LEDs with wavelengths of 755-785 nm, which are invisible to cats (Guenther and Zenner, 1993), were attached to the skin projections of the base of the fifth metacarpal on the right paw and the vertebral border of the right scapula. The frequency of sampling was 200 Hz. Position, velocity, and acceleration were determined and averaged across all selected strides.

Surgical Procedures

After each cat was trained, surgery was performed under aseptic conditions using isoflurane anesthesia. In two cats, bipolar EMG electrodes (flexible Teflon-insulated stainless-steel wires) were implanted bilaterally into three forelimb muscles: m. triceps brahii (Tri, elbow extensor), m. extensor digitorum communis (EDC, wrist and phalanges dorsal flexor), and m. extensor carpi ulnaris (ECU- wrist dorsal flexor); and into three hindlimb muscles: m. gastrocnemius lateralis (GL, ankle extensor), m. soleus (SO, ankle extensor), m. vastus lateralis (VL, knee extensor). The electrode placements were verified by stimulation through the implanted wires before closure of the incision. The wires were led subcutaneously and connected to sockets on the head base.

To access the left motor cortex, a portion of the os frontale, os ethmoidale, and dura above the left motor cortex over an area of approximately 1 $cm^2$ were removed. The motor cortex was visually identified by surface features and photographed (Fig. 2.1A). The aperture was then covered by a 1 mm-thick acrylic plate, in which approximately 200 holes, 0.36 mm in diameter, had been drilled and filled with a mixture of bone wax

and petroleum jelly. Recording microelectrodes were later inserted into the motor cortex of the awake animal through the holes in the plate.

Two 26 gauge hypodermic guide tubes were implanted vertically above the medullary pyramids with tips approximately at the Horsley-Clarke coordinates (P7.5, L0.5) and (P7.5, L1.5), and the depth of H0. They were later used for physiologically guided insertion of stimulating electrodes into the pyramidal tract (Prilutsky et al., 2005). These electrodes were used to identify pyramidal tract neurons (PTNs) in the awake animal.

Sampling of Neuronal and EMG Activity

After recovering from surgery over a period of five or six days, the cat was placed on a table and encouraged to take a "sphinx" position on a comforting pad. After resting in this position for a few minutes, the base attached to the skull during surgery was fastened to an external frame, thereby approximating the natural resting position of the cat's head. After a few sessions of an increased duration, all cats would calmly rest in the sphinx position with their head restrained. The cats did not seem to be in any discomfort and would often fall asleep.

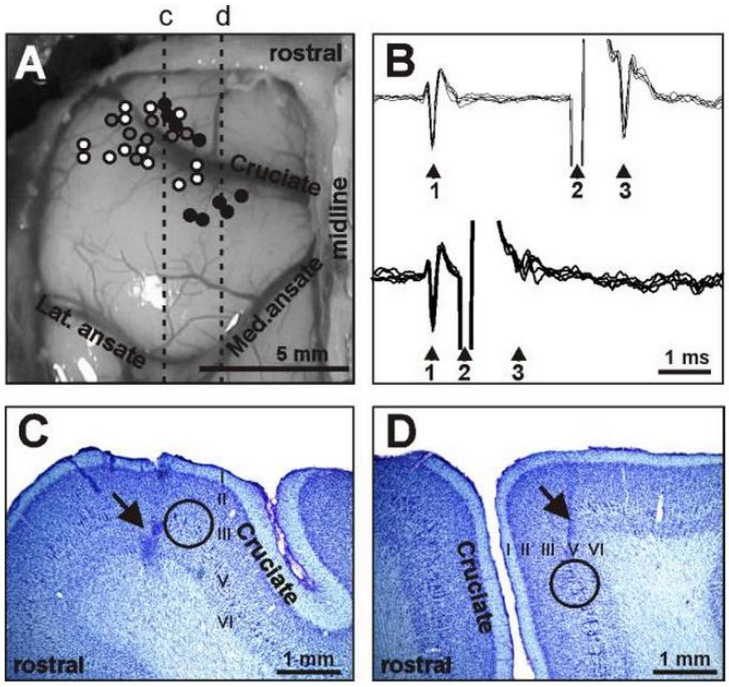


**Fig. 3.1:** Location and identification of neurons. ***A***: Areas of recording in the left motor cortex. Microelectrode entry points into the cortex were compiled from all cats and are shown as circles on the photograph of the cat 2-cortex: cat 1, 2, and 3 entry points are depicted by grey, white, and black circles, respectively. Positions of parasagittal sections, whose photomicrographs are shown in ***C*** and ***D,*** are indicated by dotted lines *c* and *d*, respectively. ***B***: A collision test determines whether a neuron's response to pyramidal tract stimulation is antidromic. Top: the neuron spontaneously discharges (arrowhead 1) and the pyramidal tract is stimulated approximately 3 ms later (arrowhead 2). The neuron responds with a latency of approximately 1 ms (arrowhead 3). Bottom: the neuron spontaneously discharges (arrowhead 1) and the pyramidal tract is stimulated about 0.5 ms later (arrowhead 2). The neuron does not respond (arrowhead 3) because at 0.5 ms the spontaneous spike was still en-route to pyramidal tract, therefore causing the collision, or nullification, of the response. The test confirms the response of the neuron was antidromic, and therefore the neuron proves to be a pyramidal tract projecting neuron (PTN). ***C***: Photomicrograph of a parasagittal section through the lateral pre-cruciate motor cortex (section *c*). An arrow points to a reference electrolytic lesion in the forelimb representation of the motor cortex. ***D***: Photomicrograph of a parasagittal section through the medial motor cortex (section *d*). An arrow points to a reference electrolytic lesion in the hindlimb representation of the motor cortex. In ***C*** and ***D***: Layers of the cortex are numbered. Groups of giant cells in layer V, which are characteristic for area 4γ and are visible throughout both the pre- and post-cruciate cortex, are encircled. Cresyl violet stain.

While the animal was at rest on the table, the motor cortex was searched for well-isolated neurons. Neuronal activity was recorded extracellularly using either platinum-tungsten quartz-insulated microelectrodes (40 μm outer diameter) pulled to a fine tip and mechanically sharpened (Reitboeck, 1983), or commercially available tungsten varnish-insulated electrodes (Frederick Haer & Co; Bowdoin, ME). The impedance of both types of electrodes was 2–4 MΩ at 1000 Hz. A custom made light-weight (2.5g), manual, single-axis micro-manipulator chronically mounted to animal's skull was used to advance the microelectrode. Signals from the microelectrode were pre-amplified with a custom-made miniature preamplifier positioned on the cat's head, and then further amplified with CyberAmp 380 (Axon Instruments). After amplification, signals were filtered (0.3-10 kHz band pass), digitized with a sampling frequency of 30 kHz, displayed on a screen, fed to an audio monitor, and recorded to the hard disk of a computer by means of a data acquisition hardware and software package (Power-1401/Spike-2 System, Cambridge Electronic Design, Cambridge, UK). An example of a recording is shown in Fig. 3.2A, D.

Signals from EMG pre-amplifiers were amplified and filtered (30-1,500 Hz) using a CyberAmp 380 amplifier, digitized with a sampling frequency of 3 kHz, displayed on the screen, and recorded to the disc of a computer using the same data-acquisition software as for neurons. After digitizing, the EMG signals were rectified and smoothed by filters with a time constant of 50 ms. An example of an untreated data recording is shown in Fig. 3.2A and D.

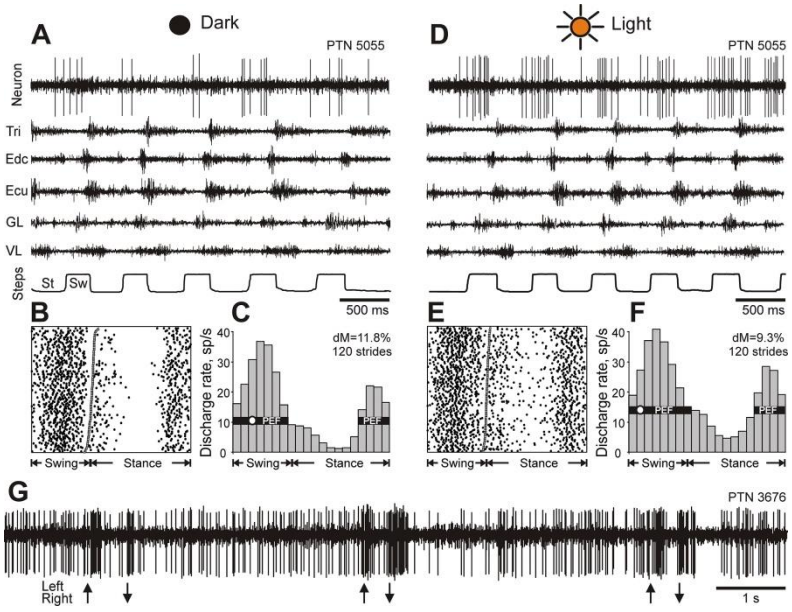


**Figure 3.2.** A typical example of the activity of a neuron (PTN 5055) and selected right fore- and hindlimb muscles during locomotion in the darkness and light. (A) Activity of the neuron and muscles during locomotion in the darkness. Tri, m. triceps brahii (elbow extensor); Edc, m. extensor digitorum communis (wrist and phalanges dorsal flexor); ECU, m. extensor carpi ulnaris (wrist dorsal flexor); GL, m. gastrocnemius lateralis (ankle extensor), VL, m. vastus lateralis (knee extensor). The bottom trace shows the stance (St) and swing (Sw) phases of the step cycle of the right forelimb that is contralateral to the recording site in the cortex. (B and C) The activity of the same neuron during locomotion in the darkness is presented as a raster of 120 step cycles (B) and as histograms (C). The duration of step cycles is normalized to 100%. In the raster, the end of swing and the beginning of the stance in each cycle is indicated by an open triangle. In the histogram, the horizontal black bar shows the period of elevated firing (PEF) and the circle indicates the preferred phase as defined in Section 2.5. The value of dM is stated. (D–F) Activities of the same neuron and muscles during locomotion in illuminated room. (G) Responses of a neuron to movements of an object in front of the cat (PTN 3676, located in the rostral cruciate sulcus). Arrows pointed up indicate movements from cat's right to left, arrows pointed down indicate movements from cat's left to right.

## Identification of Neurons

A waveform analysis was employed to identify and isolate the spikes of a single neuron using the Power-1401/Spike-2 system waveform-matching algorithm. In addition, all encountered neurons were tested for antidromic activation using pulses of

graded intensity (0.2 ms duration, up to 0.5 mA) delivered through the bipolar stimulating electrodes to the medullary pyramidal tract. The identification of antidromic responses was determined by the test for the collision of spikes (Bishop et al., 1962; Fuller and Schlag, 1976), which is illustrated in Fig. 3.1B. For the purpose of conduction velocity calculation, the distance between electrodes in the medullary pyramidal tract and at recording sites in the peri-cruciate cortex was estimated at 51 mm. Neurons were classified as fast- or slow-conducting PTNs based on the criteria of Takahashi (Takahashi, 1965): PTNs with conduction velocity of 21 m/s or higher were considered to be fast-conducting, while those with conduction velocities below this threshold were considered to be slow-conducting. Neurons were tested for antidromic activation before, during, and after locomotion.

Recordings were obtained from the rostral and lateral sigmoid gyrus (forelimb representation area) as well as from the postcruciate cortex within the fold of the cruciate sulcus (forelimb and hindlimb representations) (Fig. 3.1A, C and D). These areas are considered to be the motor cortex based on a considerable body of data obtained by means of inactivation, stimulation, and recording techniques (Armstrong and Drew, 1984b, 1985a, b; Beloozerova and Sirota, 1993a; Drew, 1993; Martin and Ghez, 1985, 1993; Nieoullon and Rispal-Padel, 1976; Phillips and Porter, 1977; Vicario et al., 1983). A parasagittal section through the frontal cortex with a reference electrolytic lesion next to giant pyramidal cells in cortical layer V, which are characteristic of motor cortex area 4γ, is shown in Fig. 3.1C (pre-cruciate cortex) and 3.1D (post-cruciate cortex). To determine the locations of the fore- and hindlimb representations within the motor cortex, somatic receptive fields were mapped while the animals were resting on a pad with their head restrained. Somatosensory stimulation was produced by palpation of muscles and

tendons and by passive movements of joints. Neurons responsive to passive movements of joints were further assessed for directional preference.

Responses to visual stimulation were tested by presenting moving stimuli: circles 2.5 cm in diameter, stripes 5 cm wide and 100 cm long, fields 50 x 50 cm, and complex three-dimensional stimuli such as toys, laboratory objects, and hands. Two-dimensional stimuli were black. All stimuli were presented against the natural laboratory background and were moved in different directions in the frontal plane at the distance of approximately 50 cm in front of the animal and also toward the animal and away from it with the speed of 0.5–1.0 m/s.

Analysis of Neural Activity

To compare the activity of neurons during locomotion in the light and darkness we used only the strides in which average durations in the two conditions differed by less than 10%. These strides were selected from three strides made in the middle of walkway. Earlier we showed that in the middle of the corridor cats walk with nearly constant speed (Beloozerova et al. 2010). For forelimb-related cells, the onset of the swing phase of the right forelimb was taken as the beginning of the step cycle. The duration of each cycle was divided into twenty equal bins. For hindlimb-related neurons, the beginning of the $16^{th}$ bin of the forelimb cycle, which corresponds to the beginning of the swing phase of the right hind limb, was taken as the onset of the hind limb step cycle. A phase histogram of the discharge rate of each neuron in the step cycle was then generated and averaged over all selected cycles (Fig. 3.2B, C, E, and F). Phase histograms were smoothed by recalculating the values of the bins according to the equation $F_n' = 0.25 \times F_{n-1} + 0.5 \times F_n + 0.25 \times F_{n+1}$, when $F_n$ is the bin's original value. The first bin was considered to follow the last bin, and the last bin was considered to precede the first 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 the minimal histogram bin, and N is the total number of spikes in the histogram. Neurons with dM>4% were judged to be stride-related. This was based on an analysis of fluctuations in the activity of neurons in the resting animal. For this analysis, the activities of 100 neurons recorded while the cat was sitting with its head restrained were processed as if the cat was walking (Marlinski et al., 2012). The timing of steps made by the same cat during the preceding walking test was used to construct the histogram. In stride-related neurons, the period of elevated firing (PEF) was defined as the portion of the cycle in which the activity level exceeds the minimal activity by 25% of the difference between the maximal and minimal frequencies in the neuronal discharge histogram (Fig. 3.2C and F). PEFs were smoothed by renouncing all one-bin peaks and troughs (a total of 1% of bins were altered throughout the database). The "preferred phase" of discharge of each neuron with a single PEF was assessed using circular statistics (Batshelet, 1981; Fisher, 1993; Drew and Doucet, 1991; see also Beloozerova et al., 2003; Sirota et al., 2005).

For comparisons of the discharge rate of individual neurons in different conditions and between groups of neurons, the two-tailed Student's t-test was used to determine statistical significance. When comparing dM, preferred phase, and duration of PEF, differences equal to or greater than 20%, 10%, and 20%, respectively, were considered significant. These criteria were established based on the results of a bootstrapping analysis (Efron and Tibshirani, 1993; Stout and Beloozerova, 2012), which compared differences in discharges between various reshufflings of strides of the same locomotion task. It showed that natural PTN activity fluctuations remain within these limits with 95% confidence. Unless noted otherwise, for all mean values, the standard error of the

mean (SEM) is given. When data were categorical, a nonparametric Fisher's two-tailed test was used

## Histological Procedures

At the termination of experiments, cats were deeply anaesthetized with pentobarbital sodium. Several reference lesions were made in the regions of the motor cortex from which neurons were sampled. Cats were then perfused with isotonic saline followed by a 3% paraformaldehyde solution. Frozen brain sections of 50 μm thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet. Zoning of the cortex was performed according to established criteria (Hassler and Muhs-Clement, 1964; Ghosh, 1997a; Weyand et al., 1999). The positions of recording tracks in the motor cortex were estimated in relation to the reference lesions (Fig. 3.1C and D). The positions of stimulation electrodes in the medullar pyramids were verified.

# Results

## Kinematics of Walking in the Light and Darkness are Similar

During the recording of each individual neuron, cats walked between 10 and 80 (typically 20-50) times down each of the chamber's corridors. From these runs, 20–200 strides (72±34 in the light and 73±37 in the darkness, mean ± SD) made in the middle of the corridor (with average durations in the light and dark differing by less than 10%) were selected for analysis. One of the cats walked relatively quickly (cat 1) and the other two walked more slowly (cats 2 and 3). The average durations of strides selected were 600 ms and 740-780 ms, respectively (Fig. 3.3A). This corresponded to a walking speed of 0.83 m/s and 0.65-0.68 m/s, respectively. Although different cats walked with a

slightly different speed overall, each of them had approximately the same speed during walking under the two illumination conditions. Only cat 2 had a statistically significantly different average duration of strides during locomotion under normal illumination and in the dark, and the difference was 20 ms or 2.7% of the stride cycle. The ratio of the stance duration to the cycle duration (the stride duty factor) varied only slightly between cats (Fig. 3.3B), and was 60-65% on average. There was a slight difference of 1.5% for the average stride duty factor in cat 3 between illumination conditions.

Movements of the right paw and scapula were recorded in cats 2 and 3 while they walked around the chamber under illumination protocol similar to that used during neuronal recordings. Representative examples of vertical paw and scapula positions, and horizontal and vertical velocities during locomotion in the two conditions are shown in Figs. 3.3C and D. For both cats, movements of the paw were similar in all parameters tested (Fig. 3.3C). Position of the scapula was 2.3±1.3 mm higher throughout most of the step cycle during walking in the illuminated room as compared to walking in the darkness; however, in the middle of the stance phase scapula position was always similar between the conditions (Fig. 3.3D, top panel). Vertical velocities of the scapula were also similar (Fig. 3.3D, middle panel). In approximately one third of the trials, horizontal velocities were slightly higher (by 7-10%) during walking in the illuminated room as compared to walking in the darkness (Fig. 3.3D, lower panel).

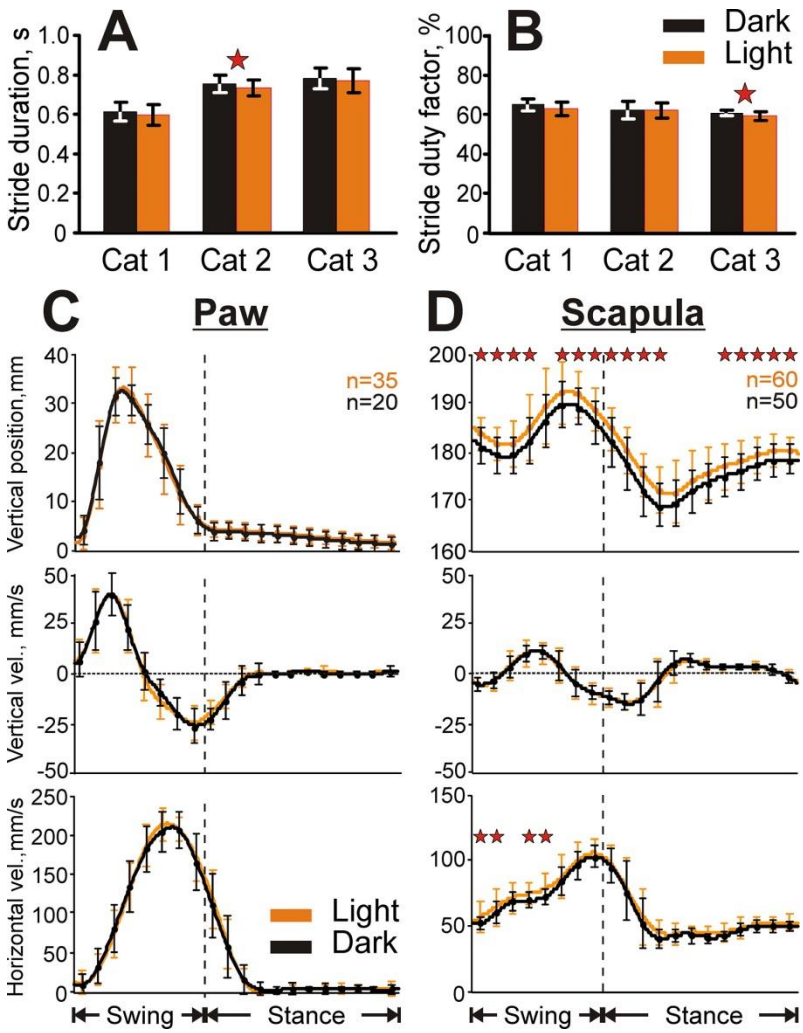


**Fig. 3.3** Kinematics of locomotion in the darkness and light. (A) The average duration of the stride of each cat in the two light conditions. (B) The average stride duty factor (the percent of the total cycle in which the right forelimb is in the stance phase). (C and D) Vertical position (top panel), and vertical (middle panel) and horizontal (bottom panel) velocity of the right paw (C) and scapula (D). Error bars are SDs. Stars denote significant differences in parameters between the conditions (Student's unpaired t test, $p < 0.05$).

## Activities of Muscles during Walking in the Light and Darkness

In cats 1 and 2, the activity of three forelimb and three hindlimb muscles was recorded on each side of the body. Muscles showed only very minor differences in the activity during locomotion under different lightening conditions (Fig. 3.4). Elbow extensor *m. triceps brachii* was typically active slightly longer during walking in the light (Student's unpaired *t* test, $p<0.05$), by 5-10% of the cycle. Wrist and phalanges dorsal flexor *m. extensor digitorum communis* often began later in the cycle during walking in the light (Student's unpaired *t* test, $p<0.05$), by 5-10%, but finished at the same time as during walking in the darkness. Ankle extensor *m. soleus* occasionally showed differences analogous to those seen in the *m. extensor digitorum communis,* however in other trials was active uniformly between conditions. Wrist dorsal flexor *m. extensor carpi ulnaris* during walking in the light was more active at the peak (Student's unpaired *t* test, $p<0.05$) generating 3.8±1.5% more output (mean±SD). At the same time, the activity of the ankle extensor and knee flexor *m. lateral gastrocnemius* and that of the knee extensor *m. vastus lateralis* were typically indistinguishable during locomotion in the darkness and light.

## Characteristics of Neurons

Neuronal data was collected from a total of 29 tracks through the motor cortex of three cats (7 tracks in cat 1, 13 in cat 2, and 9 in cat 3; Fig. 3.1A). This data was considered together to analyze the activity of 146 neurons (19 from cat 1, 77 from cat 2, and 50 from cat 3). Based on cytoarchitectural features, it was determined that all neurons were located in layer V of the motor cortex area 4γ. Fifty-one cells responded to stimulation of the pyramidal tract (17 from cat 1, 22 from cat 2, and 12 from cat 3). The latencies of responses ranged from 0.8 to 4.6 ms. Estimated conduction velocities were

between 11 and 64 m/s. Among responding neurons 63% (32/51) responded at 2.0 ms or faster, conducting at 25 m/s or faster, and thus were “fast-conducting” PTNs, while 37% (19/51) were “slow-conducting” (Takahashi, 1965).

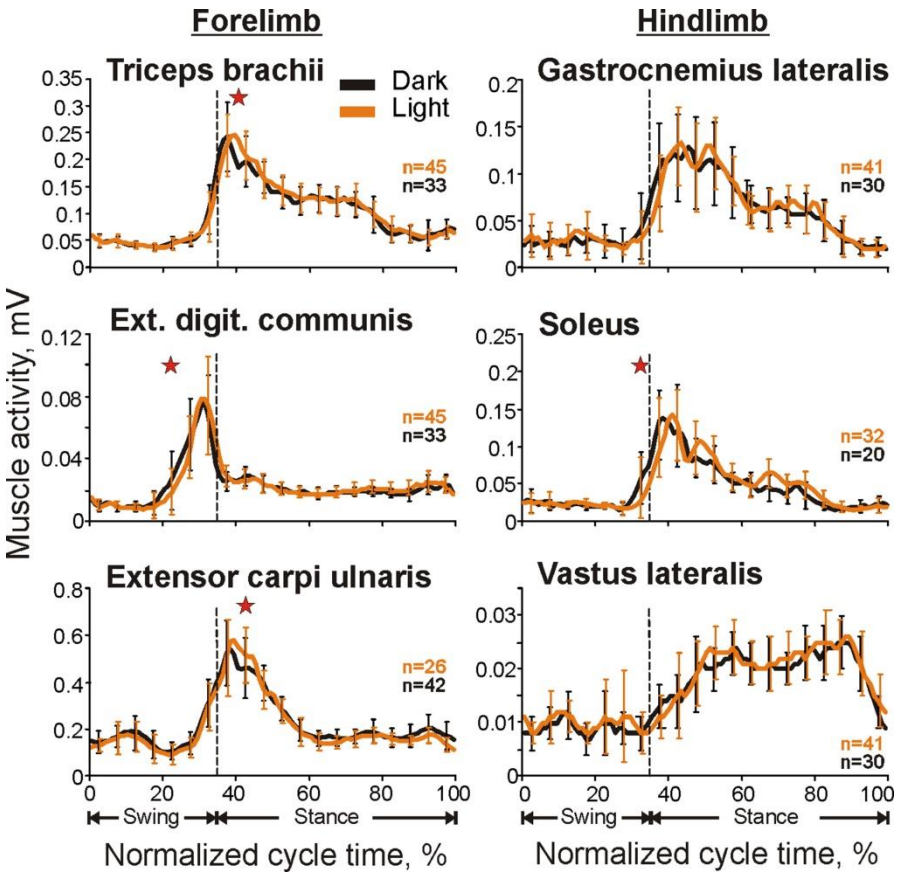


**Figure 3.4.** Typical examples of EMG activity of selected limb muscles during locomotion in the darkness and light. Each panel shows a representative activity of a muscle, which was averaged over 20–45 strides of each locomotion task, all recorded during one session (see Section 2.5 for stride selection). Error bars are SDs. Stars denote significant differences between the conditions (Student's unpaired t test, $p < 0.05$).

Responses of 114 neurons to somatosensory stimulation were tested. A somatosensory receptive field was found in 87% (99/114) of neurons. All receptive fields were located on the contralateral (right) side of the body and all but one were excitatory. Most neurons (77/99) had a receptive field on the forelimb, while 22 neurons responded

to stimulation of the hindlimb. From neurons responding to stimulation of the forelimb, 23 were activated by passive movements of the shoulder or palpation of back or neck muscles, 31 responded to movements in the elbow joint or palpation of arm muscles, and 23 were activated by movements of the wrist or palpation of muscles on the forearm or paw. Several neurons responded to movement in two joints or the entire forelimb. Neurons activated by a passive movement in a joint often had a preferred direction. One third (n=8) of shoulder-related cells responded to adduction of the shoulder, while others responded to either abduction (n=4), flexion (n=4), or extension (n=2) of the joint. From elbow-related cells, 7 neurons responded to flexion and 3 were activated by extension. From wrist-related neurons, 7 responded to wrist ventral flexion while 4 responded to dorsal flexion.

One half of neurons activated by stimulation of the hindlimb responded to movement or palpation of the ankle. They often were also directionally specific with 5 neurons responding only to ankle flexion and 3 only to ankle extension. From the other hindlimb-related cells, 4 responded to movements in the knee, 2 to the hip, and 2 to the paw.

From the group of 15 unresponsive somatosensory neurons, nine responded to visual stimuli. Four of these cells were activated by an object moving across the field of view from the periphery to the central field of vision and three were activated by approach of an object. An example of a neuron responding to movements of an object in front of the cat is shown in Fig. 3.2G. All visually responsive cells were located within the forelimb representation of the area 4γ, and eight of the nine were found in the post-cruciate fold of the sigmoid gyrus.

A typical example of activity of a neuron during walking in the light and dark is shown in Fig. 3.2A-F. The neuron was recorded from the forelimb representation region but it was not responsive to either somatosensory or visual stimulation. During locomotion in the dark, the neuron's activity was modulated in the rhythm of strides: it was high at the end of stance and during most of the swing phases, while low in the beginning and middle of the stance phase. When the lights were turned on, the neuron's activity increased throughout all of the step cycle. The raster plots in Fig. 3.2B and E show the activity of the neuron across 120 individual strides during locomotion in the dark (B) and light (E). The pattern of activity was very consistent across strides of each locomotion task, but during walking in the light there was more activity during the middle of the stance phase compared to walking in the dark. The activity is summed in Fig. 3.2C and F showing histograms of neuron firing rates across the step cycle during locomotion in the dark (C) and light (F). The period of elevated firing (PEF, see definition in Methods) is indicated by a black horizontal bar; it was contained within the swing and late stance phase of the step during both locomotion tasks. The preferred phase (indicated by a circle in Fig. 3.2C and E) was in the beginning of the swing phase during both locomotion tasks.

Activity of Neurons during Locomotion in the Darkness

The mean activity of the population during walking in the darkness was 10.7±0.6 spikes/s. The discharge rate of 90% (131/146) of cells was modulated in the rhythm of strides: it was higher in one phase of the stride and lower in another phase. The great majority of neurons (79%, 104/131) exhibited a single PEF, while 21% (27/131) had two PEFs. The average depth of modulation in one- and two-PEF populations was similar: 10.6±0.6 and 9.7±0.7, respectively. In both populations, PEFs and preferred phases were

fairly evenly distributed across the step cycle (Fig. 3.5A1 and B1). The average duration of the PEF was 60±1.5% and 58±2.5% of the cycle in one- and two-PEF groups, respectively. In 70% (19/27) of neurons with two PEFs, the PEFs differed in their duration by 10-35% of the cycle, and in eight of the neurons the longest PEF had also the highest discharge rate, on average by 12±8 spikes/s (mean ± SD). Neurons with two PEFs of a similar duration typically were also more active during one of their PEFs.

Because of a largely even distribution of PEFs and their relatively long duration in both one- and two-PEF populations, PEFs of different neurons overlapped and approximately 60% of neurons were simultaneously active at any time of the cycle in either of the groups (Fig. 3.5A3 and B3). However, in the one-PEF group, there were slightly more neurons with preferred phases during swing (Fig. 3.5A1 and 3) and they were more active than those with preferred phases during stance (Fig. 3.5A2); as a result, the whole population of one-PEF cells was slightly, but statistically significantly, more active during the swing phase as compared to stance (Student's unpaired t test, $p<0.05$; Fig. 3.5A4). The activity of two-PEF neurons as a group was steady over the cycle (Fig. 3.5B2 and 4)

Neurons with Different Receptive Fields Differed in Their Step-Related Activity

Forelimb-related cells as a group had a steady activity over the cycle that measured 11-13 spikes/s. Within the forelimb-related population, wrist-related neurons were most active and modulated (Student's unpaired t test, $p<0.05$), but as a group they engaged later in the cycle as compared to both elbow and shoulder-related cells, firing right before the foot made contact with the floor (Fig. 3.6A2, A4 vs. B2, B4 vs. C2, C4). In stark contrast, most hindlimb-related neurons discharged during the swing or beginning of the stance phase (Fig. 3.6D1 and D3). Thus, their population's activity was sharply

modulated with a peak during swing that reached approximately, 20 spike/s (Fig. 3.6 D4). Visually responsive neurons (n=9) were as active as the rest of population, however only five displayed activity that was step cycle related. This was a significantly smaller proportion of step-related cells as compared to the entire population (Fisher's two-tailed test, p=0.0001)

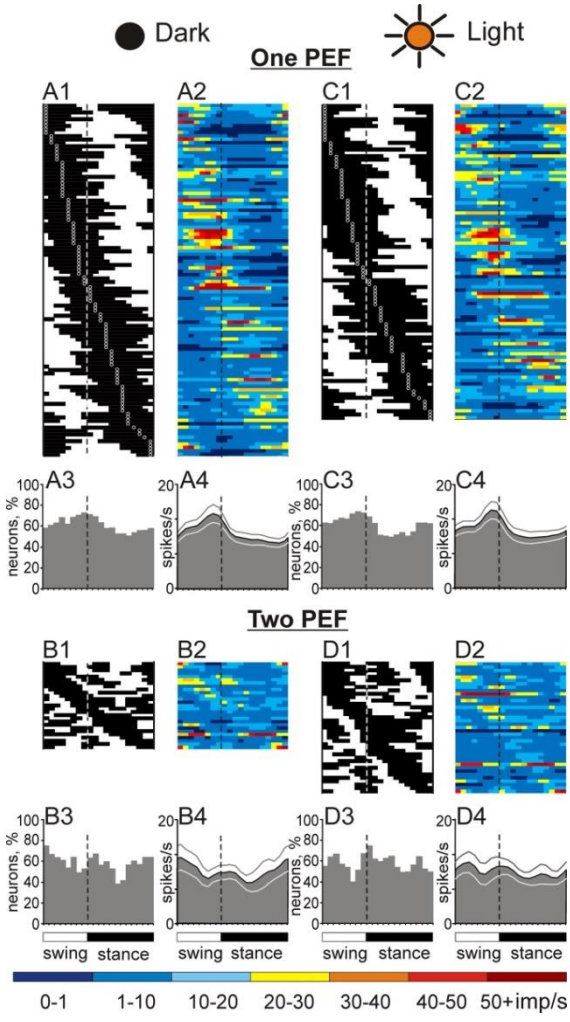


**Figure 3.5.** Population characteristics of one- and two-PEF neurons during locomotion in the darkness and light. (A1 and C1) Phase distribution of PEFs of all one-PEF neurons during locomotion in the dark (A1) and under normal illumination (C1). Each row represents the PEF of one cell. A circular mark on each PEF denotes the cell's preferred phase. Neurons are rank-ordered so that those with a preferred phase earlier in the cycle are plotted on the top of the graph. Vertical interrupted lines indicate the end of swing and beginning of stance phase. (A2 and C2) Corresponding phase distributions of discharge frequencies. The average discharge frequency in each 1/20th portion of the cycle is color-coded according to the scale shown at the bottom of the figure. (A3 and C3) Proportion of active one-PEF neurons (neurons in their PEF) in different phases of the step cycle during walking in the darkness (A3) and light (C3). (A4 and C4) The mean discharge rate of one-PEF neurons during walking in the darkness (A4) and under normal illumination (C4). Thin lines show SEM. (B1–4 and D1–4) Characteristics of two-PEF neurons locomotion in the darkness (B1–4) and light (D1–4). Designations are similar to those in (A) and (C).

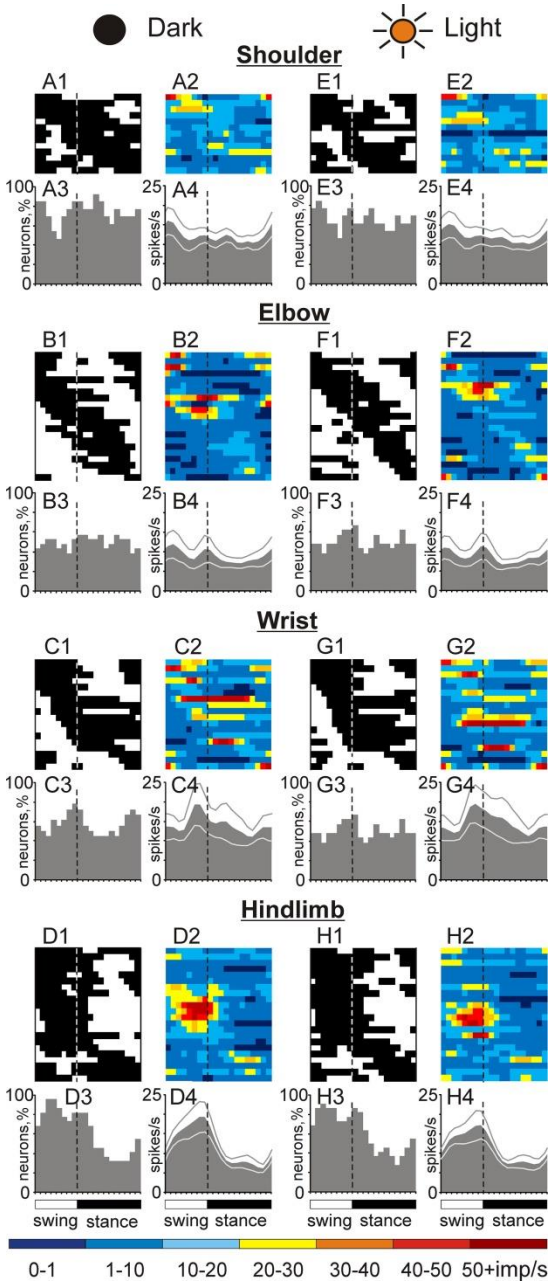


**Figure 3.6**. Population characteristics of neurons with different somatosensory receptive fields during locomotion in the darkness and light. (A and E) Activity of neurons responsive to movements in the shoulder joint, and/or palpation of back or neck muscles during locomotion in the darkness (A) and under normal illumination (E). (A1 and E1) Phase distribution of PEFs. (A2 and E2) Corresponding phase distribution of discharge frequencies. The average discharge frequency in each 1/20th portion of the cycle is color-coded according to the scale shown at the bottom of the figure. (A3 and E3) Proportion of active neurons (neurons in their PEFs) in different phases of the step cycle. (A4 and E4) The mean discharge rate. Thin lines show SEM. Vertical interrupted lines denote end of swing and beginning of stance phase. (B and F) Activity of neurons responsive to passive movement of the elbow joint or palpation of arm muscles. (C and G) Activity of neurons responsive to passive movement in the wrist joint or palpation of muscles on the forearm or paw. (D and H) Activity of neurons responsive to stimulation of the hindlimb.

Effect of Light on the Activity of Neurons during Locomotion

The activity of 49% (71/146) of neurons was different during locomotion in the light as compared to the darkness. The mean discharge rate was different in 19% (28/146) of neurons: it was higher in 15 cells by 30±3% and lower in 13 cells by an approximately similar amount (Student's unpaired t test, $p<0.05$; Fig. 3.7A). The depth of frequency modulation was different in 24% (32/131) of neurons. In the light, it was higher in 11 cells by 32±4%, and lower in 21 cells by 50±7% (Fig. 3.7B). The duration of the PEF was different in 19% (25/131) of neurons. In the light, it was shorter in 13 and longer in 12 cells by 20-40% of the cycle (Fig. 3.7C).

Number of PEFs was different in 26% (34/131) of cells. A fifth of neurons with one-PEF in the dark (21%, 22/104) had two PEFs in the light. This typically occurred via an appearance of a second trough within the PEF (n=7; Fig. 3.8A and B), an appearance of a new PEF within the trough (n=6; Fig. 3.8C and D), or an increase of activity within a part of the PEF (n=5; Fig. 3.8E and F). On the other hand, 31% (8/26) of neurons with two-PEFs in the dark had just one PEF in the light. This typically occurred by an increase of activity within one of the troughs so that two of the PEFs became fused together (n=4), or by reduction in the activity within one of the PEFs (n=3; Fig. 3.8G and H). As a result of these differences in the number of PEFs in individual neurons, during walking in the light, there were less one-PEF and more two-PEF neurons than during walking in the darkness (Fig. 6A1,2 and B1,2 vs. C1,2 and D1,2). In addition to neurons having a different number of PEFs in different lighting conditions, the activity of two cells was step cycle-modulated only in the light, whereas the activity of two others was only modulated in the darkness. In the population of neurons that had one PEF during both conditions (n=86), the preferred phase was very stable with only three neurons having a different preferred phase in the light.

The great majority of neurons (80%, 57/71) had only one or two parameters of their activity different between lighting conditions: most often the average discharge rate and the duration of the PEF, or the duration of the PEF and the number of PEFs, or the average discharge rate and the depth of modulation. Some neurons (20%, 14/71), however, differed in three, four, or even all five parameters measured. Figure 8D shows distribution of neurons with a statistically significant difference in at least one parameter of the activity between dark and light conditions across the three-dimensional space of (i) change in the average discharge rate vs. (ii) change in the duration of the PEF vs. (iii) change in the depth of modulation. One can see that the light substantially affected the activity of motor cortical neurons. Moreover, there was a relationship between changes in the average discharge rate, depth of modulation, and width of the PEF: as the discharge rate or the width of the PEF increased, the depth of modulation tended to decrease (Fig. 3.7E and F). There were some differences, however, in how different neurons responded to the light during locomotion.

Neurons with different Numbers of PEFs

Two-PEF neurons responded stronger to light as compared to both unmodulated and one-PEF cells. In the light, a much larger portion of two-PEFs neurons (25%, 6/26) had a lower average discharge rate than in the darkness as compared to one-PEF and unmodulated neurons, of which only 5% and 7% had a lower rate in the light (6/105 and 1/14, respectively; Fisher's two-tailed test, $p=0.01$). In addition, a larger portion of two-PEF cells (21%, 5/26) had a greater depth of locomotion-related frequency modulation in the light than in the darkness as compared to one-PEF neurons, from which only 6% (6/105) had a greater modulation depth in the light (Fisher's two-tailed test, $p=0.042$).

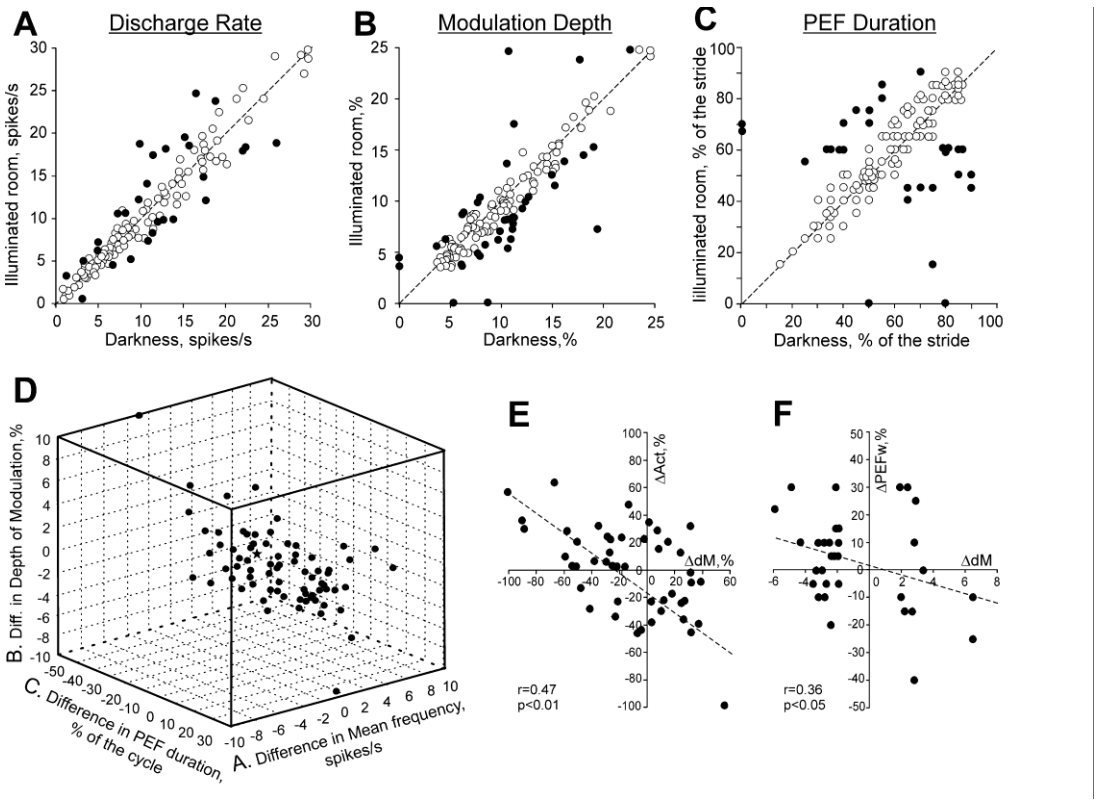


**Figure 3.7**: Comparison of activity characteristics of individual neurons between locomotion in the darkness and light. (A) Mean discharge frequency averaged over the stride. (B) Depth of frequency modulation, dM. (C) Duration of PEF; for two-PEF neurons, the combined duration of two PEFs is given. (A–C) The abscissa and ordinate of each point show the values of a characteristic of a neuron during locomotion in the darkness and light, respectively. Neurons whose characteristics were statistically significantly different during the two tasks (see Section 2.5) are shown as filled circles; others are shown as open circles. (D) Neurons with a statistically significant difference in at least one of the above parameters of the activity between two conditions. The abscissa, ordinate, and applicate of each point show the difference in a discharge characteristic of a neuron between dark and light conditions. The difference is positive if the value of the parameter was larger during locomotion in the light. The star in the middle of the cube denotes the zero point. (E) Negative correlation between the relative change in the depth of modulation, dM, and activity, Act. (F) Negative correlation between the change in the depth of modulation and duration (width) of PEF, PEFw. In (E) and (F): the abscissa and ordinate of each point show the difference in a discharge characteristic of a neuron between two illumination conditions. The difference is positive if the value of the parameter was larger during locomotion in the light. Only neurons with statistically significant difference in the dM between two walking conditions are shown. The coefficient of correlation (r) is indicated.

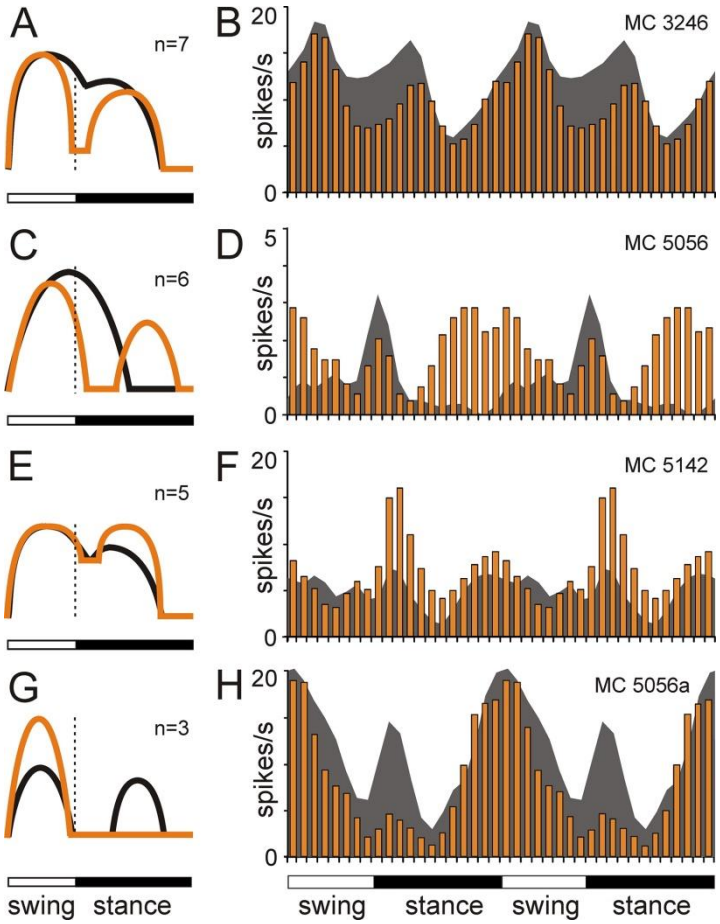


**Figure 3.8.** Differences in discharge patterns of neurons during locomotion in the darkness and light. (A) A schematic presentation of the most frequently observed type of difference in the discharge pattern. In the light, a subtle trough within the one PEF seen during walking in the darkness deepens and divides the PEF into two. The black line shows activity during walking in the darkness, and orange line shows the activity during walking in the light. The vertical dotted line shows end of swing and beginning of stance phase. (B) An example activity of a neuron exhibiting this behavior. The dark gray area histogram shows the activity of the neuron during locomotion in the darkness. The orange bar histogram shows the activity during locomotion in the light. To promote visualization of the difference in activities between two tasks, the stride cycle is shown twice. (C and D): same as (A and B) but showing the second most frequent type of discharge pattern difference. The transition from one- to two-PRF discharge pattern occurred because in the light a new PEF appeared within the former trough. (E and F): same as above but showing the third most frequent type of discharge pattern change in the light from one to two PEFs per cycle: by an increase in activity within a part of the PEF. (G and H) Same as above but showing the discharge pattern change from two- to one-PEFs per cycle.

Neurons with different Receptive Fields

A larger portion of hindlimb-related cells (36%, 8/22) had different discharge rates during walking in the darkness and light as compared to forelimb-related cells (14%, 11/76; Fisher's two-tailed test, p=0.032). In addition, all but two of the hindlimb-related neurons discharged at a lower frequency in the light than in the dark, unlike forelimb-related cells, which most often were more active in the light (Fisher's two-tailed test, p=0.015).

Of the nine visually responsive cells, two had a higher discharge rate and one had a lower rate in the light than in the darkness. Of five cells with a discharge rate that was step-phase related in the darkness, two had a lower depth of modulation in the light. Differences in the number of PEFs were inconsistent: the number of PEFs was smaller in two and greater in one cell, and there was no difference in two other cells. There was no difference to the duration of the PEF in any of visually responsive cells between light and dark conditions.

Fast- and Slow- Conducting PTNs

The discharge rate of 45% (23/51) of PTNs was different during locomotion in the light and darkness. Nearly a quarter of fast-conducting PTNs, 22% (7/32), had a lower activity in the light. This was different from the behavior of slow-conducting PTNs, which never had a lower discharge rate in the light (Fisher's two-tailed test, p=0.037). Overall, considering fast and slow-conducting cells together, PTNs had a tendency to discharge at different rates during locomotion in the light and darkness more often than unidentified neurons. In contrast to the unidentified neurons, which often had a shorter PEF in the light than in the darkness, the PEF of PTNs almost never was shorter in the light (Fisher's two-tailed test, p=0.01). PTNs tended to have two or more parameters of

the activity different between light and dark conditions, while in unidentified neurons, most often only one parameter was different.

Despite significant differences in the locomotion-related activity between light and dark conditions displayed by nearly half of the neurons considered in this study, the average discharge rate, depth of modulation, and duration of PEF for the entire motor cortex population remained similar (Fig. 3.5). This was because, between the conditions, a roughly similar number of neurons changed their activities in opposing ways.

Effect of Light Wears Off Gradually

The activity of 46 neurons was analyzed separately for the first, second, and third steps made after the lights were turned off, and for the first step made after the lights were turned on. After the removal of the light, approximately half of neurons (54%, 25/46) changed their average discharge rate and/or depth of locomotion-related frequency modulation not abruptly but in a gradual manner. A representative example is shown in Fig. 10A. In the light, PTN 5046 discharged intensely during the second half of the swing phase and was much less active throughout remainder of the step cycle (Fig. 3.9Aa and Ab). The activity of the neuron during the first step made without the light was very similar (Fig. 3.9Ac and Ad). During the second step taken in the dark, however, the activity of the PTN near the end of the swing phase increased by approximately 10 spikes/s, while the activity during the rest of the stride remained unchanged (Fig. 3.9Ae and Af). During the third step in the darkness, the activity of the neuron during the stance phase decreased, forming a clear gap in the activity immediately after the peak at the end of the swing phase (Fig. 3.9Ag and Ah). The activity of the neuron during the first step taken after lights were turned on was very similar to its activity averaged over all steps in the light (Fig. 3.9Ai and Aj).

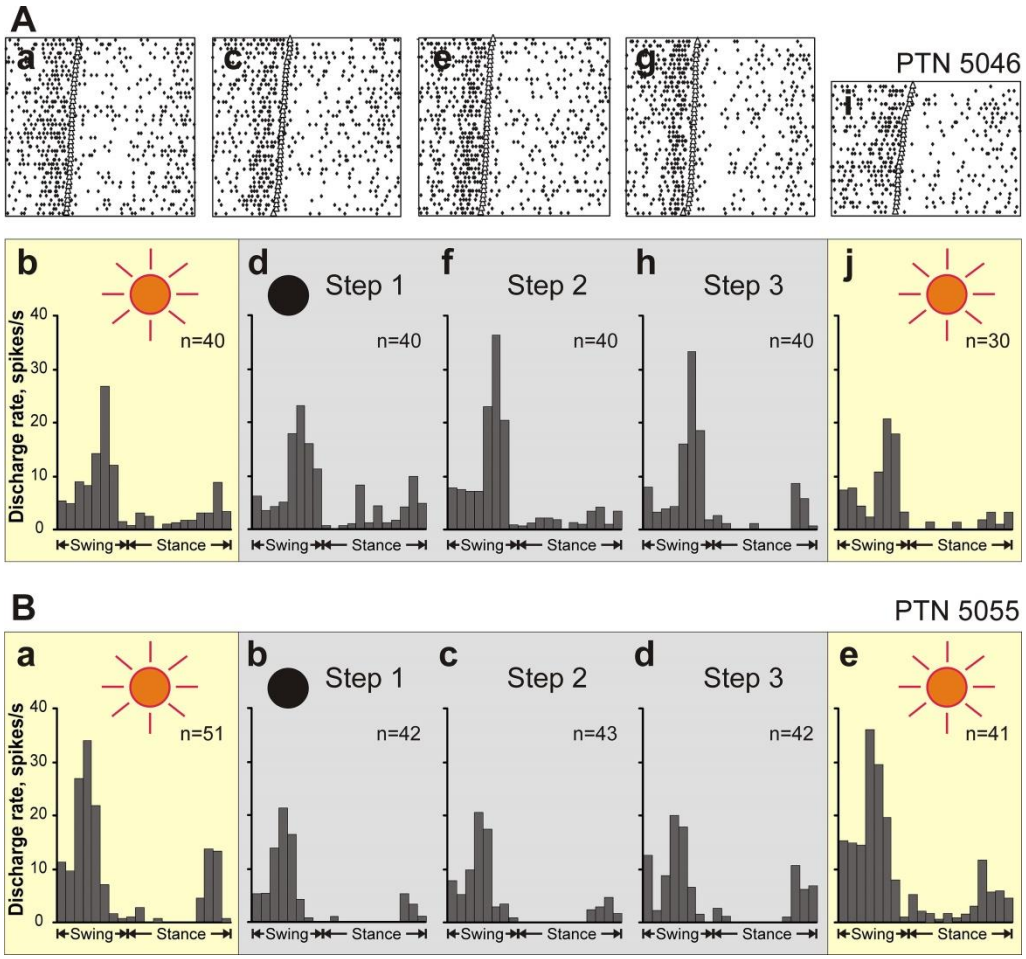


**Figure 3.9** Changes in the activity and modulation over the period of darkness. (A) An example of a neuron (PTN 5046) that changed the discharge rate and modulation depth during the period of the darkness. (Aa and Ab) The activity of the neuron during locomotion in the light is presented as a raster of 40 step cycles (a) and as a histogram (b). The duration of step cycles is normalized to 100%. In the raster, the end of swing and the beginning of the stance in each cycle is indicated by an open triangle. (Ac and Ad) The activity of the same neuron during the first step made after lights were turned off (Step 1) is shown as a raster of 40 step cycles (c) and as a histogram (d). (Ae and Af) The activity of the same neuron during the second step after lights were turned off (Step 2) is shown as a raster of 40 step cycles (e) and as a histogram (f). (Ag and Ah) The activity of the neuron during the third step in the darkness (Step 3) is presented as a raster of 40 step cycles (g) and as a histogram (h). (Ai and Aj) The activity of the neuron during the first step made after lights were turned back on is shown as a raster of 30 step cycles (i) and as a histogram (j). (B) Histograms of the activity of another neuron (PTN 5055) that had different activities between light and dark conditions but did not change the activity during the period of the darkness.

Out of 25 neurons that changed their activity gradually after removal of the light, 15 changed their average discharge rate, typically increasing it by 3.7±0.8 spikes/s, either between the 1st and the 2nd or between the 2nd and the 3rd step in the darkness. Fourteen cells changed the depth of modulation by increasing or decreasing it by 3.8±0.4 %. This too could occur either between the 1st and the 2nd or between the 2nd and the 3rd step in the darkness. Among those there were four neurons that changed both the discharge rate and the depth of modulation as did PTN 5046 shown in Fig. 3.9A. In contrast, 21 neurons out of 46 tested had already changed their activity during the first step in the dark and did not alter it more during the period of the darkness maintaining the change until after the lights came back on (Fig. 3.9B).

## Discussion

Characteristics of neuronal discharges in the motor cortex during locomotion found in this study are consistent with earlier reports (Armstrong and Drew, 1984; Beloozerova and Sirota, 1985, 1993a; 1993b; Drew, 1993; Prilutsky et al., 2005; Stout and Beloozerova, 2012; Widaewicz et al., 1994). In accordance with previous findings, the activity of nearly all neurons in layer V was modulated in the rhythm of strides, with the great majority exhibiting one PEF per cycle. PEFs of forelimb-related neurons were distributed almost evenly throughout the stride, while those of hindlimb-related neurons were concentrated in the swing phase, so the group of hindlimb-related cells was substantially more active during swing (Fig. 3.6).

This is the first investigation of the activity of the motor cortex during locomotion with and without light and thus the ability of subjects to see. Comparison of motor cortex population activity during locomotion in the darkness and light, both within this study (Figs. 3.6 and 3.7) and with data on locomotion in the light that were previously

published (Armstrong and Drew, 1984a, b; Beloozerova and Sirota, 1993a; 1993b; Drew, 1993; Prilutsky et al., 2005; Stout and Beloozerova, 2012) showed that they are largely similar. However, a comparison of activities of individual neurons during locomotion in the darkness and light revealed that the light has a significant effect on discharges of cells in the motor cortex. In 49% of neurons the mean discharge rate, depth of locomotion-related frequency modulation, width of PEF, and/or the number of PEFs were affected by the light (Figs. 3.7-3.9).

We believe that the differences in firing behavior of motor cortical neurons between the two lighting conditions are due to the differences in available visual information, not to a difference in the stride kinematics. We are confident in this assessment in part because during data analysis, we specifically selected and compared strides in the darkness and light that closely matched in duration and duty factor (Fig. 3.3A and B). In addition, the three-dimensional kinematics analysis showed that during these selected steps, the movements of the forepaw were similar (Fig. 3.3C) between the two conditions and only very minor differences existed in the movements of the scapula (Fig. 3.3D). Moreover, from six muscles tested, including three from the hindlimb, two did not show any appreciable differences in EMG timing or amplitude between the two lighting conditions, and four had only minor differences (Fig. 3.4). One may suggest that observed differences in neuronal discharges in the motor cortex during locomotion in the darkness and light reflect different levels of attention to the task by the animal in different conditions. While this cannot be completely ruled out within our experimental paradigm, we wish to note that during our testing, the main focus of subjects' attention remained unchanged regardless of the illumination condition: cats' behavior was driven solely by their desire to complete a round of walking around the chamber to receive a food reward.

The conclusion that the light and thus the ability to see, affects the activity of neurons in the motor cortex during locomotion is consistent with both anatomical data and previous reports on widespread distribution of visual responsiveness in frontal cortex in anesthetized or awake but paralyzed cats (Garcia-Rill and Dobrovsky, 1971, 1974; Weyand et al., 1999). In particular, Weyand and colleagues (1999), working with awake paralyzed cats, showed that in area 4γ, the area we recorded from, cells are reliably responsive to visual stimuli, and respond best to the light on/off stimulus. The motor cortex receives visual information via two groups of routes: cortical and subcortical. The input from the parietal cortex, areas 5 and 7, is typically viewed as the main avenue. Indeed, both of these areas respond strongly to visual stimuli and, especially area 5, project intensively to the motor cortex (Babb et al., 1984; Andujar and Drew, 2007; Ghosh, 1997). In our preliminary study, however, we found that neurons of layer III in area 5b, which are the only neurons projecting to the motor cortex from this area, have rather low activity during locomotion, both in terms of proportion of neurons involved (58%) as well as their average discharge rates (2.5 spikes/s) and have relatively similar activity during vision-independent locomotion on a flat surface and vision-involved locomotion along a horizontal ladder (Beloozerova et al., 2011). Therefore it appears unlikely that the rather sparse activity of visually unresponsive parietal-to-motor cortex projection is actually fit to assure quite pronounced visual responses in the motor cortex during locomotion. On the other hand, when we investigated locomotion-related activity of the ventrolateral thalamus, which links the motor cortex with the lateral cerebellum, we found that upon transition from a visually-independent to a visually-demanding locomotion task, the activity of 79% of neurons in the ventrolateral thalamus change (Marlinski et al., 2012). The most typical changes were: an increase in the magnitude of the stride-related frequency modulation, a more precise focusing of the

discharge in a particular phase of the stride, and a change in the number of PEFs per cycle. In addition, the average discharge rate of neurons in the ventrolateral thalamus is roughly twice as high as that of neurons in the motor cortex. Taking into account the rich visual inputs of the lateral cerebellum, from which the ventrolateral thalamus receives one of its main inputs (rev. in Stein and Glickstein, 1992; Glickstein, 2000) we concluded that the ventrolateral thalamus must play an important role in transmitting processed visual signals to the motor cortex, and this is discussed in detail in chapter 5 below.

Our finding that the mean discharge rate in 19% of motor cortical neurons, the depth of stride-related frequency modulation in 24%, the duration of the PEF in 19%, and/or the number of PEFs in 26% of them was different between walking in the darkness and light points to three mechanisms by which visual information can affect discharges in the motor cortex: a "tonic" mechanism of changing the average activity rate (19% of neurons), an "in-phase" mechanism of altering the magnitude of already existing activity modulation (24% of neurons), and an "out-of-phase" mechanism that alters the pattern of stride-related activity modulation by changing the duration of the PEF or even the number of PEFs (19% and 26% of neurons, respectively). The tonic mechanism was seen more often in the hindlimb-related neurons and typically reduced their discharge rate in the light. A reduction of cortical influence on the spinal hindlimb-related networks might render them more susceptible to other influences, including propriospinal inputs from forelimb-related circuits. This will make the hind limbs "listen" more to what the forelimbs are doing when the cat walks in the light. The fact that the "out-of-phase" mechanism is so common suggests that the visual information received in the motor cortex during locomotion often fundamentally structures locomotion-related discharges of its neurons. For example, the activity of two-PEF neurons, which presumably discharged their second PEF in the darkness in response to

an input from a second limb controller (Zelenin et al., 2011), was often depressed, their depth of frequency modulation enhanced, and their discharge pattern often re-written to a one-PEF pattern in the light (Figs 3.8G and H). In parallel, discharges of PTNs, with a one-PEF or two-PEF pattern alike, were shaped to become more locomotion-phase specific.

It was demonstrated in several studies that simple locomotion on a flat surface does not require participation of the motor cortex to be successful while locomotion over complex terrains strictly does (Beloozerova and Sirota, 1993a; Chambers and Liu, 1957; Drew, 1996; Friel et al., 2007; Liddell and Phillips, 1944; Metz and Whishaw, 2002; Trendelenburg, 1911). It was previously suggested that the locomotion-related modulation of the activity of motor cortical neurons during simple locomotion has an informational character (Beloozerova and Sirota, 1993a). This modulation sets, for each individual neuron, the allowable phase of the cycle when, should a need arise, a descending influence is permissible. It thus allows the motor cortex to affect subcortical and spinal locomotor mechanisms during corrections of movements in complex situations without interruption of the on-going locomotor rhythm. Results of the current study show that these permissible time windows are adjusted based on the availability of visual information.

It has been previously shown that during some motor tasks the activity of selected subpopulations of the motor cortex does not correlate with the activity of muscles or movement mechanics and appears to have other control targets (e.g., Beloozerova and Sirota, 1993b; Beloozerova et al., 2005, 2006; Kurtzer, 2005). In addition to determining the allowable phase of the response during voluntary gait modifications as suggested above, another possible function of these neurons may be to contribute to the activation and reconfiguration of the brain stem–spinal locomotor

networks. Such a function seems to be necessary when one considers the enormous variety of modifications to locomotion available to animals and humans. Among other possible functions, not related directly to the production of motor output, the motor cortical neurons could participate in the modulation of afferent signals' transmission to different motor centers.

In addition to these possible roles of the activity of the motor cortex during locomotion, the fact that it took a step or two for the activity of about half of neurons to wear off the effect of the light when the lights were extinguished suggests that during locomotion the activity of neurons in the motor cortex may encode memories of the visual scene. It is interesting, however, that the activity of few cells that responded to visual stimuli at rest, during locomotion in the darkness was often step cycle-unrelated, and that these cells reacted inconsistently and rather weakly to introduction of the light. These observations suggest that these motor cortical neurons are members of a separate network, which is perhaps devoted to detection of particular visual features of the environment.

In conclusion, in this study we extend previous observations of visual responsiveness of cells in the motor cortex obtained in anesthetized and awake but paralyzed cats to freely walking cats, and demonstrate that during locomotion the responsiveness to light in the motor cortex is very common, typically stride phase-depended, and often significantly modifies the locomotion-related pattern of the activity observed during walking in the darkness.

# 4. ACTIVITY OF SOMATOSENSORY RESPONSIVE NEURONS IN HIGH SUBDIVISIONS OF SI CORTEX DURING LOCOMOTION

Published in the Journal of Neuroscience (Favorov et al. 2015)

## Abstract

Responses of neurons in the primary somatosensory cortex (SI) during movements are poorly understood, including even during such simple task as walking on a flat surface. In this study we have analyzed spike discharges of neurons in the rostral bank of the ansate sulcus (areas 1-2) in two cats while cats walked on a flat surface or on a horizontal ladder, a complex task requiring accurate stepping. All neurons (n=82) that had receptive fields on the contralateral forelimb, during simple locomotion exhibited frequency modulation of their activity that was phase-locked to the stride cycle. Neurons with proximal receptive fields (RFs: upper arm/shoulder) and pyramidal tract-projecting neurons (PTNs) with fast-conducting axons tended to fire at peak rates in the middle of the swing phase while neurons with RFs on the distal limb (wrist/paw) and slow-conducting PTNs typically showed peak firing at the transition between swing and stance phases. Eleven out of 12 neurons with tactile receptive fields on the volar forepaw began firing towards the end of swing, with peak activity occurring at the moment of foot contact with floor, thereby preceding the evoked sensory volley from touch receptors. Requirement to step accurately on the ladder affected 91% of the neurons suggesting their involvement in control of accuracy of stepping. During both tasks, neurons exhibited a wide variety of spike distributions within the stride cycle, suggesting that during either simple or ladder locomotion they represent the cycling somatosensory events in their activity both predictively before and reflectively after these events take place.

**Acknowledgements:** This study was supported by NIH R21 NS-078619 (to OVF), ASU–BNI Interdisciplinary Graduate Program in Neuroscience (to WUN), and NIH R01 NS-058659 (to INB).

## Introduction

Primary somatosensory cortical area (SI), comprising cytoarchitectonic areas 3a, 3b, 1 and 2, is structurally and functionally a heterogeneous cortical region. While neurons in area 3b possess receptive fields (RFs) closely resembling those of the thalamic neurons, neurons in area 1 and especially in area 2, which receive their major input from area 3b, possess larger RFs and exhibit greater selectivity for higher-order stimulus features (Iwamura, 1998). This indicates that areas 1 and 2 occupy hierarchically high levels in cortical processing of somatosensory information (Felleman and Van Essen, 1991). It was shown that neurons in area 2 are involved in haptic perception during object manipulation (Koch and Fester, 1989; Iwamura, 1998; Gardner et al., 2007) however, due to technical difficulties in monitoring and controlling spatiotemporal patterns of physical interactions between the hand and manipulated objects, the nature of information-processing tasks performed by area 2 neurons, their contribution to perception, or even most basically the somatosensory representation of events by these neurons remain essentially unknown.

Locomotion is a behaviorally prevalent form of object-body interactions. During routine locomotion, a regularly repeating pattern of proprioceptive input to SI is periodically supplemented by tactile input coming from skin areas of the volar feet. It is believed that this tactile input enables perception of the properties of the walking surface. The study by Fitzsimmons and colleagues (2009) in rhesus macaques walking with their hind limbs on a treadmill showed that information about foot contact with the

ground can be readily extracted from spike firing patterns of 40-150 neurons simultaneously recorded in SI areas 1-2, along with information about speed of walking, step length, positions and angles of the hip, knee and ankle joints, and leg muscle contractions. In sitting monkeys, however, it appears that, in addition to the somatosensory information, SI receives an efference copy signals from the motor system. The major evidence for this is the fact that many SI neurons raise their spike discharge activity up to few hundreds of milliseconds prior to the onset of an actively generated limb movement, preceding even EMG activity (e.g., Soso and Fetz 1980; Nelson, 1987; Lebedev et al., 1994; London and Miller, 2013). Furthermore, in the vibrissae system, it was shown that both the somatosensory and efference copy signals contribute to activation of neurons in the SI during whisking (Fee et al., 1997; Kleinfeld and Deschênes, 2011). Because of difficulties associated with obtaining stable single neuron recording in walking subjects, however, besides the study of Fitzsimmons and colleagues in monkeys (2009) and few studies in rats (Chapin and Woodward, 1982a,b), which investigated treadmill locomotion, little is known about what takes place in SI during walking.

Here we have analyzed spiking activity of 82 neurons from the forelimb representations of two cats that walked in an experimental chamber on a flat surface or on crosspieces of a horizontal ladder, a task that required accurate placing of paws.

## Methods

Extracellular recordings from single neurons in areas 1 and 2 of the SI cortex were obtained during chronic experiments in cats. Two adult females were used. Please refer to the chapter 2 for details of surgical preparation and recording techniques. Only the specific techniques are briefly described here.

Locomotor Tasks

Two locomotor tasks were used: walking on a flat surface (Fig. 4.1A), and walking on crosspieces of a horizontal ladder (Fig. 4.1B). Biomechanics and limb muscle activities in the cat during walking on the flat surface and on the horizontal ladder in a similar experimental setup have been previously described in details (Beloozerova et al., 2010). It was shown that walking along the ladder is similar to walking on the flat surface in nearly all kinematic and EMG parameters tested except that the variability of stride lengths is much smaller during ladder walking because ladders' crosspieces narrowly determine where the cat steps. It was shown in several studies that while walking on the flat surface does not require vision and can be accomplished without the forebrain, walking on a ladder relies on vision and requires thalamo-cortical processing to be successful (Trendelenburg, 1911; Liddell and Phillips 1944; Chambers and Liu 1957; Beloozerova and Sirota 1993a, 2003; Sherk and Fowler 2001; Metz and Whishaw 2002; Friel et al. 2007; Marigold and Patla 2008; Rivers et al. 2014).

Cats were trained to walk in an experimental chamber with two connected parallel corridors (2.5 x 0.3 m each) that is described in the methods section (Chapter 2). In one corridor the walking surface was flat, while 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 flat floor (Beloozerova and Sirota, 1993, Beloozerova et al., 2010). The crosspieces had flat tops, and were 5 cm wide, which was slightly greater than the 3 cm diameter support area of the cat foot. Therefore cats had had full support for their paws.

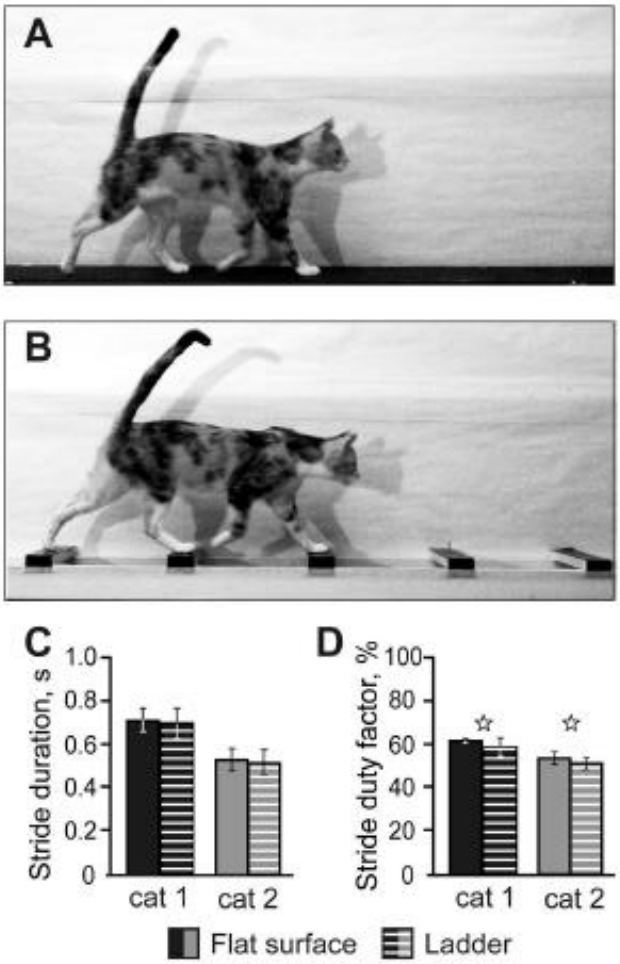


**Figure 4.1.** Locomotor tasks. The experimental box was divided into two corridors. In one corridor the floor was flat (**A**), while the other corridor contained a horizontal ladder (**B**). **C:** Duration of strides during flat surface and ladder walking**. D:** Stride duty factor (proportion of the stance phase in the step cycle) during flat surface and ladder walking**.** Stars indicate statistically significant difference ($p$<0.05, $t$-test).

Surgical Procedures

The surgical procedure was similar to that described in the general methods section (Chapter 2). On the left side of the head, the dorsal surface of the sigmoid gyrus was exposed by removal of approximately 1.6 cm$^2$ of bone and dura mater. The region of the SI cortex was visually identified based on surface features and photographed (Fig. 4.2A, B). The exposure was covered with a 1 mm thick acrylic plate. The plate was pre-perforated with holes of 0.36 mm in diameter spaced by 0.5 mm, and the holes were filled with bone wax. It allowed for later insertion of recording electrodes in the SI

cortex. In addition, two 26 gauge hypodermic guide tubes were implanted vertically above the medullary pyramids with tips approximately at the Horsley-Clarke coordinates (P7.5, L0.5) and (P7.5, L1.5), and the depth of H0. They were later used for physiologically-guided insertion of stimulating electrodes into the pyramidal tract (Prilutsky et al., 2005). These electrodes were used for identification of pyramidal tract neurons (PTNs) in the awake animal.

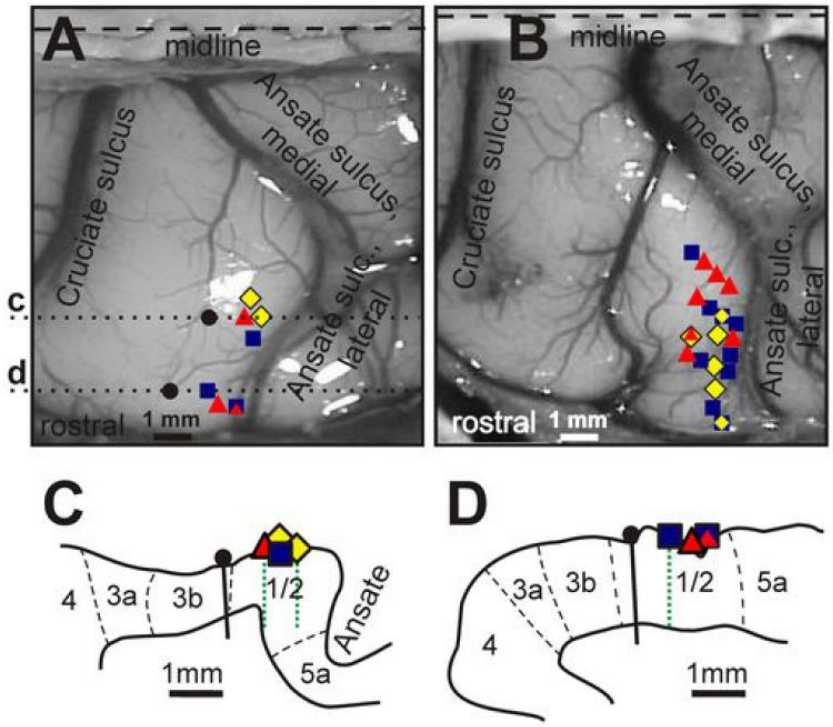


**Figure 4.2.** Location of neurons: **A, B:** Areas of recording in the forelimb representation of the left SI in cats 1 (**A**) and 2 (**B**). Microelectrode entry points into the cortex are shown by colored symbols. ■ – Penetrations where the majority of recorded neurons had RFs on the shoulder, arm and/or forearm and were not responsive to stimulation of the wrist or paw; ♦ – Penetrations where most recorded neurons had RFs on the arm and/or forearm, and also responded to stimulation of the wrist and/or paw; ▲ – Penetrations where most neurons had RFs on the wrist and/or paw. Symbols with combined colors indicate points were approximately equal

## Single-Unit Recording

Extracellular recordings from the rostral bank of the lateral ansate sulcus were obtained using tungsten varnish-insulated microelectrodes (120 μm in outer diameter; FHC Inc., Bowdoin, ME) using procedures similar to those in the study described in chapter 2. An example of a raw recording from a neuron during locomotion is shown in Figure 4A.

## Identification of Neurons and Classification of their Receptive Fields

The waveform analysis was employed to discriminate and identify the spikes of individual neurons using the Power-1401/Spike-2 system waveform-matching algorithm. Only the neurons with stable spike shape were used for analysis. In addition, all neurons were tested for antidromic activation from the pyramidal tract as described in chapter 3. A stable response latency and consistent positive collision response were required for neurons to be classified as PTNs. For calculation of the conduction velocity, the distance between recording electrodes in the SI and stimulation electrodes in the pyramidal tract was estimated as 50 mm. This calculation accounted for the curvature of the pathway and also for the spread of the current and refractory period at the site of stimulation.

The somatic RFs of neurons were examined in animals resting with their head restrained. Somatosensory stimulation was produced by lightly stroking fur, tapping the skin with small glass rods, palpation of the muscle bellies and tendons, as well as by passive joint movements. The spatial extent of an RF was determined by identifying the entire area from which action potentials could be elicited by the most effective mode of stimulation.

Locomotion Data Analysis

From each walk down the corridor, two or three strides made in the middle of the walkway were selected for the analysis. It was previously shown that the strides in the middle of the corridor are normally made at a nearly constant speed with no net acceleration or deceleration, and that their average stride length during flat surface and ladder locomotion is identical (Beloozerova et al., 2010). To compare the activity of neurons during two locomotor tasks we used only the strides for which the average duration in the two tasks differed by less than 10%

The onset of swing of the right forelimb (contralateral to the SI recording site) was taken as the beginning of the step cycle. The duration of each step cycle was divided into 20 equal bins, and a phase histogram of the discharge rate of the neuron in the cycle was generated and averaged over all selected cycles (please see chapter 2 for more detailed description). The stride-to-stride variability of the mean discharge rate of individual neurons was calculated as $V$ = (Standard Deviation)$^2$/(Mean rate over the stride cycle). The "depth" of modulation, *dM*, PEF (Fig. 4.4), and the Preferred Phase of one-PEF neurons were calculated as described above.

The effects of locomotor task on parameters of strides, discharge rate of individual neurons, and mean parameters of group activities of neurons were tested using two-tailed Student's *t*-test. When comparing depth of frequency modulation dM of individual neurons, their preferred phases of activity, and duration of PEF during the two walking tasks, differences equal to or greater than ±2, ±10%, and ±20%, respectively, were considered significant (see chapter 2 for details).

Non-parametric $\chi^2$ test or *t* test for proportions were used for comparison of categorical data. For all tests, the significance level was set at $p = 0.05$. Unless indicated otherwise, for all mean values the standard error of the mean (SEM) is given.

Histological Procedures

In deeply anesthetized cats, reference loci were made near the recording sites, and the brains were obtained from the perfused cats (see methods chapter). The 50 $\mu$m sections were stained for Nissl substance with cresyl violet. The position of stimulation electrodes in the medullar pyramids was verified. The positions of recording tracks in the cortex were estimated in relation to the reference lesions and with regard to cytoarchitectonic boundaries separating areas 3b, 1, 2, and 5a (identified by criteria of Hassler and Muhs-Clement, 1964; Dykes et al., 1980; McKenna et al., 1981) (Fig. 4.2). Namely, in parasagittal sections of the lateral portion of the posterior sigmoid gyrus, moving in the rostrocaudal direction, one can recognize sequentially areas 3b, 1 and 2. In the area 3b, granular layer IV is readily detected as having a thick band of small, densely packed cells bounded below by cell-poor layer V and above by sublayer IIIc containing darkly stained pyramidal cells (Ito and Craig, 2003, Leclerc et al., 1994). Area 1 is characterized by a clear laminar arrangement and a radial appearance involving all laminae (Ghosh, 1997a), and the presence of large pyramidal cells in widened layer V (Leclerc et al., 1994). In the forelimb area, a clear-cut boundary between areas 1 and 2 could reliably be determined (McKenna et al., 1981). Area 2 usually occupies most of the rostral wall of the lateral ansate. It possesses a thick layer I, wide laminae IIIc and V with many large pyramidal cells in layer Vb (Ghosh, 1997a), and gradually declining width of layer VI. In the lateral ansate, it extends towards the fundus of the sulcus where it borders area 5a. In area 5, laminae III and V are narrower, and lamina IV is well defined

(Andujar and Drew, 2007; Ghosh, 1997a). The overall area pattern over the flattened (planar reconstruction of) hemisphere was most recently reported in general (Clasca et al., 2000), and in greater detail by Andujar and Drew (2007).

## Results

### Location and Characteristics of Neurons

This study was confined to SI neurons that were located in the rostral bank of the lateral ansate sulcus and had RFs on the contralateral forelimb. Eighty two such neurons were isolated and studied in 26 microelectrode penetrations: 25 neurons from cat 1 and 57 from cat 2. Figures 2 A and B show, relative to the ansate sulcus, the entry points of microelectrode penetrations, in which neurons were collected in cat 1 (A) and cat 2 (B). Histological examination showed that all electrode tracks were confined to the cytoarchitectonic areas 1 and 2 (Fig. 4.2 C, D).

The studied neurons had diverse receptive fields (RFs). The size of the RF varied from about 2 $cm^2$ to as large as nearly entire lateral or medial aspect of the limb (Fig. 4.3). The most commonly encountered RFs, found in 15% of the neurons, were small cutaneous fields confined to the ventral surface of the forepaw (Fig. 4.3A, C). The rest of neurons had RFs of varying sizes and positions throughout the forelimb (Fig. 4.3B, D-F). Overall, the majority of neurons (58%) received their peripheral input from cutaneous mechanoreceptors, 29% of neurons received their input from mechanoreceptors innervating joints or deep tissues, and 13% of neurons received mixed skin and deep inputs. The skin RFs were more commonly found in distal forelimb areas compared to proximal forelimb areas, where deep RFs were more common. The mixed submodality RFs included both distal and proximal forelimb regions more evenly (Fig. 4.3G). The

majority of neurons were excited by stimulation of their RF, while only five cells were inhibited.

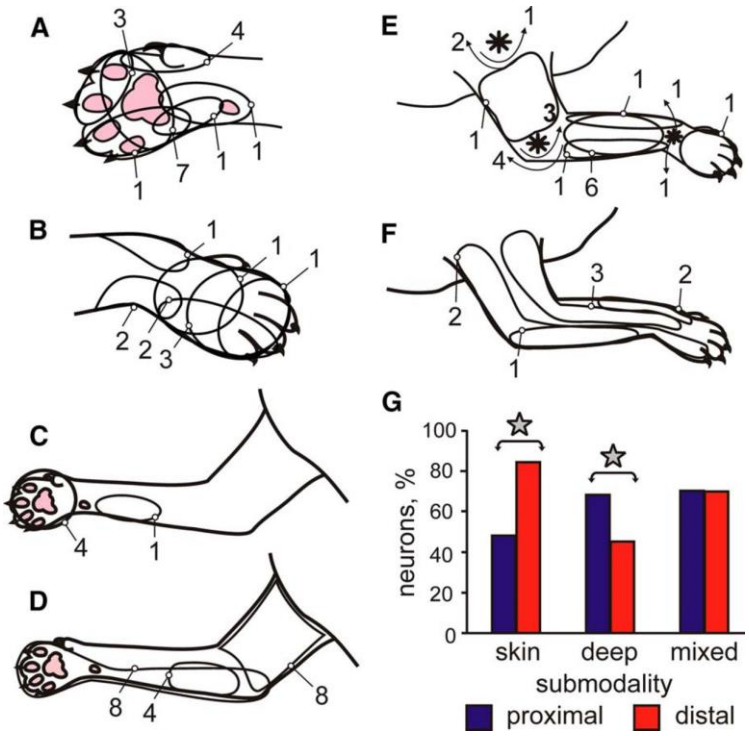


Figure 4.3. Receptive fields of neurons. A - F: Outlines show locations of RFs of individual neurons on the forelimb. Numbers indicate the number of neurons that had an RF in each location. In E, asterisks denote shoulder, elbow, and wrist joints, and arrows show direction of the joint movement, to which the cells responded. G: proportions of neurons with distal (wrist/paw) vs. proximal (upper arm/forearm) RFs among neurons with skin, deep, and mixed RFs. Stars indicate statistically significant difference ($p<0.05$, t-test).

Twenty four cells (29%) responded antidromically to electrical stimulation of the pyramidal tract. The latencies of responses varied in the range of 0.8-3.0 ms. Estimated conduction velocities were between 17 and 60 m/s. Approximately one half of neurons (13/24) responded at 2.0 ms or faster, conducting at 25 m/s or faster, and thus were classified as “fast conducting” PTNs; the other half was “slow conducting” PTNs (Brookhart and Morris, 1948; Bishop et al. 1953; Takahashi, 1965). Sixteen out of 26

penetrations (62%) yielded at least one PTN, and these tracks were distributed throughout the studied cortical area without a clear pattern.

Characteristics of Locomotion and an Example of Firing Behavior of a Neuron

During recording of each neuron, cats walked between 10 and 50 (typically 20-30) times down each of the chamber's corridors (25±8, mean±SD), and the number of strides selected for the analysis according to the criteria described in the Methods section ranged between 20 and 80 (45±15) for both flat surface and ladder walking. For different neurons, the average duration of selected strides was between 450 and 800 ms, which corresponded to the speed of walking of 0.8-1.1 m/s. Although cat 2 walked faster than cat 1, for each cat the average duration of the strides during two tasks was the same ($p>0.05$, t-test; Fig. 4.1B). The ratio of stance duration to the duration of the cycle (the stride duty factor) was 0.45–0.64. In both cats, the duty factor was smaller during the ladder task ($p<0.05$, t-test), but the difference was small: 4-5% (Fig. 4.1C). The gait that cats used during locomotion was a walk with the support formula of 2-3-2-3-2-3-2-3, which indicates the number of limbs supporting the body during different phases of the stride cycle (Hildebrand, 1965).

An example of a neuron's spiking activity during standing, walking on the flat surface and along the ladder is shown in Figure 4. This fast-conducting PTN had a slowly-adapting cutaneous RF covering the glabrous skin of the entire central pad, which should be fully in contact with the ground and thus well stimulated when the cat was standing. Nevertheless, this neuron had low spike firing activity while standing (6.3 spikes/s; marked by a dashed horizontal line in Fig. 4.4C, E). During locomotion, however, the neuron's average activity rose substantially to 18.8 spikes/s on the flat surface and 18.2 spikes/s on the ladder and was prominently modulated with respect to

the step cycle. During walking on the flat surface, the neuron was active during most of the swing and first half of the stance phase reaching frequency of 57 spikes/s at the beginning of stance and was less active during the end of the stance and early swing phases. During walking along the ladder, the neuron's firing activity was even more strongly modulated. The neuron became still more active during transition from swing to stance, now reaching discharge frequency of 80 spikes/s while its activity during first half of the swing phase decreased. The rasters in Figs. 4.4B and D show the activity of the neuron across 40 individual strides during flat surface walking (B) and 37 strides during ladder walking (D). The pattern of activity was very consistent across strides of each locomotor task. The activity is summed in Figs. 4.4C and E showing a histogram of the neuron's firing rate across the step cycle during flat surface (C) and ladder (E) walking. The period of elevated firing (PEF, see definition in Methods) is indicated by a black horizontal bar: it spanned most of the swing phase and the first third of the stance phase during flat surface walking but was confined to the swing-to-stance transition phase and was only half as long during walking along the ladder. The preferred phase (indicated by a circle in Figures 4.4C and E) was at the very end of the swing phase during flat surface walking and at the very beginning of the stance phase during ladder walking.

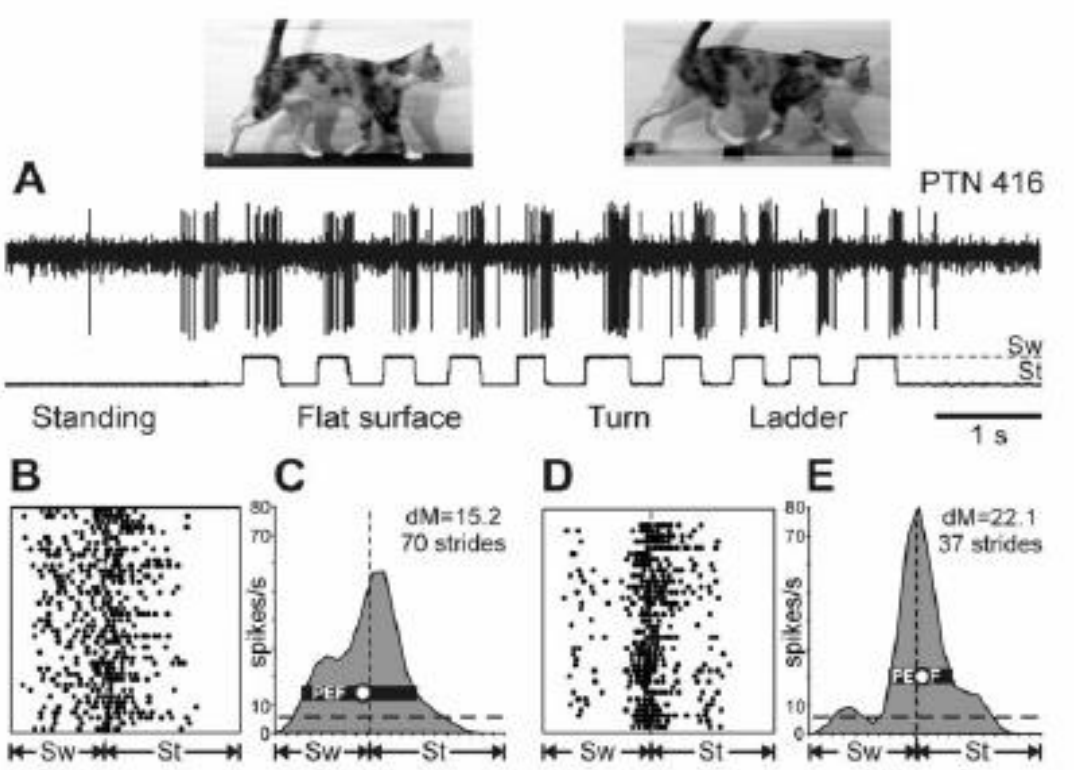


**Figure 4.4.** Exemplary activity of a neuron during standing and walking on the flat surface and horizontal ladder. **A:** Activity of PTN 416 during standing and walking. The bottom trace shows the swing (Sw) and stance (St) phases of the step cycle of the right forelimb that is contralateral to the recording site in the cortex. **B, C:** Activity of the same neuron during flat surface locomotion is presented as a raster of 40 step cycles (**B**) and as a histogram of 70 step cycles (**C**). In the rasters, spike occurrences are indicated with dots. In the raster and histogram, the duration of step cycles is normalized to 100% and, in the histogram, the interrupted horizontal line shows the level of activity during standing. The horizontal black bar shows the PEF and the circle indicates the preferred phase as defined in the Materials and Methods. ***D***, ***E***, Activity of the same neuron during ladder locomotion presented as a raster (***D***) and as a histogram (***E***) of 37 step cycles. In ***B***–***E***, vertical interrupted lines indicate the end of stance and the beginning of the swing phase.

Effect of Locomotion on Mean Spike Discharge Rates

While the cat was standing, awaiting the reward after each trip around the chamber, all neurons were active. The mean discharge rate varied from 0.5 to 50 spikes/s and was 13.3±1.2 spikes/s on average. The activity of neurons with RFs confined to the wrist and paw was lower than that of cells with more proximal RFs (11.7±1.3 *vs.*

17.4±2.5 spikes/s, $p<0.05$, *t*-test). The mean discharge rate of neurons with skin and deep RFs however, was similar, as was the mean firing rate of fast- and slow-conducting PTNs ($p>0.05$, *t*-test).

When the cat was walking on the flat surface, the mean activity of the entire population of neurons was similar to that of during standing (16.6±1.4 *vs*.13.3±1.2 spikes/s, $p>0.05$, *t*-test). However, locomotion had diverse statistically significant effects on the mean discharge rates of individual neurons and groups of neurons. During locomotion, 44% (36/82) of cells increased their mean discharge rate, on average by three fold, while 22% (18/82) decreased it by as much as 80% compared to standing (Fig. 4.5A). The majority of neurons with RFs on the wrist and/or paw increased the discharge rate, and this group's average rate during walking was higher than during standing by 9.3 spikes/s. In contrast, neurons with RFs on the upper arm or forearm discharged with similar mean rates during standing and walking. Both fast- and slow-conducting, PTNs more often changed their discharge rate with the start of locomotion than the rest of the population ($p<0.05$, *t*-test) and typically increased rather than decreased it. Neurons with deep RFs tended to be more active during walking than those with skin fields, but the difference did not reach the level of statistical significance ($p=0.068$). Across all cells, neurons with relatively low activity during standing (<10 spikes/s) nearly always increased it with the start of locomotion, while more active neurons could either decrease or increase the activity. The stride-to-stride variability of the mean discharge rate across the cycle, *V*, was 1.68±0.89 (mean ± SD).

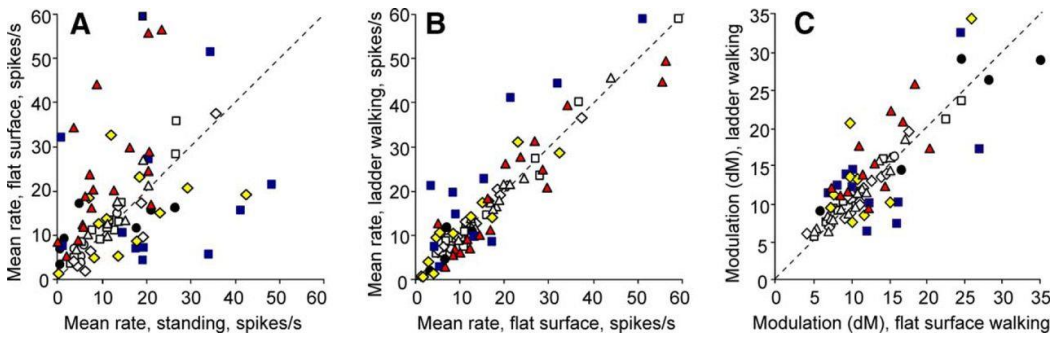


**Figure 4.5.** Comparison of activity characteristics of individual neurons between different tasks. **A**: Mean discharge frequency averaged over all selected strides of flat surface walking is plotted against the mean frequency observed during standing. **B**: Mean discharge frequency averaged over all selected strides of walking along the ladder is plotted against that of flat surface walking. **C:** Mean depth of frequency modulation during walking along the ladder is plotted against that of flat surface walking. In **A-C,** the abscissa of each point shows the values observed during one task and ordinate shows values seen during the other task. Differently shaped symbols represent neurons with RFs on different segments of the forelimb: ■ – neurons with RFs on the arm and/or forearm, which did not respond to stimulation of the wrist or paw; ♦ – neurons with RFs on the arm and/or forearm that were also responsive to stimulation of the wrist and/or paw; ▲ –neurons with RFs confined to the wrist and/or paw; ● – neurons whose RF encompassed most of the limb as well as neurons with incompletely mapped RFs. Neurons whose characteristics were statistically significantly different during the two tasks (see Methods) are shown with filled symbols, while the other

Stride-Synchronized Modulation of Spike Discharges

During locomotion, the discharge of all 82 neurons was modulated with respect to the stride: it was greater in one phase of the stride and smaller in another phase. There were two major forms of modulation: 78% (64/82) of cells had one period of elevated firing (PEF, see Methods) per stride and 22% (18/82) had two PEFs. Figures 6A and B show phase positions within the step cycle of PEFs and preferred phases of all one-PEF neurons. PEFs of different neurons were distributed throughout the cycle. Their duration varied between neurons in the range of 15-90% of the cycle, and was 52±2.5 % of the stride time on average. At any given time of the cycle, 40-75% of the cells were in their PEF (Fig. 4.6C). However, more neurons had PEFs and preferred phases during late

swing or early stance than in the opposite phase (Fig. 4.6A-C). As a result, the average discharge rate of one-PEF population was peaking at around 25 spikes/s at the end of swing/beginning of stance phase while dropping in half to about 12 spikes/s during mid-stance (Fig. 4.6D; $p<0.05$, *t*-test). Among individual neurons, the magnitude of the stride-related modulation varied. 17% (14/82) of neurons were completely silent for a part of the step cycle; the majority, however, were active throughout the cycle, while their discharge rate was modulated. The average coefficient of modulation, *dM*, across one-PEF population was 13.3±6.6 (mean ± SD)

The activity of a typical neuron with two PEFs per stride is shown in Figure 7A. This neuron had a slowly-adapting cutaneous RF on the paw. It was active during second half of the stance and first half of the swing phase reaching frequency of 52 spikes/s at beginning of swing while discharging only around 10-20 spikes/s during mid-swing and mid-stance. The neuron's activity, however, was as high as 40 spikes/s during transition from the swing to stance phases, thus forming the second, albeit a shorter, PEF. The magnitude of the activity modulation, *dM*, was 9.1. During walking along the ladder, the activity of the neuron became even more strongly modulated (*dM* =17.8, Figs. 7G and H). Although the neuron became less active during its longer PEF, the activity during the shorter PEF increased. In addition, the activity during both inter-PEF intervals further decreased, as much as almost to zero during the late swing phase. The total duration of PEF was smaller on the ladder by 20% of the stride cycle.

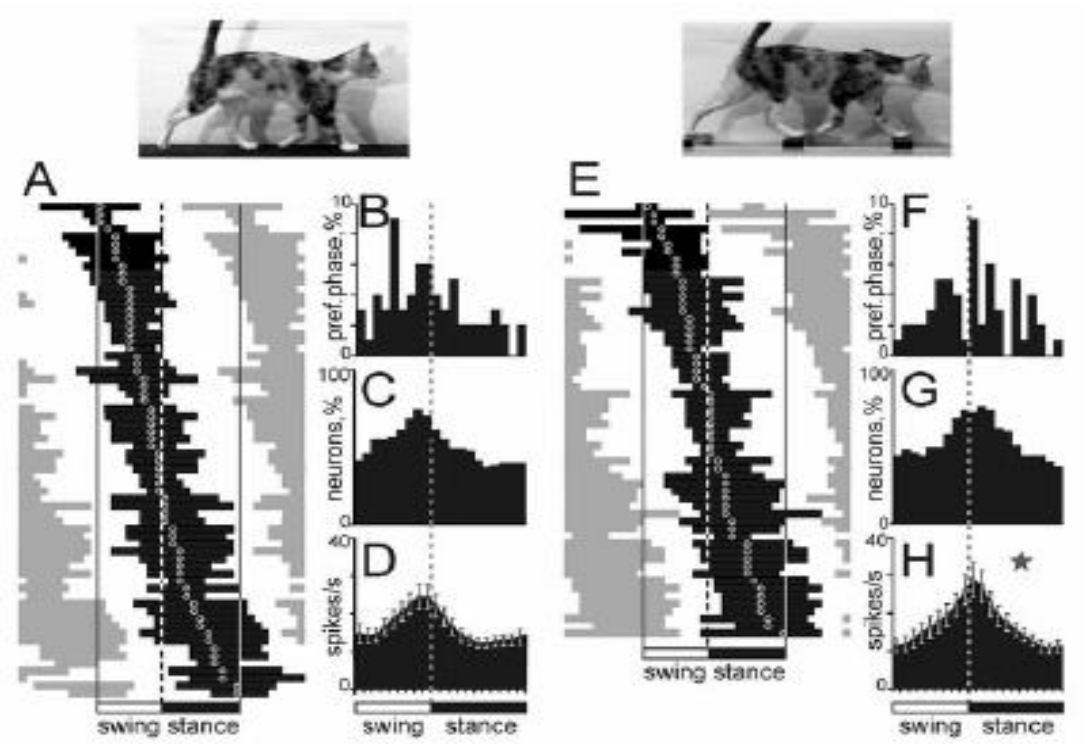


**Figure 4.6.** Population characteristics of one-PEF neurons. **A, E:** Phase distribution of PEFs during walking on the flat surface (**A**) and along the ladder (**E**). Each row represents PEF of one cell. A circular mark on a PEF denotes the preferred phase. Neurons are rank-ordered so that those with a PEF earlier in the cycle are plotted on the top of the graph. The top row shows PEF and preferred phase of the neuron, the activity of which is shown in details in Figure 4. Vertical interrupted lines indicate end of stance and beginning of swing phase. **B, F:** Distribution of preferred phases. **C, G:** Proportion of active neurons (neurons in their PEF) in different phases of the step cycle. **D, H:** The mean discharge rate at different phases of the step cycle. Error bars show SEM. Star in **H** indicates the phase during which the activity of neurons during ladder walking is higher than during flat surface walking ($p<0.05$, *t*-test).

Figure 4.7C shows phase positions within the step cycle of PEFs of all two-PEF neurons, with PEFs of the example neuron shown on the top of the graph. PEFs of different neurons were distributed throughout the cycle. Their duration varied among neurons in the range of 35-80% of the cycle and was 57±4% of the stride time on average. Thirteen of 18 neurons had PEFs that differed in duration by 10% or more of the stride cycle, “long” and “short” PEFs. The duration of the long PEF ranged from 20% to55% of the cycle, and that of the short PEF was 10-35%. In the activity of all but one of

these neurons, the longer PEF was also the stronger one, as the peak discharge rate in it exceeded that in the shorter PEF by 20±15 spikes/s. Across all two-PEF population at any given time of the cycle, 45-75% of the cells were in their PEF (Fig. 4.7E). As a group, two-PEF neurons had two approximately equal activity peaks: one at the very end of the swing phase, which was similar to the peak of the one-PEF neuronal population and the other peak near the transition from the stance to swing phase (Fig. 4.7F). Among individual neurons, the magnitude of the stride-related modulation varied, with the average $dM$ = 10.1±0.6 slightly lower than in the one-PEF group ($p$<0.05, $t$-test).

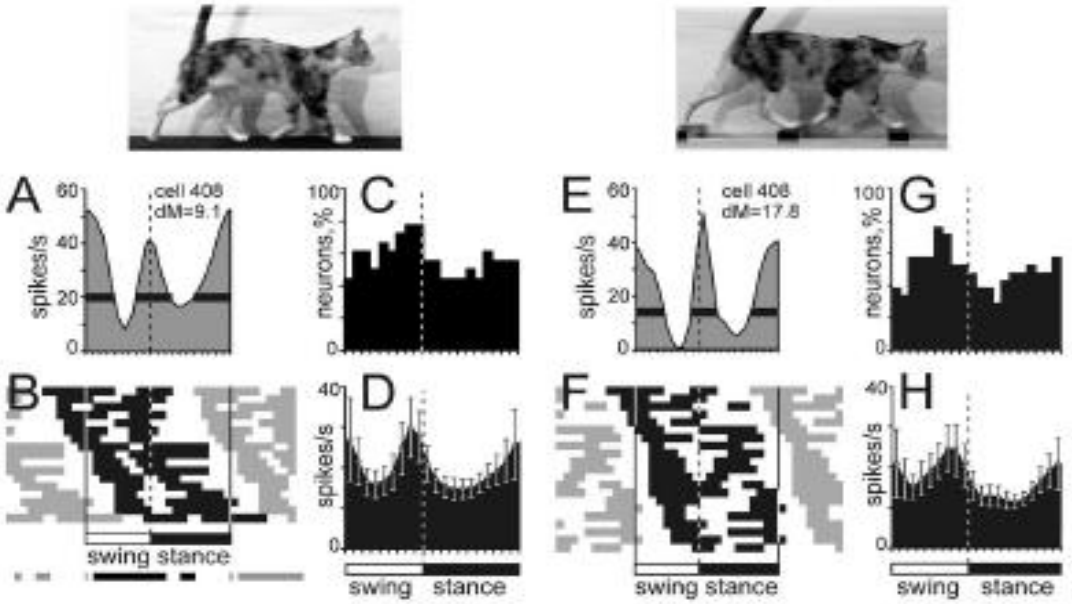


**Figure 4.7.** Population characteristics of two-PEF neurons. **A, E:** Example activity of a neuron during walking on the flat surface (**A, B**) and along the horizontal ladder (**G, H**) presented as histograms (**A, G**) and rasters (**B, H** [22 strides and 16 strides, respectively]). In **A, B** and **G, H** designations are as in Figure 4. **C, I:** Phase distribution of PEFs during walking on the flat surface (**C**) and along the ladder (**I**). The top row shows PEFs of the neuron, the activity of which is shown in **A, B** and **G, H**. **E, K:** Proportion of active neurons (neurons in their PEF) in different phases of the step cycle. **F, L:** The mean discharge rate at different phases of the step cycle. Error bars show SEM

While one- and two-PEF spike discharge patterns were the most conspicuous features of the discharge frequency modulation during walking, stride activity profiles – i.e., the frequency of spike firing as a function of the phase of the stride cycle – also varied greatly among neurons. Activity profiles of all cells could be divided into 6 groups based on the relationship of their PEFs to the transitions between the swing and stance phases of the stride cycle. In each panel of Figure 8, activity profiles of all cells belonging to a particular group are shown superimposed in a single plot. Neurons in Group 1 (32% of cells) were preferentially active during the swing phase of the stride cycle. Neurons in Group 2 (17%) had their activity centered on the moment of the foot contact with the ground. Neurons in Group 3 (18%) were preferentially active during the stance phase of the stride cycle. Neurons in Group 4 (5%) had their activity peaked around the time the foot broke contact off the ground or, in one neuron, the activity was preferentially suppressed around this time. Neurons in Groups 5 and 6 had bimodal activity profiles. Neurons in Group 6 (6%) were preferentially active during transitions between the swing and stance phases, whereas neurons in Group 5 (17%) had the opposite tendency of reducing their firing during those transitions.

## Association between RF Properties and Patterns of Activity Modulation during Locomotion on the Flat Surface

Each of the six cell groups described above included neurons with diverse RF properties. All groups had comparable ratios of cells with skin and deep RFs, showing that these groups had no preference for one or the other RF submodality. Preferences, however, were seen with regard to RF location on the limb. Neurons with proximal RFs restricted to the shoulder, upper arm and forearm (“exclusively proximal” RFs) as a group discharged intensively throughout all of the swing phase with an average

population peak at around 20 spikes/s, and were only half as active during mid-stance (Fig. 4.9 A). Neurons with RFs that included some proximal (upper arm and/or forearm) and some distal (wrist, paw) forelimb regions ("proximal+distal" RFs) discharged more vigorously during the swing phase as well, but reached the peak of their activity only in the middle of this phase (Fig. 4.9 B). Neurons with RFs confined to the wrist and/or paw ("exclusively distal" RFs) were activated still later in the cycle reaching the activity peak during transition from the swing to stance phases (Fig. 4.9 C). In addition, these neurons were approximately 50% more active at their peak than either ones of more proximal groups. Thus, it appeared that, during the stride cycle, the activity of neurons with proximal RFs tended to lead the activity of neurons with more distal RFs. This observation was supported by results of the analysis of distribution of distal *vs.* proximal RFs among the six types of activity profiles shown in Figure 8. Namely, neuronal group 2, which included cells most active when the foot was coming in contact with the ground, consisted almost exclusively from neurons with distal RFs ($p<0.01$, *Z*-test for proportions). In contrast, group 5, the group of two-peaked neurons active both in the mid-swing and mid-stance, was dominated by cells with RFs encompassing both distal and proximal limb. Neurons that had cutaneous RFs restricted to the ventral surface of the forepaw presented a special opportunity to gauge how much stride-related activity patterns of neurons in areas 1 and 2 reflect the stride-related spike discharge patterns of the mechanoreceptors that innervate these neurons. The pattern of firing of cutaneous mechanoreceptors on the ventral forepaw during locomotion can be expected to be greatly dominated by the forepaw coming into contact with the ground during the stance phase of the stride and the time-course of the force exerted by the foot on the ground during the stance phase (Fig. 10 A). According to these considerations, neurons of areas 1 and 2 with cutaneous RFs on the ventral forepaw should start generating prominent

spike discharges about 20 ms after the moment the foot touches the ground (given that the shortest transmission latency from cat's forepaw to middle layer cells in area 3b is ~15 ms; Diamond, 1989) and behave as neurons of Group 3 (see Fig. 8).

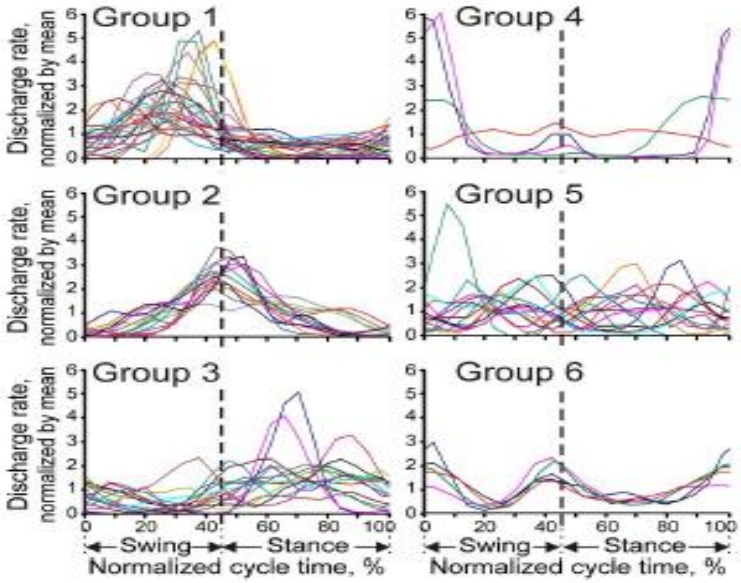


**Figure 4.8.** Six groups of stride-related activity patterns. Each plot shows superimposed the stride activity profiles of all studied cells belonging to a particular group

Twelve neurons isolated in this study had cutaneous RFs restricted to the ventral surface of the forepaw, and their stride activity profiles are plotted in Figure 10 B. Surprisingly, only one neuron had its activity raised during the stance phase as the Group 3 type (neuron 433 shown on top left), resembling at least to some degree the expected pattern of its peripheral drive. The majority of cells (n = 8) had their activity centered on the moment of the foot coming in contact with the ground (the Group 2 type). Two other neurons were of the Group-6 type, firing preferentially around the time of foot making contact with the ground and also breaking contact. Finally, one cell fired throughout the stride cycle, but more in the swing phase than in the stance phase, making it the Group 1 category.

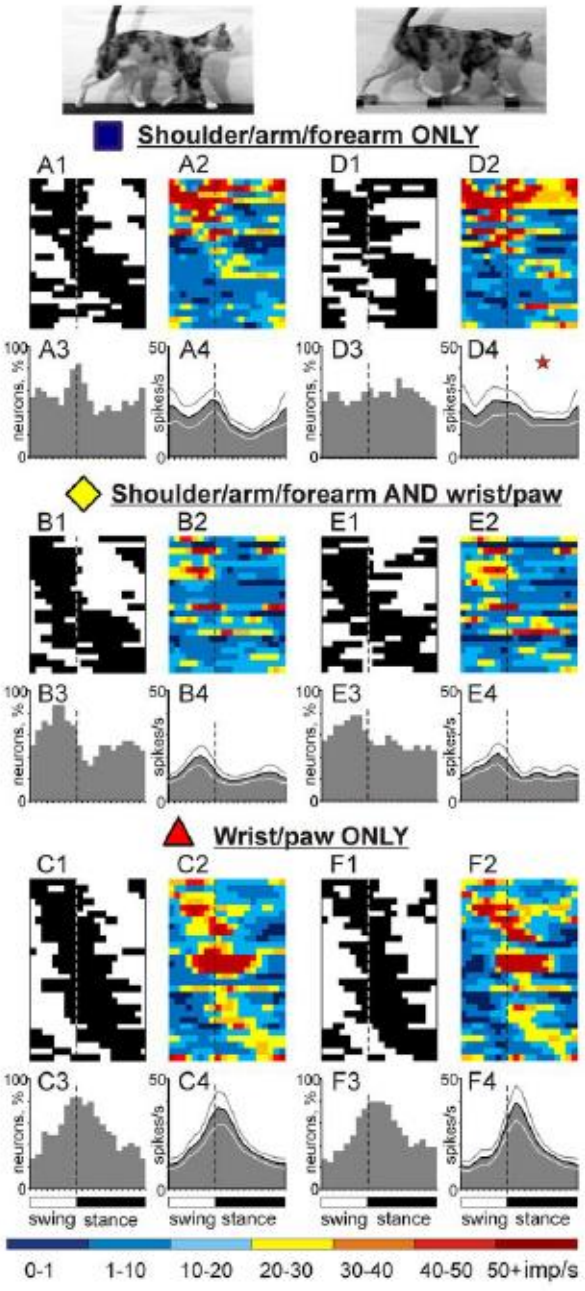


**Figure 4.9**

Stride-related activity of neurons with RFs on different forelimb segments. **A, D**: Activity of neurons with exclusively proximal RFs during walking on the flat surface (**A**) and along the ladder (**D**). In **D4**, star indicates the phase, during which the activity of neurons during ladder walking is higher than during flat surface walking ($p<0.05$, *t*-test). **B, E:** Activity of neurons with proximal+distal RFs. **C, F:** Activity of neurons with exclusively distal RFs. Other designations as in Figure 6.

The most striking feature of the behavior of these cells is that all 12 cells began to increase their firing 50-150 ms before the physical contact of the foot with the ground, although during this time – according to their RFs – these cells should not be receiving any significant afferent input. The other striking feature is that all cells, except for a

single Group-3 cell, reached the peak of their firing very near the moment of the foot touching the ground. Four cells had their firing peak just prior to the physical contact, five cells had their peak just after the physical contact and before the afferent input could reach SI, and only two cells had their peaks 25-30 ms after the physical contact. All these cells showed no evidence of any major surge in spike firing that could be attributed to activation of mechanoreceptors by the foot touching the ground. Overall, Fig. 10 C suggests that activity of neurons with RFs restricted to the ventral surface of the forepaw was driven during locomotion to a large extent by anticipation of the foot coming in contact with the ground, as opposed to reacting to such a contact.

Regardless of their RFs, PTNs with different axonal conduction velocities were different in phasing of their population activity. Although PEFs of individual fast- and slow-conducting PTNs were distributed over the stride cycle (Figs. 11 A1-3; B1-3), the average population activity of fast-conducting PTNs peaked during transition from the swing to stance phases (Fig. 11 A3,4) reaching approximately 50 spikes/s; in contrast, the activity of slow-conducting PTNs peaked earlier in the cycle: during the mid-swing phase, albeit only reaching approximately 35 spikes/s, ~50% less than the peak activity of fast-conducting PTN group. Thus, the activity of slow-conducting PTN group led that of fast-conducting PTN group.

The activity of all but one neuron recorded during walking on the flat surface was also recorded when cats walked along the horizontal ladder. Discharges of the overwhelming majority of the neurons (74/81, 91%) were different between the two walking tasks.

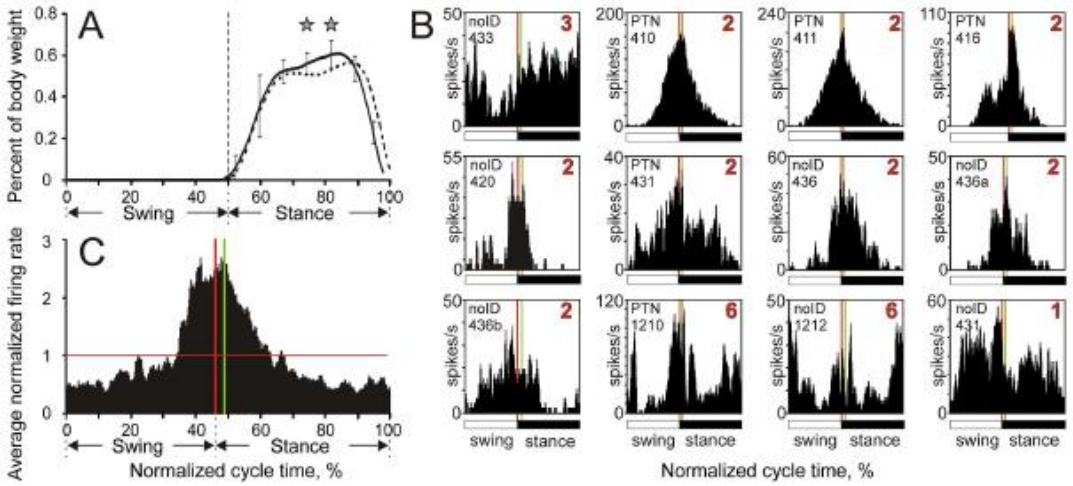


**Figure 4.10.** Stride-related activity of 12 neurons with cutaneous RFs restricted to the ventral side of the forepaw. **A:** Vertical ground reaction forces during walking on the flat surface (solid line) and horizontal ladder (interrupted line). SDs were similar between the tasks and for clarity are shown only for flat surface walking (error bars). Stars indicate statistically significant differences between the walking tasks ($p<0.05$, *t*-test). **B:** Activity profiles of the 12 cells during locomotion on the flat surface. Vertical lines indicate the moment of the foot making contact with the ground (red) and 15 ms later (green). **C:** Average activity profile of the 12 cells, each normalized by its mean firing rate during flat surface walking.

Changes to the Activity Evoked by Necessity to Step Accurately on the Horizontal Ladder

The activity of all but one neuron recorded during walking on the flat surface was also recorded when cats walked along the horizontal ladder. Discharges of the overwhelming majority of the neurons (74/81, 91%) were different between the two walking tasks.

The average discharge rate was different in 54% (44/81) of neurons. From these, half of cells (22/81, 27%) were more active during walking on the ladder than on the flat surface, on average by 73%, while the other half were less active, on average by 32% (Fig. 4.5B). Neurons with RFs on the wrist and/or paw had a tendency to decrease discharge rate, while those with RFs on the arm or forearm tended to increase it. Across all groups, fast-conducting PTNs typically decreased activity during walking on the ladder, while

slow-conducting PTNs typically increased it ($p > 0.05$, $\chi^2$ test). Because changes in the activity of different neurons were often of opposite directions, the average discharge rate of the whole studied population was similar between the walking tasks. (17.5±1.5 *vs.* 16.6±1.4 spikes/s, $p > 0.05$, *t*-test)

The stride-to-stride variability of the mean activity across the cycle, *V*, was 1.42±0.74 (mean ± SD), which was statistically significantly smaller than during walking on the flat surface ($p = 0.044$, *t*-test). Only five individual neurons, however, showed statistically significant differences in the variability of their mean discharge rates between the two tasks, and three of them were recorded sequentially along the same microelectrode penetration.

During walking along the ladder, the activity of all cells was modulated with relation to the stride. The same two major forms of modulation as during walking on the flat surface were observed: one PEF was seen in 72% of neurons (58/81) and two PEFs were found in 26% (21/81) of cells. Two additional cells had three PEFs. Proportions of cells with different number of PEFs were similar to those seen during walking on the flat surface ($p > 0.05$, *t*-test). The depth of frequency modulation, *dM*, however, was different in 46% (37/81) of neurons. During walking along the ladder, it was higher in 23 cells by 40±21%, and lower in 14 by 27±13% (mean±SD, Fig. 4.5C). Two-PEF neurons nearly always increased the depth of their frequency modulation, and the average *dM* for their group was much higher during walking along the ladder than on the flat surface (16.4±1.7 *vs.* 10.1±0.6, $p = 0.003$). Groups of neurons with different RF properties (e.g., proximal *vs.* distal, skin *vs.* deep) and PTNs with different axonal conduction velocities had approximately similar proportions of cells increasing and decreasing *dM* upon transition from the flat surface to ladder.

The duration of the PEF was different between the tasks in 15% (12/81) of neurons: during walking along the ladder, it was longer in eight and shorter in five cells by 25-50% of the cycle. Neurons with RFs confined to the wrist and paw were different from cells with exclusively proximal RFs in their strong preference not to change duration of the PEF ($p<0.05$, *Z*-test).

Neurons typically had the same number of PEFs during both locomotor tasks (62/81, 77% of cells). However, 17% (11/63) of neurons that had one-PEF pattern during flat surface walking changed to two PEFs during walking along the ladder. On the other hand, 6 of 18 (30%) neurons that had two-PEFs on the flat surface changed to only one PEF on the ladder, while two other two-PEF cells changed to three PEFs. Among 52 neurons that had one PEF during both locomotor tasks, 13 cells (25%) had it at a different time of the step cycle however, that difference was typically only 10-15% of the cycle. Neurons with RFs on the wrist and paw were different from the rest of one-PEF population in that none of them changed the preferred phase of the activity upon transition from flat surface to ladder locomotion ($p<0.05$, *Z*-test).

The great majority of neurons (74%, 60/81) had only one or two parameters of their activity different between the two walking tasks: most often the average discharge rate or the depth of frequency modulation, or both (30 cells total). In 11 additional cells, these changes were accompanied by a change in the preferred activity phase or the number of PEFs.

Changes in the activity of individual neurons upon transition from flat surface to ladder locomotion, although often opposite, led to changes in the activity of some cell groups. So, during walking along the ladder, the average discharge rate of the one-PEF group was higher than on the flat surface (17.7±1.7 *vs*. 13.3±1.3 (spikes/s, respectively;

$p<0.05$, *t*-test). The increase was due to rise of activity during most of the stance phase (compare Fig. 4.6D and H; $p<0.05$, *t*-test). Neurons with proximal RFs contributed heavily, as their population activity during the mid-stance phase was greater than on the flat surface (Fig. 4.9D). Much of this activation was produced by neurons that during walking on the flat surface had PEFs in the swing phase of the stride (in Figs. 9 A2 and D2, they are located on the top of the graphs). Fast-conducting PTNs had a tendency to shift their activity more into the stance phase; however, this tendency did not reach the level of statistical significance (Figs.11 A, D). Population activities of two-PEF neurons, neurons with distal RFs as well as slow-conducting PTNs were similar between the two walking tasks (Figs. 7 and 11 B and E).

## Discussion

We found that walking on a flat surface affected spike discharge activities of all the cells that were isolated in microelectrode penetrations through the rostral bank of the ansate sulcus and possessed RFs on the contralateral forelimb. Specifically, spike firing rate of all cells was modulated in relation to the stride, varying systematically as a function of the phase of the stride. In addition, 44% of cells increased their mean firing rate during locomotion, while 22% of cells decreased it as compared to their mean firing rate while standing. Overall, the mean firing rate of this neuron sample was 14 spikes/s during standing and 17 spikes/s during walking. These numbers are comparable to average firing rates of neurons in areas 1-2 reported by Fitzsimmons and colleagues (2009) for bipedal treadmill locomotion of macaque monkeys.

While all the cells in the study were affected by walking, they varied greatly in how they were affected. Different cells were activated at different phases of the stride cycle and 95% could be sorted into 6 groups based on the timing of their spike firing relative to

the stride cycle (Fig. 8). Some cells fired preferentially during the swing phase, other cells fired during the stance phase, yet other cells fired at the transition phases from swing to stance, or from stance to swing, or both. The specific pattern of firing was correlated with the RF location on the limb. Neurons with proximal RFs (upper arm or shoulder) tended to fire at peak rates in the middle of the swing (Group 1). They appeared to be analogous to those neurons of primates whose activity profiles during arm reaching movements are similar to that of the velocity of reaching: peaking between the onset of the reach and arrival at the target (Gardner et al., 2007); these reaching movements are largely performed by proximal muscles, especially those of the shoulder and elbow . In contrast, neurons with RFs on the distal limb (wrist/paw) typically showed peak firing at the transition between swing and stance phases, immediately prior and right after the paw contacted the floor at the beginning of stance (Group 2). These neurons, along with neurons of Groups 4 and 6, which were activated at peak rates at the making (Group 4) or making and breaking (Group 6) of paw contact with the floor, might be similar in function to the primate's neurons that fire at hand contact prior to grasping and/or release of objects held in the hand (Gardner et al., 2007). Neurons in Group 3 fired most intensively during stance, and thus might have signaled alterations in the disposition of the animal's weight during that phase. Within each group, cells differed in the timing of their peak firing. As a result, each moment in the stride cycle was characterized by a unique spatial pattern of activity in the areas 1-2 neuron population. Such instantaneous spatial patterns of activity in areas 1-2 thus can be used to infer the state of ongoing locomotion.

The pattern of firing activity was also distinct between groups of fast- and slow-conducting PTNs regardless of their RFs (Fig. 4.10). This suggests that these cortical efferent neurons have distinct roles in control of walking, as was previously also

suggested for fast- and slow-conducting PTNs of the motor cortex (Stout and Beloozerova, 2013). Fast- and slow-conducting PTNs differ in their connections to the spinal cord, such that fast-conducting PTNs preferentially influence distal muscle-related networks, while slow-conducting PTNs influence both proximal and distal muscle-related networks (Brookhart, 1952; Wiesendanger, 1981). Correspondingly, population activity of fast-conducting PTNs peaked at the transition from swing to stance when control of the distal limb was most critical, while the slow-conducting PTN group fire most actively during mid-swing where we have previously shown that the length of the limb is actively stabilized (Klishko et al., 2014).

The patterns of spike discharge activity generated by the population of neurons during walking on a flat surface or on a horizontal ladder were qualitatively similar. However, many individual neurons exhibited statistically significant quantitative differences in their mean firing rate and/or characteristics of stride-related activity modulation between these two locomotor tasks (Fig. 4.5B and C), just as there were quantitative differences in the cat's manner of walking on a flat surface *vs.* a ladder, which, besides a dramatic difference in the variability of stride lengths, included distinctions in the posture of the neck and head (e.g., Fig. 4.1A and B), flexion of distal joints, and few other variables (Beloozerova et al., 2010). These differences suggest that spatiotemporal activity patterns generated in areas 1-2 during locomotion might carry detailed information on the fine status of the skeletomuscular locomotor apparatus on a moment-by-moment basis. Indeed, using linear decoders, Fitzsimmons et al. (2009) were able to extract information about positions and angles of the hip, knee and ankle joints, leg muscle contractions, foot contact with the ground, step length and walking speed from spike firing patterns of multiple neurons recorded simultaneously in areas 1-2 of monkeys engaged in bipedal walking on a treadmill. The fact that a dramatic

decrease in the variability of the stride length during accurate stepping on the ladder compared to unconstrained walking (Beloozerova et al., 2010) is accompanied by a decrease in the variability of neuronal discharges during this cortex-controlled task, which we found here, further supports the above suggestion.

A large body of literature shows that SI activity in behaving subjects does not simply reflect the spatiotemporal patterns of activation of mechanoreceptors, but is shaped by other, central, factors, such as sensory gating by anticipatory or efference copy inputs from the motor system. In this study, the influence of such factors on SI activity during locomotion could be ascertained among those sampled neurons that had cutaneous RFs on the ventral surface of the contralateral forepaw. Afferent drive to these neurons should have been initiated and terminated at each walking step by the forepaw coming in contact and leaving the ground. However, 11 out of 12 such cells increased their firing 50-150 ms prior to the physical contact of the forepaw with the ground (Fig. 4.10B), during which time they were not expected to receive any significant peripheral drive. Half of all the spikes discharged by these cells, as a group, during a stride cycle occurred prior to the foot touching the ground, at least for the range of walking speeds that we have analyzed (Fig. 4.10C). Such anticipatory behavior suggests that these cells acted in a predictive mode (e.g., Bubic et al., 2010).

Anticipatory activity or sensory response modulation on a similar timescale was commonly observed in primate areas 1-2 prior to onset of a haptic task or even prior to simple voluntary wrist or elbow movements (Soso and Fetz, 1980; Fromm and Evarts, 1982; Nelson, 1987; Koch and Fuster, 1989; Lebedev et al., 1994; Weber et al., 2011; London and Miller, 2013). Such anticipatory cortical activity is generally thought to be driven by efference copy inputs from the motor system, intended to facilitate the processing of the expected sensory and motor events. Anticipatory activity can also be

evoked in SI without any motor action, however, by just expecting to receive a tactile stimulus (Carlsson et al., 2000). In this case, the anticipatory activity in SI is triggered, for example, by a visual cue that must be transmitted to SI by a top-down mechanism engaging higher-level sensory cortical areas. So, it is possible that a significant portion of differences in firing activity of individual neurons between flat surface and ladder walking (Fig. 4.5 B and C) arose from differences in visual cues used for guiding stepping during these tasks (Rivers et al., 2014).

However, unlike other studies, in which anticipatory activity in SI was observed while the subject was in a relaxed state, expecting a tactile stimulus or getting ready for a motor act, in this study we observed anticipatory activity while the cat was already in an active state – walking – with neurons in areas 1-2 firing spikes on each stride cycle ahead of the movement-generated stimulation of their RFs. In this situation, the pre-contact anticipatory activity may reflect the rhythm of walking that is generated in the spinal cord (Brown, 1911; Grillner and Zangger, 1979; McCrea and Rybak, 2008), and the post-contact activity may reflect information on the action that has been accomplished. In addition to the motor system and higher-level sensory areas, the anticipatory spike firing in areas 1-2 can be also driven locally by other active neurons within the areas whose RFs are stimulated earlier in the stride cycle. For example, neurons with proximal RFs may drive those with distal RFs (Figs. 9, 10), and slow-conducting PTNs may drive fast-conduction ones (Fig. 4.11). That is, given that locomotion is a regularly practiced behavioral activity in which limbs cycle through a highly reproducible temporal sequence of muscle contractions and joint movements, one can expect that in an SI neuronal population modulated by walking, those neurons that are engaged (by virtue of their RF properties) earlier in a stride cycle will have well developed lateral excitatory connections onto other neurons that are engaged a little later in the same cycle (Drew and Abbott,

2006). Additional experiments, however, are necessary to determine specific sources of the predictive activity in areas 1-2 during locomotion.

Regardless of the source, however, it is interesting that the peak of anticipatory activity among our studied neurons closely matches the timing of the actual physical contact of the forepaw with the ground (Fig. 4.10C) rather than the timing of the arrival of the contact-evoked afferent input to those neurons. This timing suggests that areas 1-2 signaled a sensory event (i.e., a foot contact) exactly when it occurred by predicting it rather than signaling it "after the fact" by responding to the event-evoked afferent input. Such a predictive manner of sensory event representation appears to be common to sensory perception in general and is referred to as "perceiving-the-present" (Changizi et al., 2008). However, this present study is the first one to reveal neuronal correlates of the predictive sensation during locomotion.

Numerous studies have shown that active limb movements suppress transmission to SI of spike discharges evoked in somatosensory afferents by peripheral stimulation, even if that stimulation was unrelated to the movement (Chapin and Woodward, 1981, 1982b; Jiang et al., 1991; Shin et al., 1994; Seki and Fetz, 2012). Such nonspecific somatosensory gating takes place all along the somatosensory pathways, including the dorsal column nuclei (Ghez and Pisa, 1972; Coulter, 1974; Chapman et al., 1988) and ventrobasal thalamus (Cocquery, 1978; Shin et al., 1993). We see a possible evidence of such sensory gating in our study in the fact that in 10 cells among the 12 that had cutaneous RFs on the ventral forepaw, the first half of the stance period (during which the contact-evoked afferent input from the foot should be quickly rising) was associated with a prominent decline – rather than increase – of spike firing. However, such a decline in firing might also be due to a more selective integration of internally generated prediction with the actual sensory information (Chapin and Woodward, 1982a; Bays et

al., 2006; London and Miller, 2013). Theoretically, such integration might emphasize "error signals;" i.e., deviations of the sensory signal from predicted, so that neural activity in SI does not represent all the afferent somatosensory information, but only the part that is not accurately predicted by the internal model (Rao and Ballard, 1999; Alink et al., 2010; Ouden et al., 2010). Alternatively, integration of prediction with the afferent information might be used to produce the representation of sensory events that is more accurate than possible from either source alone, resulting in lower average activation and a higher signal-to-noise ratio (Murray et al., 2004; Vaziri et al., 2006).

To conclude, in this study we found that the activity of neurons in areas 1-2, the high subdivisions of SI, prominently reflects highly predictable somatosensory events – such as those generated by locomotion – both shortly before and after these events take place, and that this activity differs between unrestrained locomotion and accurate target stepping.

# 5. SIGNALS FROM THE VENTROLATERAL THALAMUS TO THE MOTOR CORTEX DURING LOCOMOTION

Published in Journal of Neurophysiology (Marlinski et al. 2012)

## Abstract

The activity of the motor cortex during locomotion is profoundly modulated in the rhythm of strides. The source of modulation is not known. In this study we examined the activity of one of the major sources of afferent input to the motor cortex, the ventrolateral thalamus (VL). Experiments were conducted in chronically implanted cats using an extracellular single neuron recording technique. VL neurons projecting to the motor cortex were identified by antidromic responses. During locomotion, the activity of 92% of neurons was modulated in the rhythm of strides. 67% of cells discharged one activity burst per stride, a pattern typical for the motor cortex. The characteristics of these discharges in most VL neurons appeared to be well suited to contribute to the locomotion-related activity of the motor cortex. In addition to simple locomotion, we examined the VL activity during walking on a horizontal ladder, a task that requires vision for correct foot placement. Upon transition from simple to ladder locomotion, the activity of most VL neurons exhibited the same changes that have been reported for the motor cortex, i.e. an increase in the strength of stride-related modulation and shortening of the discharge duration. Five modes of integration of simple and ladder locomotion related information were recognized in the VL. We suggest that, in addition to contributing to the locomotion-related activity in the motor cortex during simple locomotion, the VL integrates and transmits signals needed for correct foot placement on a complex terrain to the motor cortex.

**Acknowledgements:** The authors are grateful to Dr. Galina A. Pavlova for acquisition of a part of the data and to Peter Wettenstein for exceptional engineering assistance. We are also indebted to Drs. Alan R. Gibson, Kris M. Horn, and Vladimir K. Berezovskii for help in processing and analysis of our morphological data. Fluorescent imaging was supported by National Eye Institute Grant P30 EY-12196 and performed at Harvard University Medical School. This research was supported by National Institute of Neurological Disorders and Stroke Grants R01 NS-39340 and R01 NS-058659 to Irina. N. Beloozerova.

## Introduction

This study on the activity of the ventrolateral thalamus (VL) in the walking cat was prompted by the fact that during locomotion the activity of the target of VL projection – the motor cortex – changes periodically in the rhythm of locomotor movements (Armstrong and Drew 1984; Beloozerova and Sirota 1985a, b, 1993a, 1993b; Drew 1993). The origin of this modulation is unclear. In decerebrated cats, it was shown that integrity of the cerebellum is required for all subcortical descending tracts: vestibulo-, reticulo-, and rubro-spinal, to exhibit locomotion-related modulation of their activity (Orlovsky 1970, 1972a, 1972b). Neurons of those tracts receive direct synaptic projections from the cerebellum. The motor cortex also gives rise to a major descending tract – the pyramidal tract. The motor cortex does not receive direct input from the cerebellum, but receives input from the VL, one synapse away from it. We hypothesized that during locomotion the VL passes locomotion-related information from the cerebellum to the motor cortex. The first goal of this study was to elucidate whether the pattern of activity of VL neurons during locomotion in an uncomplicated environment (simple locomotion) is suitable to

contribute to the locomotion-related activity in the motor cortex. We found that the majority of VL neurons were well fit for such a role.

It is known that VL neurons change their activity prior to voluntary movements (Evarts 1971; Kurata 2005; Neafsey et al. 1978; Schmied et al. 1979; Strick 1976; van Donkelaar et al. 1999). We hypothesized that VL is also involved in voluntary modifications of locomotion. The second goal of this study was to explore whether VL neurons contribute to an increase in modulation of the motor cortex activity that is observed during locomotion over a complex terrain (complex locomotion). On complex terrain, vision is required to adjust steps to irregularities of the walking path. During stepping under visual control the activity of the motor cortex is dramatically different from that during walking on the flat surface, and these changes are crucial for correct stepping on a complex terrain (Beloozerova and Sirota 1988, 1993a; Beloozerova et al.2010; Drew 1988, 1993). The inputs responsible for the changes of the activity in the motor cortex during complex locomotion as compared to simple walking are not clear. Area 5 of parietal cortex, which is known to integrate visual and motor information for control of limb movements (e.g. Buneo and Andersen 2006; Mountcastle 1995), could be responsible for modulation of the motor cortex activity during complex locomotion It was found, however, that the pattern of activity of area 5 neurons was quite different from that of the motor cortex and thus seemed unlikely to be the primary source of modulation (Beloozerova and Sirota 2003). Here we consider another possibility that during complex locomotion the input from the VL changes. We hypothesized that, in addition to basic locomotion-related information, the VL transmits information that is needed to control landing positions of feet during walking on a complex terrain to the motor cortex. We have found strong evidence supporting this hypothesis.

## Methods

Extracellular recordings from single VL neurons were obtained during chronic experiments in cats. One adult female (cat A) and two adult males (cats B and C) were used. The methods of experimental preparation are described in details in chapter 2. Here I discuss only more specific method to this study and others only briefly.

### Locomotion Tasks

Two locomotion tasks were used: 1) simple locomotion on a flat surface, and 2) complex locomotion on crosspieces of a horizontal ladder. It has been demonstrated in several studies that simple locomotion does not require vision, while complex locomotion does (Beloozerova and Sirota 2003; Liddell and Phillips 1944; Marigold and Patla 2008; Sherk and Fowler 2001; Trendelenburg 1911). During ladder locomotion cats step on the support surface with substantially less spatial variability and more accuracy as compared to simple locomotion (Beloozerova et al. 2010).

The walking chamber was a rectangular enclosure with two connected parallel corridors, which is described in Methods chapter (Ch. 2). One corridor had flat walking surface while the other contained a horizontal ladder (Fig. 5.1C).

### Surgical Procedures

In anesthetized cats, skull was exposed, the head implants were anchored as described in chapter 2. An arrangement of seven or nineteen 28-gauge hypodermic guide tubes was implanted above the VL. The guide tubes were soldered together at their tops. The outer diameter of the 7-tube arrangement was 1.08 mm, and that of 19-tube one was 1.8 mm. Cat A received the 7-tube implant in both left and right hemispheres, cat B

received the 19-tube implant in both left and right hemispheres, and cat C received one 7-tube implant in the left hemisphere. The tip of the arrangement was lowered to the vertical Horsley and Clarke coordinate V +7.0. In two cats (A and B), on the left side of the head, the dorsal surface of the rostral and lateral sigmoid gyri, and the rostral part of the posterior sigmoid gyrus were exposed by removal of approximately 0.6 square centimeters of bone and dura mater. The region of the motor cortex was visually

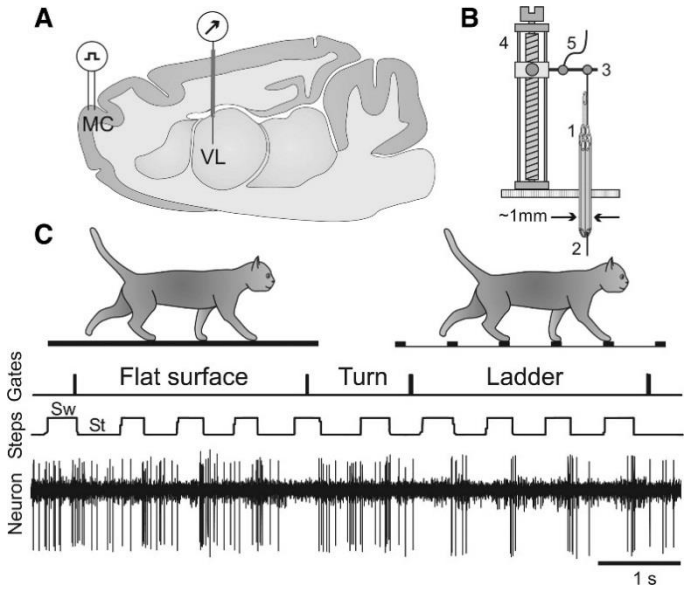


**Fig. 5.1**: Experimental paradigm. A: schematic drawing of a parasagittal section of the brain showing the position of chronically implanted guide tubes for recording electrodes above the ventrolateral thalamus (VL) and stimulating electrodes in the forelimb representation of the motor cortex (MC). B: method of insertion and advancement of electrodes into the VL. 1: A group of 28-gauge cannulas is chronically implanted in the cortex above VL. 2: An electrode is manually inserted into one of the cannulas and soldered to an arm (3) of a micromanipulator (4). A wire (5) that leads to a miniature preamplifier positioned on the head of the animal is also soldered to the arm. In this manually driven micromanipulator, 1 revolution of the screw results in 200-µm advancement of the electrode. C: locomotion tasks: walking in a chamber on a flat surface and along a horizontal ladder. The Gates trace indicates when the cat has passed the beginning and end of each of the chamber's corridors. The Steps trace shows the swing (Sw) and stance (St) phases of the right forelimb recorded with an electro-mechanic sensor. The Neuron trace shows discharge of a neuron from the VL.

identified based on surface features and photographed (Fig. 5.5A). The exposure was covered with a 1 mm thick acrylic plate. The plate was pre-perforated with holes of 0.36 mm in diameter spaced by 0.5 mm, and the holes were filled with bone wax. The plate allowed for later implantation of stimulation electrodes in the motor cortex for identification of thalamo-cortical projection neurons.

Single-Unit Recording

The motor cortex was mapped using multiple-unit recording and microstimulation techniques. During microstimulation, trains of ten 25 μA, 0.2 ms, cathodal pulses at 350 Hz were applied using a monopolar platinum-tungsten quartz insulated microelectrode with impedance of 200-500 kΩ. Stimulating electrodes were implanted into the motor cortex (MC) representation of the distal (MCd) and proximal (MCp) forelimb at approximate coordinates A 25-26, L 7-9 and L 5-6, respectively (Fig. 5A and B). Electrodes were made of platinum-iridium, Teflon-insulated wire with outer diameter 140 μm (A-M Systems Inc., Carlsberg, WA). The 0.4 mm tip of the wire was freed from isolation and tapered. Wires were individually inserted into the cortex 1 mm apart through perforations in the acrylic plate implanted above it, and fixed. Extracellular recordings from the VL were obtained using tungsten varnish-insulated microelectrodes (120 μm OD; FHC Inc., Bowdoin, ME) or platinum-tungsten quartz insulated microelectrodes (40 μm OD) pulled to a fine tip and mechanically sharpened using a diamond grinding wheel (Reitboeck 1983). The impedance of both types of electrodes was 1-3 MΩ at 1000 Hz.

Identification of Neurons

The ventrolateral thalamus is subdivided into two parts by its afferent connections (Asanuma et al. 1983; Ilinsky and Kultas-Ilinsky 1984). The major projections to the posterior part originate in cerebellar nuclei while the anterior part receives its primary input from the basal ganglia (Grofová and Rinvik 1974; Hendry et al. 1979; Larsen and McBride 1979; Rinvik and Grofová 1974; Sakai et al. 1996). In this paper, we identify divisions of the thalamic ventrolateral nuclear complex in accordance with nuclear delineation of the cat brain atlas of Reinoso-Suarez (1961). Thus, we denote the anterior division of the complex as the ventral anterior nucleus (VA). This area is analogous to the anterior part of the ventral lateral nucleus (VLa) in primates. The posterior division of the ventrolateral complex we name the ventral lateral nucleus (VL). This region is analogous to the posterior part of primate's ventral lateral nucleus (VLp). All neurons whose activity is reported in this paper were collected in the VL.

The somatic receptive fields of neurons were examined in animals resting with their head restrained. Somatosensory stimulation was produced by lightly stroking fur, palpation of the muscle bellies and tendons, as well as by passive joint movements. In the left VL of two cats, A and B, in which stimulation electrodes were implanted in the left motor cortex, neurons were tested for antidromic activation from the motor cortex. The motor cortex was stimulated with 0.2 ms single rectangular pulses of 0.1-1.0 mA intensity applied 2-3 s apart. Current was passed between each pair of implanted wires where individual wires were within 1-1.5 mm from each other. These pulses typically did not evoke any visible motor responses, and never produced any signs of discomfort or distress in the cats. The principal criterion for identification of antidromic activation of thalamo-cortical projection cells (TCs) was the test for collision of spikes (Bishop et al. 1962; Fuller and Schlag 1976) (Fig. 5.5C and D). For calculation of the conduction

velocity, the distance between recording electrodes in the VL and stimulation electrodes in the motor cortex was estimated as 31.5 mm.

Processing of Neuronal Activity

To compare the activity of neurons during two locomotion tasks we used only the strides for which the average duration in the two tasks differed by <10%. The onset of swing of the forelimb contralateral to the VL recording site was taken as the beginning of the step cycle. The stride cycle was divided into 20 to produce phase histograms.

The coefficient of stride-related frequency modulation (M), depth of modulation (dM), Period of Elevated Firing (PEF), and Preferred Phase of the activity were calculated as described above.

To determine what differences in the modulation parameters of individual neurons during simple and complex locomotion could not be explained by spontaneous fluctuation in the discharge, we compared the activity of single neurons in sets of randomly selected steps of the same task. Recordings from 50 neurons that were long enough to enable selection of at least two nonoverlapping sets of 25–40 steps per task were used. From each record the first step was assigned to *set 1*, the second to *set 2*, the third to *set 3*, etc. For each neuron, the M, the dM, the preferred phase, and the duration of the PEF were calculated for each set of steps and compared. When three or more sets were available, all possible pairwise comparisons were considered; across all neurons, over 100 comparisons were made. Values of differences occurring in <5% of these "sham" comparisons across all neurons were determined. During analysis, the differences in activity characteristics of a neuron during simple and complex locomotion that exceeded these values were considered, with 95% confidence, to be caused by the

difference in the locomotion tasks but not by spontaneous background fluctuation in the discharge rate.

For comparisons of activities of individual neurons in different tasks and between groups of neurons, a two-tailed *t*-test was used. A nonparametric $\chi^2$-test was used for comparison of categorical data. For all tests, the significance level was set at $P = 0.05$. Unless indicated otherwise, for all mean values the standard deviation (SD) is given.

## Histological Procedures

In *cat A*, 8 nl of 1% horseradish peroxidase-conjugated wheat germ agglutinin (WGA-HRP) (Sigma) was injected by pressure in the center of the area of recording in VL 48 h prior to the animal's death. The injection was made through a flexible capillary tube made of fused silica (147-μm OD, 50-μm ID; Polymicro Technologies, Phoenix, AZ) that was directly connected to the output of pressure microinjector PMI-200 (Dagan, Minneapolis, MN). On the day of termination the animal was deeply anesthetized with pentobarbital sodium, and reference electrolytic lesions were made in the areas of recording and stimulation. The cat was then perfused with 3% paraformaldehyde and a series of 10%, 20%, and 30% sucrose-phosphate buffer solutions (0.1 M, pH 7.4). The left half of the brain was frozen and sectioned at 40 μm in the parasagittal plane. Every fourth section was processed with a modified tetramethyl benzidine (TMB) reaction (Gibson et al. 1984; Mesulam 1982) and then lightly counterstained with thionine. Adjacent sections were stained with cresyl violet. In addition, the right cerebellum was sectioned in the coronal plane and processed in the same manner. Brain sections were inspected under polarized light illumination. Locations of retrogradely labeled neurons were marked on digital images of brain sections with a computerized plotting system

(Image Tracer, Translational Technology). Positions of electrode tracks were estimated with the use of the reference lesions.

In *cat B*, 1.5 μl of 10% red fluorescent microspheres (Lumafluor, Naples, FL) was injected into the most anterior part of the explored area in the VL 6 mo. prior to the animal's death. On the day of termination, the cat was deeply anesthetized with pentobarbital sodium, and reference electrolytic lesions were made in the areas of recording and stimulation. This cat was perfused with 10% paraformaldehyde. The brain was blocked into three blocks: frontal (rostral to A14), middle (between A14 and P2), and caudal (caudal to P2). The middle block containing thalamus was sectioned in the coronal plane. The frontal cortex ipsilateral to the recorded VL and the posterior part of the brain stem with the cerebellum contralateral to the recorded VL were sectioned in the parasagittal plane. Frozen 50-μm sections were made. Every fourth section was mounted on slides, cleared with acetone and xylene, and coverslipped. Observation and digital imaging of cells retrogradely labeled with red fluorescent beads were done with Neurolucida 8, Zeiss Axioscope, and AxioCamMR3 (Carl Zeiss Int.) with a rhodamine filter. For identification of brain structures the adjacent sections were stained with cresyl violet. Cat C received no tracers. The brain sections were obtained and stained with cresyl violet by the same techniques as in cat B.

## Results

### Location of Neurons

The activity of 238 VL neurons was recorded during walking both on the flat floor and along the horizontal ladder. The majority of neurons were recorded in two cats: 94 in cat A and 99 in cat B. In cat A, all but 10 cells were recorded from the left VL. In cat B,

60 neurons were recorded from the left VL, and 39 from the right VL. The remaining neurons (n=45) were recorded from cat C.

Anatomical Reconstruction

Histological examination of recording sites showed that, in cat A, recordings were obtained from the anterior portion of the VL at coordinates A 10.5-11.0, L 4.7-5.3, and V +0.5-4.0. Here and further, the vertical coordinate is given after subtraction 10 mm from the coordinate of the atlas of Reinoso-Suarez (1961) to align it with coordinates of other commonly used atlases of the cat brain. The recording site is shown on a parasagittal section of the brain in Fig. 5.2A. It was marked with an electrolytic lesion and injection of WGA-HRP. The site is situated approximately 2 mm caudally to the Nucleus caudatus - a landmark for identification of the anterior-posterior position of the section. A reconstruction of the locations of neurons that were recorded in this cat is given in Fig. 5.2D, plates 11.0-10.5.

In cat B, nearly symmetrically on the left and right sides of the thalamus, recorded cells were located in the central portion of the VL at coordinates A 9.5-10.75, L/R 3.0-5.0, and V - 0.5 -+3.5. The recording site in the left thalamus is shown on a coronal section in Fig. 5.2B. It was labeled with an electrolytic lesion and injection of red fluorescent beads (Fig. 5.4A). A landmark for identification of the anterior-posterior position of the section was the caudal putamen that at this level has a striped appearance in the cat. A reconstruction of locations of neurons, combined from the left and right VL, is shown in Fig. 5.2D, plates 10.75-9.5.

In cat C, recordings were made from the most caudal aspect of the VL at coordinates: A, 8.75-9.25, L 4.0-4.3, and V 0.5-1.5. A reference lesion that was made approximately 200 μm caudally to the most caudal recording track in this cat is shown on a coronal section

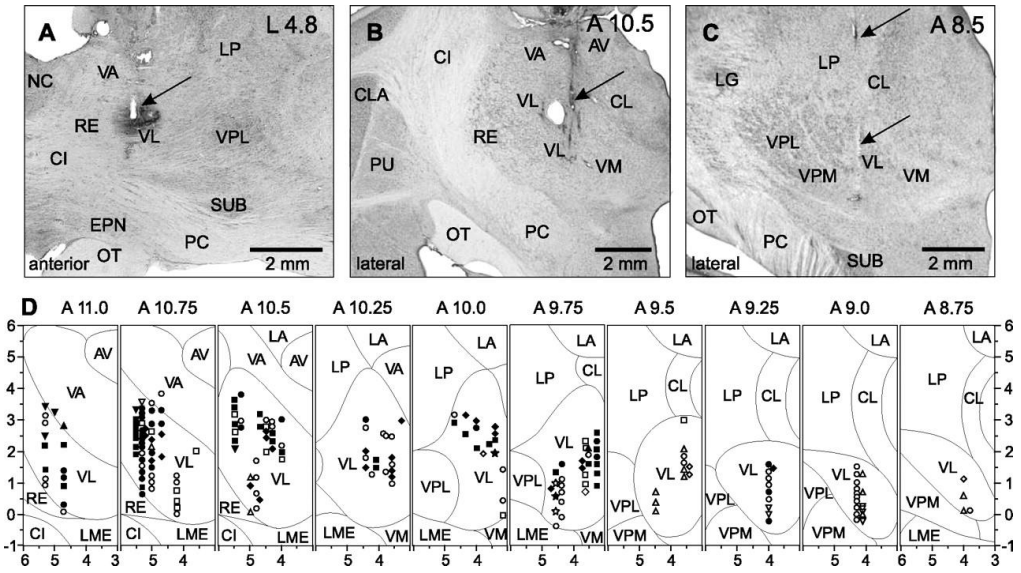


**Figure 5.2**: Sites of recording in the VL. A: recording site in cat A is shown on a photomicrograph of a parasagittal section of the thalamus. It was located in the rostral VL. Arrow points to the electrolytic lesion mark and the darkened area of tissue filled with horseradish peroxidase-conjugated wheat germ agglutinin (WGA-HRP). The site is ~2 mm caudally to the nucleus caudatus (NC) of the basal ganglia. B: recording site in cat B is shown on a photomicrograph of a coronal section of the thalamus. It was positioned in the middle of the VL. Arrow points to the electrolytic lesion mark and the darkened area where fluorescent beads were deposited. The caudal part of putamen (PU), a landmark for the anterior-posterior position of the section, is seen laterally. C: recording site in cat C is shown on a photomicrograph of a coronal section of the thalamus. It was positioned in the caudal VL. Arrows point to a track from a reference electrode. The most rostral aspect of the lateral geniculate body (LG), a landmark for the anterior-posterior position of the section, is visible laterally. D: reconstruction of positions of individual neurons recorded during locomotion in cats A, B, and C. Squares, neurons with somatosensory receptive fields on the shoulder (responding to passive movements in the shoulder joint and/or palpation of muscles on the back or neck); diamonds, cells that were activated by movements in the elbow; up-facing triangles, neurons with receptive fields on the wrist or paw; down-facing triangles, neurons whose receptive field encompassed the entire forelimb; stars, neurons responsive to vestibular stimulation; circles, neurons without somatosensory receptive fields and those whose receptive fields were not identified. Filled symbols represent neurons with axonal projections to the motor cortex (thalamo-cortical neurons, TCs); open symbols represent neurons whose projections were not identified. AV, nucleus anterio-ventralis thalami; CI, capsula interna; CL, nucleus centralis lateralis; CLA, claustrum; EPN, nucleus entopeduncularis; LA, nucleus lateralis anterior; LME, lamina medullaris externa thalami; LP, nucleus lateralis posterior; OT, optic tract; PC, pedunculus cerebri; RE, nucleus reticularis thalami; SUB, nucleus subthalamicus; VA, nucleus ventralis anterior; VL, nucleus ventralis lateralis; VM, nucleus medialis; VPL, nucleus ventralis postero-lateralis; VPM, nucleus ventralis postero-medialis.

of the thalamus in Fig. 5.2C. A landmark for identification of the anterior-posterior position of the section was the most rostral aspect of the lateral geniculate body (LG) that is visible in this section. A reconstruction of locations of neurons recorded in this cat is given in Fig. 5.2D, plates 9.25-8.75.

Receptive Fields

The somatosensory receptive fields of 168 neurons were tested. All receptive fields were found on the contralateral side of the body. One-third of neurons (32%, 53/168) responded to passive movements of the shoulder joint and/or palpation of muscles on the back or neck. Slightly more than half of these cells showed directional preference to shoulder movement and responded better either to flexion (17/168) or to extension and/or abduction of the joint (13/168). Eighty-four percent of shoulder-related cells were TCs. Fewer neurons (18%, 30/168) responded to movements in the elbow joint. Almost all of these neurons had a directional preference: half of them responded to flexion and another half to extension of the elbow. Ninety-five percent of tested cells were TCs. The number of neurons with receptive fields on the paw or wrist was relatively small (10%, 17/168). Typically, these neurons responded to pressure on the paw or to the wrist ventral flexion. Sixty-seven percent of tested cells were TCs. In addition, 15% (25/168) of neurons responded to stimulation of most of the forelimb, typically to flexion in the shoulder and extension in the elbow, or to synergistic movements in both joints. This group also included the only two neurons which activity diminished during somatosensory stimulation. Among all cells tested only one had a cutaneous receptive field responding to brushing of fur on the medial surface of the paw. We found only six cells that had somatosensory receptive fields on the hindlimb. Overall, characteristics of

somatosensory receptive fields were in agreement with previously reported data (e.g., Asanuma and Hunsperger 1975).

Neurons that responded to stimulation of different parts of the forelimb were distributed randomly in the VL: there were no clear clusters of shoulder-, elbow- or wrist-related cells (Fig. 5.2D). There were also 23% (38/168) of neurons that did not respond to any somatosensory stimulation. They were intermingled with the responsive cells. Five of these cells responded to vestibular stimulation produced by the whole animal translations or rotations; they were found laterally and caudally in the VL (Fig. 5.2D). Two other neurons without somatosensory receptive fields responded to visual stimulation.

Afferent Connections

In *cat A*, WGA-HRP was injected in the center of the area of recordings. The injection had a core ~1.5 mm in diameter (dark area in Fig. 5.2A). Numerous retrogradely labeled neurons were found in the anterior half of the lateral (dentate) nucleus and in the anterior interposed nucleus of the right (contralateral) cerebellum (Fig. 5.3A). In the dentate nucleus, labeled cells were found in its rostral part, with the highest density at P 8.0–8.5, and were not seen caudal to P 9.0. The most ventral part of the nucleus was free from labeled cells. In the anterior interposed nucleus, labeled neurons filled its entire rostro-caudal extent but were confined to the lateral half of the nucleus. Ipsilaterally to the injection site, labeled neurons were found in the lateral half of the entopeduncular nucleus (from L 7.0 to L 5.5). Most laterally, labeled cells filled the entire nucleus, but more medially cells were concentrated only in the anterior and posterior poles of it.

In cat B, red fluorescent beads were injected into the most anterior part of the explored area. Injected beads spread vertically by approximately 3 mm and filled the entire dorso-ventral extent of the VL; medio-laterally, they covered 0.6-0.7 mm of the medial part of the nucleus (L 3.5-4.2; Fig. 5.4A). Labeled cells were found throughout the entire rostro-caudal extent of the contralateral dentate nucleus (Fig. 5.3B). In the lateral half of the nucleus labeled cells were located mostly ventrally; in the medial half they were also present more dorsally, where two clusters of cells were evident: the rostral and caudal ones (Fig. 5.4D). Labeled cells were also found in the contralateral posterior

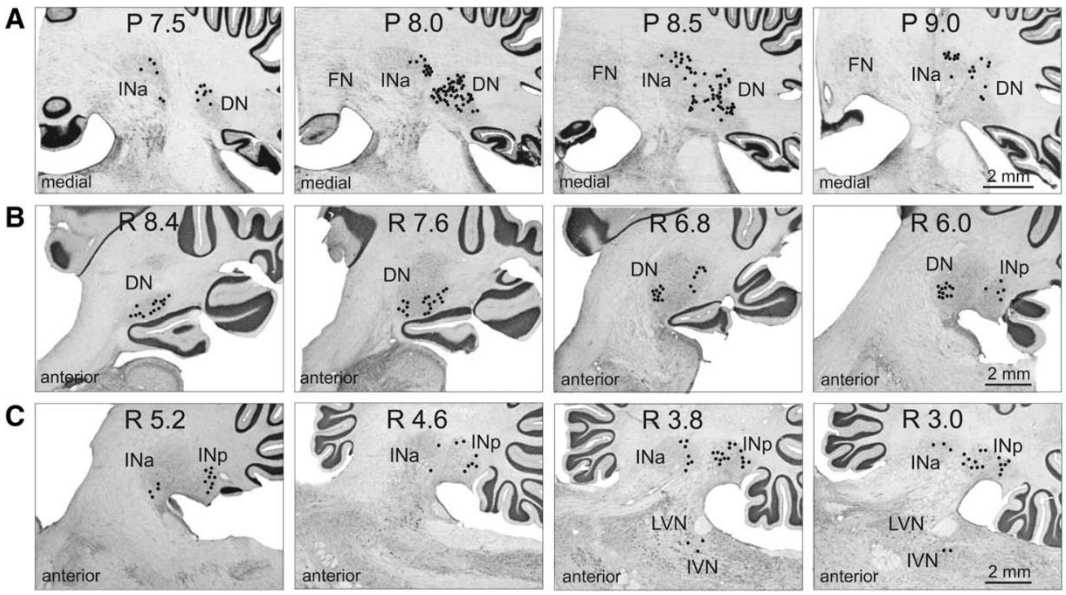


**Figure 5.3:** Cerebellar projections to the recording area in the VL. A: neurons in the anterior interposed (INa) and lateral (dentate, DN) nuclei in cat A, retrogradely labeled with WGA-HRP. Neurons are depicted with black circles on photomicrographs of coronal sections of the cerebellum contralateral to the injection site. B and C: neurons in the dentate, anterior, and posterior (INp) interposed nuclei and inferior vestibular nucleus (IVN) in cat B, retrogradely labeled with red fluorescent beads. Neurons are shown on photomicrographs of parasagittal sections of the cerebellum contralateral to the injection site. Each circle represents 1 labeled neuron. LVN, lateral vestibular nucleus.

interposed nucleus. They were distributed throughout its entire rostro-caudal extent, most intensively at the laterality of L 3.0-4.0 mm (Fig. 5.3C and 4E). A number of

labeled neurons were seen in the contralateral anterior interposed nucleus (Fig. 5.3C). Also, several cells were found throughout the contralateral fastigial nucleus (not shown), and a few were seen in the contralateral inferior vestibular nucleus (Fig. 5.3C and 5.4G). In Addition, a longitudinally extended group of labeled cells was found in the contralateral dorsal hypothalamic area. The ipsilateral entopeduncular nucleus was free from label. Numerous labeled cells were found in the ipsilateral frontal cortex, throughout layer VI of the anterior and posterior sigmoid gyri, at the laterality of L 4.0-9.0 (Fig. 5.4H), as well as more caudally, across the lateral ansate sulcus into suprasylvian gyrus up to the caudal area 5 (Fig. 5.4I). In the area 4γ, labeled cells densely filled the entire layer VI. In other cortical areas labeled cells were sparser and formed a thinner stretch through layer VI (Fig. 5.4C). In all cortical areas that contained labeled neurons in layer VI, medium-size labeled pyramidal cells were occasionally found also in the adjacent part of layer V.

Neurons Projecting to the Motor Cortex

Among the 238 neurons that were recorded during locomotion, 116 were identified as projecting to the motor cortex [thalamo-cortical neurons (TCs)]. Positions of stimulating electrodes in the left precruciate cortex of *cat B* are schematically shown in Fig. 5.5A. The electrodes were placed in the distal forelimb (paw) representation of the motor cortex (MCd) and in the proximal forelimb (elbow, shoulder) representations (MCp) (see methods). In Fig. 5.5B, a track and an electrolytic lesion made by one of the stimulation electrodes are shown on a photomicrograph. The lesion is visible in the cortical layer VI adjacent to layer V that is populated with giant pyramidal cells characteristic for area 4γ. In both cat A and cat B, all stimulating electrodes were placed

in layer VI of area 4γ in the precruciate sigmoid gyrus. An example of an antidromic response of a TC neuron to stimulation of MCd is given in Fig. 5.5C and D.

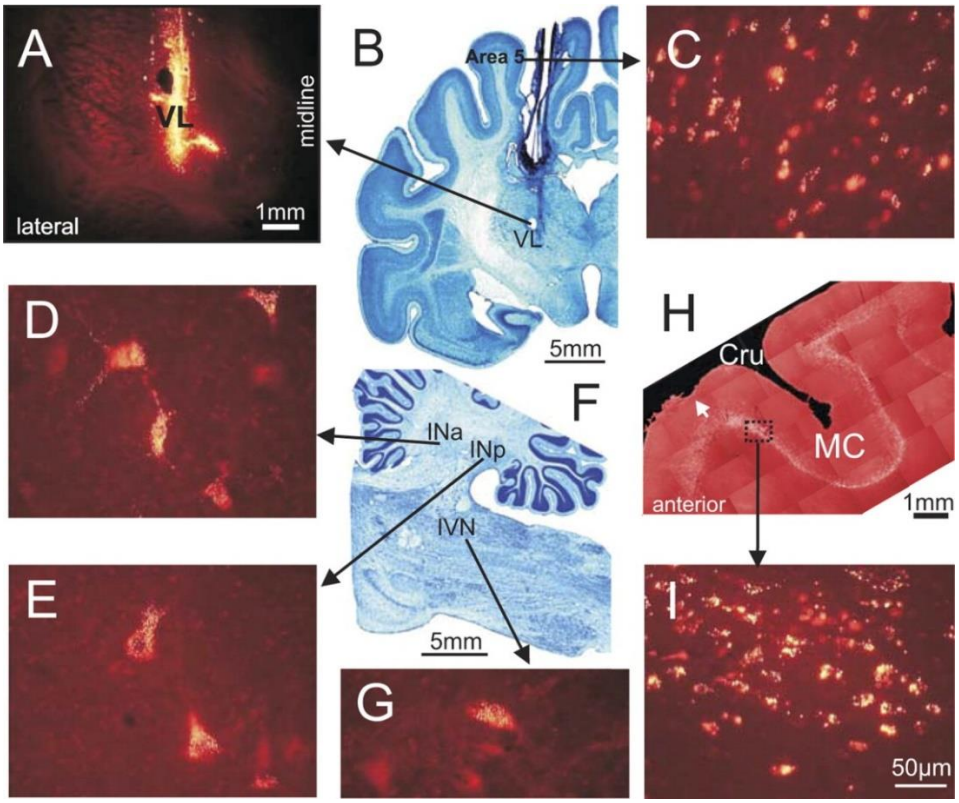


**Figure 5.4:** Cerebellar and cortical neurons labeled with fluorescent beads in cat B. A: coronal section of the thalamus containing the electrolytic lesion mark and fluorescent bead deposit site in the area of recording as seen with a rhodamine filter. B: coronal section of the brain at the same anterior-posterior level as in A, cresyl violet stain. C: labeled neurons in the caudal area 5 in the suprasylvian gyrus. D: labeled neurons in the anterior interposed nucleus (INa). E: labeled neurons in the posterior interposed nucleus (INp). F: parasagittal section of the brain stem and cerebellum, cresyl violet stain. G: labeled neurons in the inferior vestibular nucleus (IVN). H: composition of parasagittal sections of the frontal cortex showing the fold of cruciate sulcus (Cru). Sections contain neurons in layer VI labeled with red fluorescent beads. White arrow points to a small depression on the top of the cortex that was left by one of the stimulation electrodes chronically implanted in the motor cortex (MC). I: labeled neurons in the forelimb representation of the motor cortex in the anterior sigmoideus gyrus. In C, D, E, and G the scale is as in I.

The TCs were distributed fairly evenly throughout the area of recording. In Fig. 5.2D, they are represented by filled shapes. Most TC neurons responded to stimulation of either MCd or MCp only and a few responded to stimulation of both sites. Interestingly,

the vast majority (72%) of neurons projecting to MCd had receptive fields on proximal parts of the forelimb, shoulder, or elbow, and only 9% had receptive fields on the wrist or paw. Neurons projecting to the more medial cortical areas that are related to elbow and shoulder, MCp, had various receptive fields. Latencies of antidromic responses of different TCs varied in the range of 0.5–5.5 ms (Fig. 5.5E). Estimated conduction velocities ranged from 5 to 70 m/s (Fig. 5.5F). We have arbitrarily divided the TC neurons into two subgroups: responding with a latent period of 1.0 ms or faster ("fast" TCs) and responding >1.0 ms latency ("slow" TCs). Two-thirds of slow TCs were collected in cat A.

Activity during Standing

The discharge rates of VL neurons during standing varied in 0.6–63 spikes/s range and was 20.2 ± 12.5 spikes/s on average. Neurons with somatosensory receptive fields were more active than neurons without them [24.3 ± 1.5 vs. 15.4 ± 2.1 spikes/s (means ± SE), respectively; $P < 0.05$, *t*-test]. Neurons located laterally with coordinates L 4.5–5.3 (mostly collected from cat A; Fig. 5.2Aand D) were less active than those located medially at L 3.1–4.4 (mostly collected from cat B; Fig. 5.2B and D) [14.8 ± 1.2 vs. 25.6 ± 1.9 spikes/s (means ± SE); $P < 0.05$, *t*-test]. Fast-conducting TCs were more active on average than slow-conducting TCs [23.7 ± 2.2 vs. 15.4 ± 1.2 spikes/s (means ± SE); $P < 0.05$, *t*-test; Fig. 5.5G]. Moreover, there was a linear relationship between conduction velocity and the discharge rate in the standing animal (Fig. 5.5H).

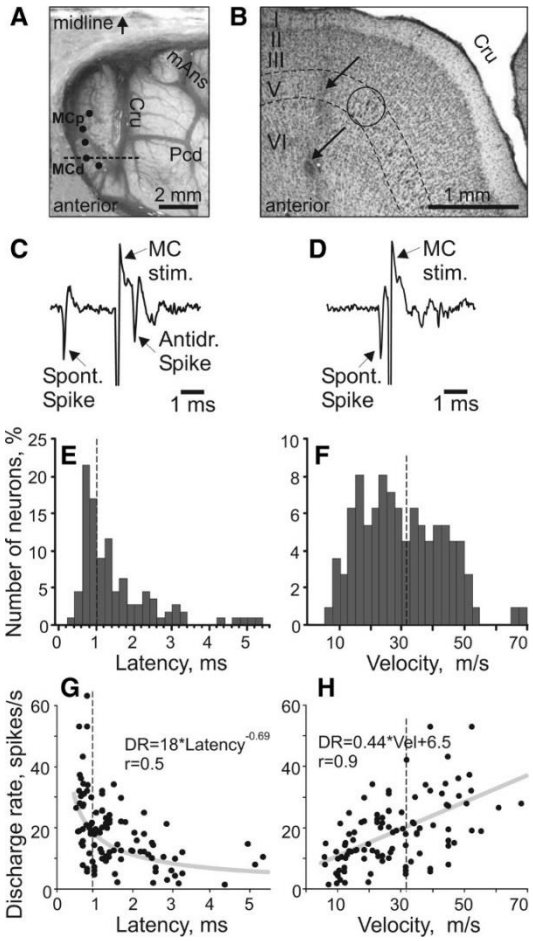


**Figure 5.5:** Axonal conduction velocities of thalamo-cortical projection neurons (TCs). A: photograph of the dorsal surface of the left frontal cortex of cat B. Entrance points of stimulation electrodes into the precruciate sulcus are schematically shown by black dots. Electrodes were placed in the paw (the motor cortex distal forelimb representation, MCd) and the elbow and shoulder (the motor cortex proximal forelimb representation, MCp) as determined by multiunit recording and microstimulation procedures. The position of the parasagittal section, whose photomicrograph is shown in B, is indicated by a dashed line. Cru, cruciate sulcus; Pcd, postcruciate dimple; mAns, medial ansate sulcus. B: photomicrograph of a parasagittal section through the rostral precruciate gyrus stained with cresyl violet. Layers of cortex are numbered. Layer V, which contains giant pyramidal cells, is highlighted by dashed lines. One of the clusters of giant cells in layer V that are characteristic for area 4γ is circled. Arrows point to a track and an electrolytic lesion made by a stimulation electrode that was placed in layer VI. C: stimulation of the motor cortex evoked a spike in a TC neuron with a latency of 0.8 ms. D: to determine whether this spike was elicited antidromically, on the next trial a spontaneous spike of the neuron was used to trigger cortical stimulation with 0.4-ms delay. Stimulation delivered with a delay smaller than the time needed for a spontaneous spike to reach the site of stimulation (that is, approximately equal to the latent time of an antidromic spike) was not followed by

a response. This indicated a collision of ortho- and antidromically conducted spikes and confirmed the antidromic nature of the evoked spike. E: distribution of latencies of antidromic responses to stimulation of the motor cortex of all 116 TC neurons whose activity was recorded during locomotion. F: distribution of estimated conduction velocities. In E and F dashed lines separate “fast” (latencies 0.4–1.0 ms) and “slow” (latencies 1.1–6.0 ms)-conducting neurons. G: relationship between the mean discharge rate of individual neurons in the resting animal and their antidromic latency, approximated with a power function. H: relationship between the mean discharge rate of individual neurons in the resting animal and axonal conduction velocity, approximated with a linear regression. r, Coefficient of correlation.

Characteristics of Locomotion

During recording from each neuron, cats ran between 15 and 100 (typically 25–50) times down each of the corridors. From these trials, 15–150 strides (50 ± 30) of each locomotion task were selected for the analysis according to criteria outlined in methods. For different neurons, the average duration of selected strides was between 600 and 750 ms. The ratio of stance duration to the duration of the cycle (the stride duty factor) was 0.57–0.60. The gait that cats used during walking on both the flat surface and along the ladder was a walk with the support formula 2-3-2-3-2-3-2-3 (Hildebrand 1965). Further details of biomechanics and muscle activities in cats walking on the flat surface and along the horizontal ladder in this experimental setup can be found in Beloozerova et al. (2010).

Population activity during simple and complex locomotion

Upon transition from standing to walking the discharge rate of many neurons changed. It increased, up to 10-fold, in 31% of neurons or decreased, down to less than one-half, in 27% of them. Because changes in the activity were opposite in different neurons, the average discharge rate of the whole population did not change. We found no correlation between receptive fields of the neurons and changes in the discharge rate with the start of locomotion. The average activity of neurons without somatosensory

receptive fields, however, was lower than that of somatosensory responsive cells ($P < 0.05$, *t*-test). Neurons with receptive fields on different parts of the forelimb had similar average activity. The average discharge rate of neurons during walking along the horizontal ladder was similar to that observed during standing and walking on the flat surface. Fast-conducting TCs were more active than slow-conducting TCs during all tasks, however ($P < 0.05$, *t*-test).

During simple locomotion, the activity of 92% of neurons (220/238), including 91% of TCs (105/116), was modulated in the rhythm of strides: it increased in one phase of the stride and decreased in another phase. Two basic patterns of modulation were recognized: one or two PEFs (see methods). Most common was the one-PEF pattern seen in 67% (148/220) of neurons, including 63% (66/105) TCs. Two PEFs were observed in 31% (69/220) of cells, including 35% (37/105) TCs. During locomotion along the ladder, the activity of 96% of neurons (229/238) was also modulated: the one-PEF pattern was seen in 63% (145/229) of neurons and the two-PEF pattern in 34% (78/229) of neurons. In addition, there were three neurons with three PEFs during simple locomotion and seven such neurons during ladder locomotion; we did not consider activity of these cells further. Most neurons had a similar modulation pattern during both locomotor tasks, but in some neurons the pattern changed. We will first compare activities of one- and two-PEF populations during simple and complex locomotion and then look into activities of individual cells.

Neurons with one PEF per stride

During simple locomotion a representative neuron shown in Fig. 5.6 discharged throughout all phases of the stride except for the middle of stance, when it was practically silent (Fig. 5.6A and B). The discharge within the PEF varied in intensity,

forming three small subpeaks; the maximum discharge rate was 80 spikes/s. In Fig. 5.6B the PEF is indicated by a solid black horizontal line, and the preferred phase of the activity is shown by a circle. During ladder locomotion, rather than discharging throughout most of the stride cycle, the neuron was active almost exclusively around the swing-stance transition (Fig. 5.6G and H) but peaked near the same preferred phase as during simple locomotion. Its firing rate reached 118 spikes/s, significantly higher than during simple locomotion ($P < 0.05$, *t*-test), whereas the activity in the trough during stance remained low. Consequently, the strength of modulation was larger during ladder than during simple locomotion. The duration of the PEF shortened by one-half.

Figure 5.6*F* and *L*, show phase positions within the step cycle of PEFs and preferred phases of all neurons during simple and ladder locomotion, respectively. One can see that, during both tasks, PEFs of different neurons were distributed fairly evenly over the cycle. Duration of PEFs differed between neurons in the range of 20–90% of the cycle and lasted ~65% of the cycle on average during either task. In the vast majority of neurons, the discharge rate within the PEF varied. In 27% of neurons at least two subpeaks with activity above the average discharge rate were observed; this level is twice as high as the threshold discharge rate for PEF recognition. The strength of the stride-related modulation of the discharge varied between neurons. About 20% of neurons were completely silent for a part of the step cycle; the majority, however, were active throughout the cycle, while their discharge rate was modulated. During simple locomotion the coefficient of modulation, M was 81± 1%, and the coefficient dM was 8 ± 0.2%. During ladder locomotion the strength of modulation was larger: M was 87 ± 1%, and dM was 9.6 ± 0.3% (means ± SE); the increase in both M and dM was highly significant ($P < 0.0001$, *t*-test). Because of even distribution of PEFs throughout the cycle and their relatively long duration, PEFs overlapped each other, and 60–70% of

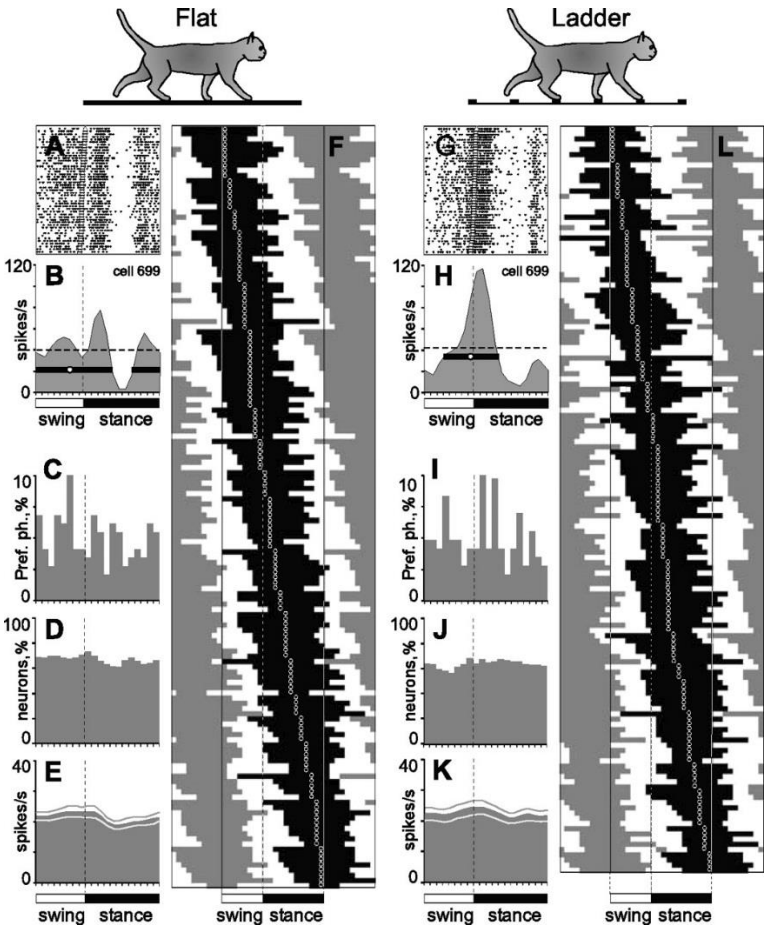


**Figure 5.6:** Population characteristics of one-period of elevated firing (PEF) neurons. A and B and G and H: example of activity of a typical neuron (group I neuron, see Fig. 13) during walking on the flat surface (A, B) and along the horizontal ladder (G, H). The activity is presented as a raster of 50 step cycles (A, G) and a histogram (B, H). In the rasters, the duration of strides is normalized to 100%, and the rasters are rank ordered according to the duration of the swing phase. The end of swing and the beginning of the stance in each cycle is indicated by an open triangle. In histograms, the horizontal interrupted line indicates the average discharge frequency during standing. The horizontal black bar shows the PEF, and the circle indicates the preferred phase (as defined in METHODS). C and I: distribution of preferred phases of activity of all one-PEF neurons during simple (C) and ladder (I) locomotion. D and J: proportion of active neurons (neurons in their PEF) in different phases of the step cycle during simple (D) and ladder (J) locomotion. E and K: mean discharge rate of neurons during simple (E) and ladder (K) locomotion. Thin lines show SE. F and L: phase distribution of PEFs during simple (F) and ladder (L) locomotion. Each horizontal bar represents the PEF location of 1 neuron (shown in black in 1 cycle only) relative to the step cycle. Neurons are rank ordered so that those active earlier in the cycle are plotted at top of graph. Vertical solid lines highlight 1 cycle. Vertical interrupted lines denote end of swing and beginning of stance phase.

neurons were simultaneously active at any time of the cycle during either task (Fig. 5.6D and J). As a result, despite the substantial modulation of the activity in most of the individual neurons, the averaged discharge rate of the population was around 20–25 spikes/s throughout the cycle during both simple and ladder locomotion (Fig. 5.6E and K).

We did not find any simple correlation between neuronal responses to somatosensory stimulation in the quiescent animal and preferred phases of their activity during locomotion. During simple locomotion, the preferred phases of most neurons responsive to passive extension of the shoulder or elbow were timed to the periods of extension of the shoulder or elbow, respectively. In contrast, most neurons responsive to passive flexion of the shoulder or ventral flexion of the wrist had preferred phases not timed to periods of flexion in these joints. Furthermore, an overwhelming majority of neurons without somatosensory receptive fields had profoundly modulated activity. Similar inconsistency was found during ladder locomotion.

In contrast to this inconsistency, neurons with receptive fields involving different joints tended to have their PEF in specific phases of the step cycle (Fig. 5.7). During simple locomotion, neurons responsive to passive movements in the shoulder joint and/or palpation of back, chest, or neck muscles more often had PEF during the swing and early stance phases (Fig. 5.7A). During ladder locomotion, however, these neurons more often had their PEF during stance, and their population activity during stance was higher than that during simple locomotion (Fig. 5.7D). Neurons responsive to passive movement of the elbow more often had their PEF in the late stance and early swing during both tasks (Fig. 5.7, B and E). Most neurons responsive to stimulation of the paw or movement in the wrist joint had their PEF in the beginning and middle of stance (Fig.

5.7C and F). PEFs and preferred phases of neurons without receptive fields were evenly distributed over the cycle.

The one-PEF neurons were distributed over the entire area of recording in the VL without apparent clustering; they projected to both distal and proximal forelimb representations in the motor cortex via both fast- and slow-conducting TCs. When grouped according to the site of projection in the motor cortex (distal vs. proximal), or the conduction velocity (fast vs. slow), during simple locomotion the neuronal populations did not show any differences in the strength of modulation or duration of PEF. However, the neurons projecting to the cortical paw representation, as well as slow-conducting TCs, had subtle but statistically significant higher activity during swing compared with stance, while their counterparts did not. Furthermore, the neurons with preferred phases in the first half of swing (SW1) or in the first half of stance (ST1) had higher activity during their respective preferred periods, compared with the neurons with preferred phases in the second half of swing (SW2) or in the second half of stance (ST2) [36.8 ± 2.2 vs. 27.3 ± 1.5 spikes/s (means ± SE); $P < 0.05$, *t*-test].

During ladder locomotion, fast TCs were more active during swing compared with stance ($P < 0.05$, *t*-test), whereas slow TCs tended to be more active in the opposite phase. The step-related modulation of neurons with shoulder or elbow receptive fields was stronger than that of neurons with wrist/paw receptive fields: The coefficient M was 87–90% vs. 75%, and dM was 9.4–9.8% vs. 6.4% ($P < 0.005$, *t*-test). In addition, neurons with preferred phases in the first half of swing (SW1) became still more active during swing ($P < 0.05$, *t*-test), while the activity of neurons with preferred phases in the second half of swing (SW2) did not change, and their number decreased by 35%.

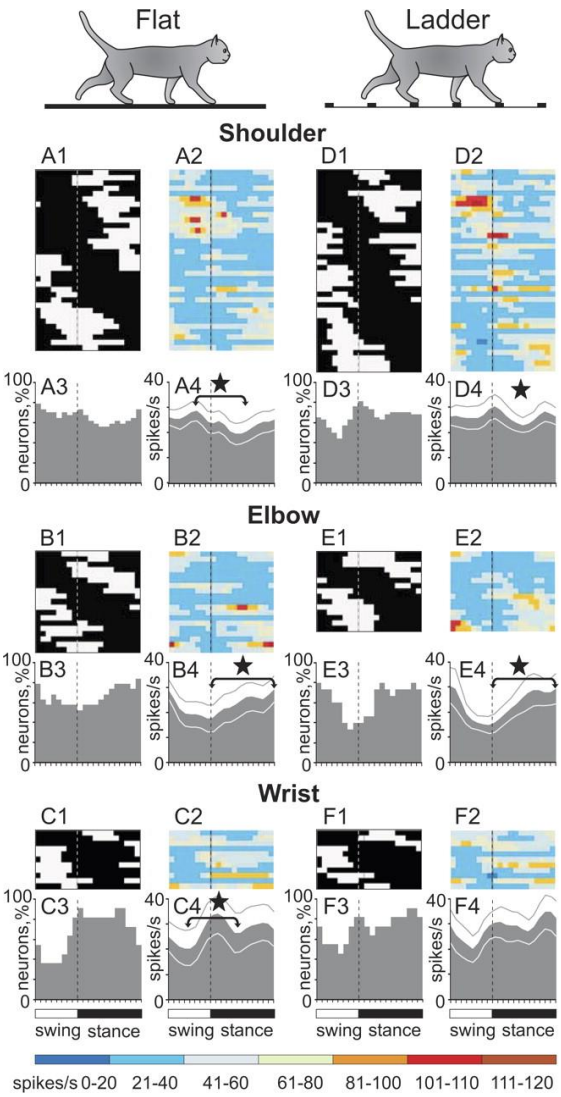


**Figure 5.7:** One-PEF neurons with receptive fields on different joints tend to discharge during different phases of the stride. A and D: activity of neurons responsive to movements in the shoulder joint and/or palpation of back, chest, or neck muscles during simple (A) and ladder (D) locomotion. A1 and D1: phase distribution of PEFs. A2 and D2: corresponding phase distribution of discharge frequencies. Average discharge frequency in each 1/20th portion of the cycle is color-coded according to the scale shown at bottom. A3 and D3: proportion of active neurons (neurons in their PEFs) in different phases of the step cycle during simple (A3) and ladder (D3) locomotion. A4 and D4: mean discharge rate during simple (A4) and ladder (D4) locomotion. Thin lines show SE. Vertical interrupted lines denote end of swing and beginning of stance phase. B and E: activity of neurons responsive to passive movement of the elbow joint. C and F: activity of neurons responsive to stimulation of the paw or movement in the wrist joint. ★Significant differences between the activity of populations during different periods of the cycle.

Neurons with Two-PEF per Stride

An example of activity of a representative neuron with two PEFs is shown in Fig. 5.8. During simple locomotion this neuron was active during both swing-stance and stance-swing transitions (Fig. 5.8A). The discharge rate reached 25 and 30 spikes/s in two different peaks (Fig. 5.8B). On the ladder, the neuron was still active during swing-stance and stance-swing transitions, but its two PEFs deviated from each other in both amplitude and duration. The PEF during swing-stance transition became markedly longer and had higher discharge rate than the other PEF. In addition, one of the troughs deepened. As a result, the strength of modulation was larger during ladder locomotion, while the total duration of PEFs was shorter.

Figure 5.8E and J show the distributions of PEFs in all two-PEF neurons. By the phase and duration of their PEFs, neurons could be loosely subdivided into three groups. The first group included cells that had a long PEF during swing and a short PEF during stance—the "swing" neurons (33% of cells). PEFs of these neurons are concentrated in the upper third of the graphs. The second group consisted of cells that had a long PEF during stance and a short PEF during swing—the "stance" neurons (25% of cells). PEFs of these neurons are concentrated in the lower part of the graphs. In both swing and stance neurons, during simple locomotion the longer PEF was typically (in 70% of cells) also the stronger one, as the discharge rate in it exceeded that in the shorter PEF by 20 ± 15 spikes/s. The duration of the longer PEF ranged from 25% to 70% of the cycle, and the duration of the shorter PEF ranged from 10% to 30%. During ladder locomotion the activity of stance neurons differed in the discharge intensity during swing: in many of these neurons the discharge rate during the shorter PEF in swing was as high as during the longer PEF in stance. By contrast, in the majority (73%) of swing neurons during ladder locomotion the activity during the longer PEF in swing was still higher.

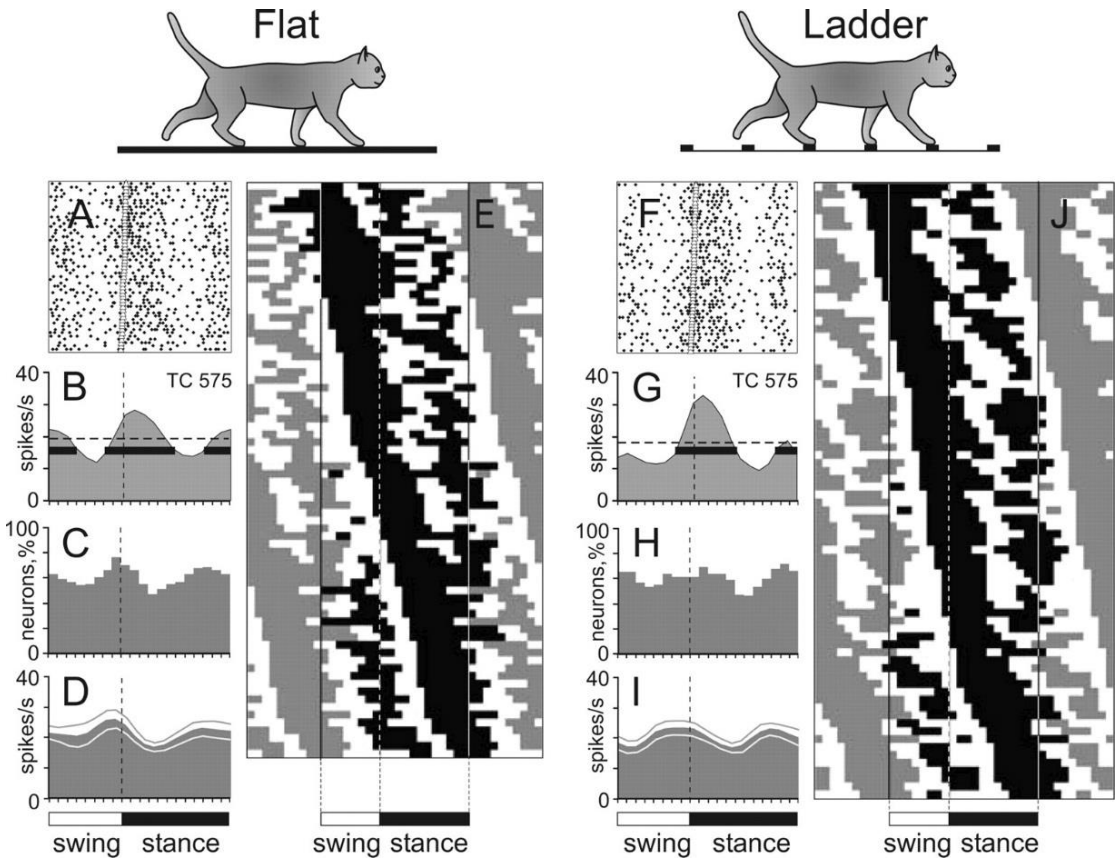


**Figure 5.8:** Population characteristics of two-PEF neurons. A and B and F and G: example activity of a typical neuron (group II neuron, see Fig. 13) during walking on the flat surface (A, B) and along the horizontal ladder (F, G). Activity is presented as a raster of 50 step cycles (A, F) and as a histogram (B, G). In rasters, the end of swing and the beginning of the stance phase in each cycle is indicated by an open triangle. In histograms, the horizontal interrupted line indicates the average discharge frequency during standing; the horizontal black bar shows PEFs. C and H: proportion of active neurons (neurons in their PEF) at different phases of step cycle during simple (C) and ladder (H) locomotion. D and I: mean discharge rate during simple (D) and ladder (I) locomotion. Thin lines show SE. E and J: phase distribution of PEFs during simple (E) and ladder (J) locomotion. Each trace shows PEFs of 1 neuron (shown in black in 1 cycle only). Vertical solid lines highlight 1 cycle. Vertical interrupted lines denote the end of swing and beginning of stance phase.

The third and most populous group included neurons whose two PEFs were of approximately similar duration, ranging from 15% to 40% of the cycle each (40% of cells). In these “transition” neurons, the first PEF typically started in mid-swing and lasted into stance, while the second PEF started in mid-stance and continued into swing. In Fig. 5.8E and J, PEFs of these neurons are concentrated in the middle of the graphs.

The average duration of each PEF was 30 ± 7% of the step cycle, approximately half as long as in the one-PEF group. Although in transition neurons the two PEFs were of a similar duration, in 68% of them the discharge in one of the PEFs was more intense, by 15 ± 12 spikes/s, on average.

Because of a rather even distribution of PEFs of different neurons throughout the cycle, and their long duration, the PEFs overlapped each other, and 50–70% of neurons were simultaneously active in any phase of the cycle (Fig. 5.8C and H). The discharge rate of the population of two-PEF neurons was slightly modulated around the value of 20 spikes/s with two small peaks during the swing-stance and stance-swing transition periods (Fig. 5.8D and I).

During simple locomotion, the strength of stride-related frequency modulation, M, was 79 ± 12% and dM was 8.2 ± 3.4%. Both values were similar to those in the one-PEF group. Upon transition to ladder locomotion, however, in sharp contrast with the one-PEF group, the strength of modulation in the two-PEF population did not increase.

In two-PEF cells the active phase during locomotion usually differed from the phase that could be expected based on somatosensory responses in the resting animal, similar to that in one-PEF neurons. Unlike one-PEF neurons, however, the activities of two-PEF cells with receptive fields involving different joints were similarly distributed across the stride: all groups had two subtle maxima at swing-stance and stance-swing transitions.

Two-PEF neurons were distributed over the entire area of sampling in the VL and intermingled with one-PEF cells. Both fast- and slow-conducting TCs with two PEFs projected to both the distal and proximal forelimb representations in the motor cortex. Groups of neurons assembled according either to the site of projection to the motor

cortex (distal vs. proximal) or to conduction velocity (fast vs. slow) during simple locomotion were similar in their activity phases, strength of modulation, and duration of PEFs. During ladder locomotion, however, the step-related modulation of neurons projecting to the paw area was stronger than that of neurons projecting to the elbow or shoulder areas: the coefficient M was 84 ± 2% vs. 77 ± 3%, and dM was 9.3 ± 0.6% vs. 7.0 ± 0.7% (mean ± SE) ($P < 0.05$, *t*-test). Also, the PEFs of neurons projecting to the paw/wrist area were slightly shorter, lasting for 55 ± 2% rather than 65 ± 3% of the cycle ($P < 0.05$, *t*-test). During either task, swing, stance, and transition neurons were similar in their average and peak discharge rates, strength of modulation, and durations of PEF. Swing neurons, however, were more active during swing than stance neurons during stance (30 ± 2.7 vs. 20 ± 1.2 spikes/s; $P < 0.05$, *t*-test).

Individual neurons discharge differently during two locomotion tasks

Upon transition from simple locomotion to walking along the horizontal ladder, individual neurons changed their activity to even a greater extent than the populations did as a whole: 79% of neurons changed at least one characteristic of the activity. First, the mean discharge rate changed in 42% (100/238) of neurons, increasing in 19% (46/238) of neurons and decreasing in 23% (54/238) (Fig. 5.9A). The increase was on average 63 ± 55%, and the decrease was 34 ± 16%. The peak discharge rates averaged over one histogram bin (1/20th of the cycle) also changed in many neurons upon transition from simple to ladder locomotion. They increased in 30% of cells (68 of 230 neurons whose activity was step related in at least one of the tasks) and decreased in 14% (33/230). The average increase was 20.5 ± 9.0 (73 ± 53%) and the decrease was 16.5 ± 6.5 (32 ± 15%) spikes/s, respectively.

Second, the strength of frequency modulation changed in an even larger fraction of cells than the mean or peak rates: it increased in 44% of cells (96 of 217 neurons modulated in both tasks; $P < 0.01$, $\chi^2$-test) and decreased in 11% (24/217). The dM increases ranged from 20% to 200% of the value observed during simple locomotion and were 57 ± 37% on average, while the decreases were 33 ± 10%. In contrast to the scatterplot of mean frequencies (Fig. 5.9A), data points in the plot for dM (Fig. 5.9B) were more widely distributed along the *y*-axis. The increase in M was 15 ± 8% on average.

Third, the duration of the PEF changed in 44% of neurons (96/217), decreasing in 24% (52/217) and increasing in 20% (44/217) of them by 15–50% of the cycle. In many neurons, the duration of the PEF differed considerably between simple and ladder locomotion, and there was no correlation between these two values ($r = 0.2$; Fig. 5.9C).

Fourth, the preferred phase of activity changed in 26% of one-PEF neurons (in 38 of 145 cells modulated with 1 PEF in both tasks; see Fig. 5.11A), and the phase positions of individual PEFs changed in some two-PEF cells. In addition, the number of PEFs per cycle changed in many neurons. Close to half (43%, 30/69) of neurons with a two-PEF pattern during simple locomotion had one PEF during ladder locomotion (see Fig. 5.12A and B). Also, 15% (22/148) of neurons that had one PEF during simple locomotion had two PEFs on the ladder (Fig. 5.12C and D).

Finally, 10 neurons were involved in the locomotion-related activity during the ladder task only, and three neurons lost their modulation during walking on the ladder. We will consider further the changes in the discharges of individual neurons separately for the cells active in the same phase during both locomotion tasks and for the cells that were active in different phases.

Neurons Active in the Same Phase during Both Tasks

The preferred phases of activity in half of one-PEF neurons (80/148, 54%) did not change upon transition from simple to ladder locomotion, whereas other parameters of the discharge could change (Fig. 5.10). Most striking was the change in the strength of stride-related modulation. In 24 (30%) neurons its value increased by 50 ± 30%. Along with this increase, either the average discharge rate or the duration of PEF often changed. Both tended to decrease as the depth of modulation increased. At the same time, the peak discharge rate and the average activity within the PEF typically increased (by 10–35 spikes/s) or did not change. There were also five cells in which the duration of the PEF decreased without a change in the strength of modulation. Thus, during complex locomotion, the activity of 36% (29/80) neurons with one PEF during both locomotion tasks became more task-related as well as more time restricted, better tuned to a particular phase of the step cycle, and more precise (compare Fig. 5.6A, B, G, and H); we have termed these cells group I neurons. Group I neurons projected to both distal and proximal forelimb representations in the motor cortex and had a variety of conduction velocities and receptive fields. Two-thirds of them were located medially in the VL. The preferred phases of activity of group I neurons were distributed evenly across the stride. Another 10% (8/80) of neurons decreased their modulation depth during ladder locomotion by 30 ± 8% on average. Besides these, 24% (19/80) of cells changed their discharge rate without altering the depth of modulation or PEF duration, and another 24% (19/80) discharged similarly during two locomotion tasks.

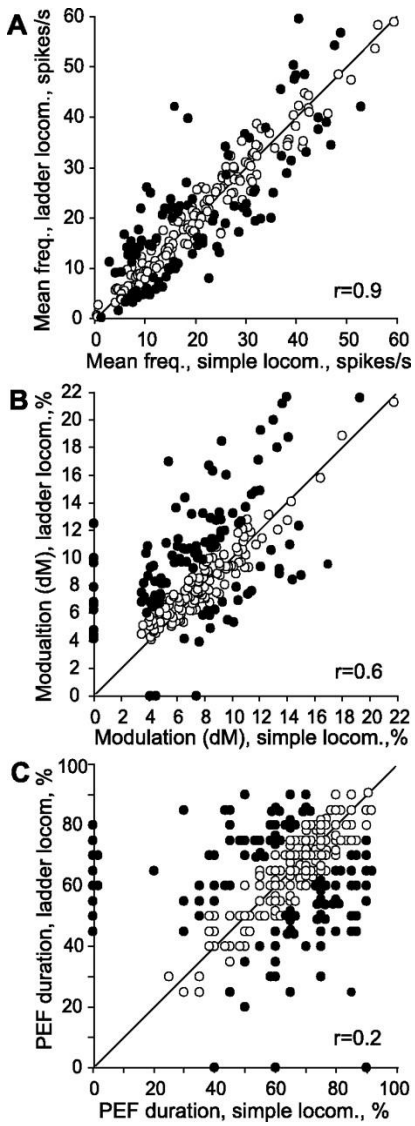


**Figure 5.9:** Comparison of activity characteristics of individual neurons during simple and ladder locomotion. The x- and y-axes of each point show the values of a characteristic of a neuron during simple and ladder locomotion, respectively. Neurons whose characteristics were statistically significantly different during the 2 tasks are shown by filled circles; the others are shown by open circles. A: mean discharge frequency averaged over the stride. B: coefficient of frequency modulation, dM. C: duration of PEF. For two-PEF neurons, the combined duration of two PEFs is given. The coefficient of correlation (r) is indicated.

Half of two-PEF neurons (38/69, 55%) also did not change their phases of activity upon transition from simple to complex locomotion (e.g., Fig. 5.8A, B, F, and G). Discharges of 36% (25/69) of these were different during the two tasks in other aspects, however. Similar to one-PEF cells, 16 (23%) of these neurons had stronger modulation, shorter PEFs, or both during ladder locomotion, while mean activity could either increase or decrease. We have named these group II neurons. All but one of these neurons projected to the distal forelimb representation in the motor cortex. Group

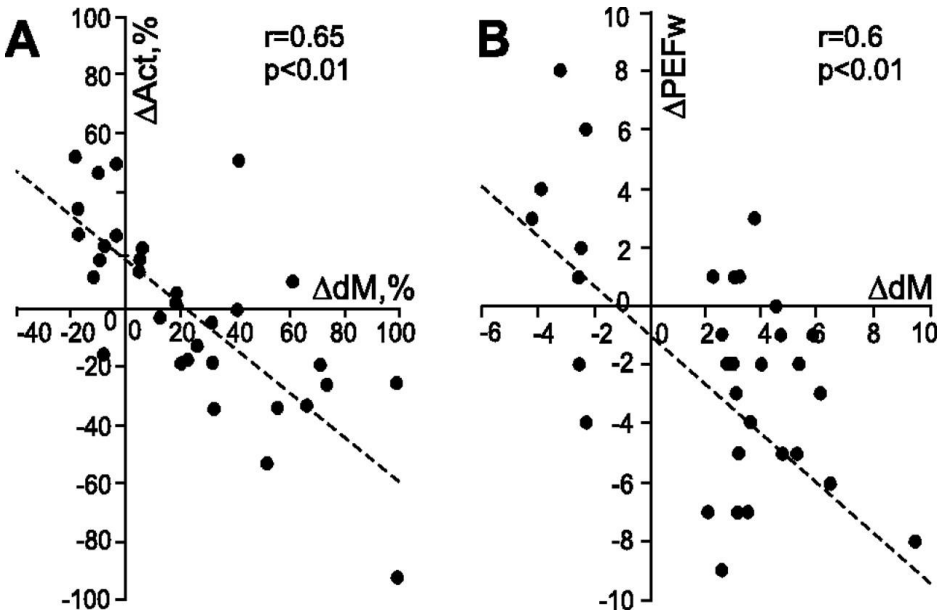


**Figure 5.10:** Changes in activity characteristics of neurons that were active in similar phases of the stride and had one PEF during both simple and ladder locomotion. A: negative correlation between change in the depth of modulation (dM) and mean discharge frequency (Act). B: negative correlation between the change in the depth of modulation and duration of PEF (PEFw). In A and B the x-axis and y-axis of each point show the difference in a discharge characteristic of a neuron between simple and ladder locomotion tasks. The difference is positive if the value of the parameter was larger during ladder locomotion. Only neurons with statistically significant difference in the depth of modulation between two tasks are shown. The coefficient of correlation (r) is indicated.

II neurons had a variety of axonal conduction velocities, and their receptive fields could be on either distal or proximal segments of the forelimb. Swing, stance, and transition neurons were all represented in this group.

Neurons Active in Different Phases during Two Tasks

A change in the phase of activity of a neuron upon transition from simple to complex locomotion could occur either because of a phase shift of the same discharge pattern within the cycle or because of re-formation of the pattern, so that the neuron had a one-PEF pattern in one locomotion task and a two-PEF pattern during another task.

Phase shifts of the discharge pattern were seen almost exclusively in one-PEF neurons. The preferred phase in 38 (26%, 38/148) one-PEF neurons changed between two tasks in this manner (Fig. 5.11). We have named these group III neurons. The average phase shift in group III neurons was 20 ± 9% of the cycle (Fig. 5.11A). Approximately similar numbers of neurons discharged earlier and later in the cycle during ladder versus simple locomotion (Fig. 5.11A). Figure 5.11C–F illustrates two most common types of phase shifts. Aside from the preferred phase shift, the most striking modification in the activity of group III neurons was a change in the strength of stride-related modulation. It increased in 50% (19/38) of neurons by 65 ± 30% on average. This was similar to the behavior of group I neurons. In group III, however, the average discharge rate was typically similar during both locomotion tasks, while the duration of PEF was also shorter in more than half of the cells (Fig. 5.11*B*). Thus, during complex locomotion, the activity of very many group III neurons became also more strongly stride related and often more focused on a specific phase, precise, compared with simple locomotion. Group III neurons had a variety of receptive fields, but elbow extension was disproportionally represented. The great majority of group III neurons projected to the distal forelimb area of the motor cortex; 75% of group III TCs were “slow,” and two-thirds of group III cells were located laterally within the nucleus.

Some neurons with one PEF during simple locomotion changed the preferred phase of activity on the ladder because of acquisition of a second PEF (22/148, 15%). We have named these group IV neurons. Fig. 5.12A–D, illustrates the two most common types of phase shifts. There was no particular phase in which the new PEF would tend to appear, and during ladder locomotion the number of *group IV* cells active in each phase of the cycle remained the same as during simple locomotion. And once again, besides the

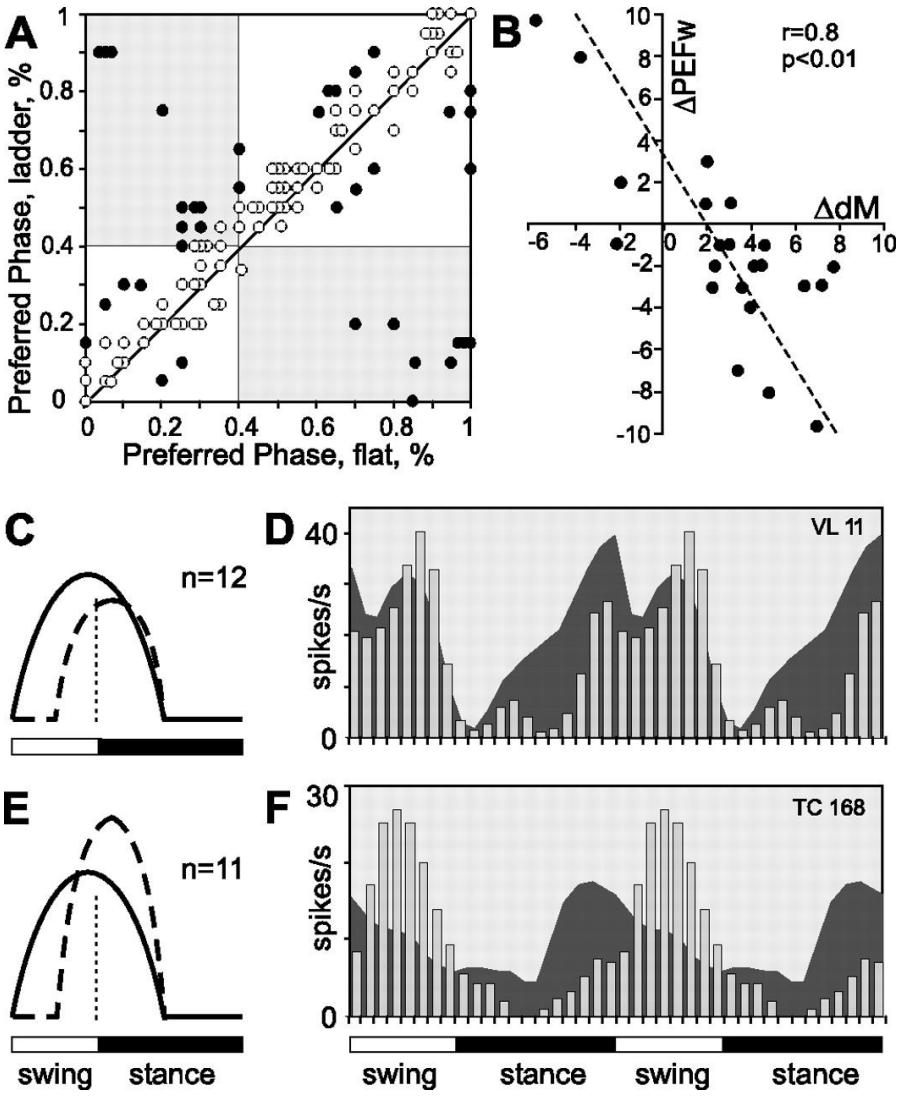


**Figure 5.11:** Changes in activity characteristics of neurons that had one PEF during both simple and ladder locomotion but were active in different phases of the stride (group III neurons). A: comparison of preferred phases of activity in individual neurons during simple and ladder locomotion. The x-axis and y-axis of each point show the preferred phase of a neuron during simple and ladder locomotion, respectively. Neurons whose characteristics were statistically significantly different during the two tasks are shown with filled circles; the others are shown with open circles. Areas that correspond to the swing phase during one task but stance phase during the other task are shaded. B: negative correlation between the change in the depth of modulation and the duration of PEF. The x-axis and y-axis of each point show the difference in a discharge characteristic of a neuron between simple and ladder locomotion. The difference is positive if the value of the parameter was larger during ladder locomotion. Only neurons with statistically significant differences in the depth of modulation between two tasks are shown. The coefficient of correlation (r) is indicated. C: schematic presentation of the most frequently observed type of the change in the discharge pattern of group III neurons upon transition from simple to ladder locomotion: the shift in the preferred phase occurs because the activity in a

part of the PEF observed during simple locomotion decreases during ladder locomotion. Solid line shows the activity during simple locomotion; interrupted line shows the activity during ladder task. Vertical dashed line shows the transition between swing and stance. D: example of activity of a typical group III neuron. Area histogram shows the activity of the neuron during simple locomotion. Bar histogram shows its activity during ladder task. To promote visualization of the difference between activities in two tasks, the cycle is repeated twice. E and F: same as C and D but showing the second most frequent type of the activity change in group III neurons when the shift in the preferred phase occurs because the activity in a part of the PEF observed during simple locomotion decreases during ladder locomotion, and this reduction is accompanied by an increase in the discharge rate during the remaining part of the PEF.

change in the pattern of modulation, the other major modification in the activity of these neurons was an increase of modulation: in 45% of group IV neurons, it increased by 74 ± 43% on average. Group IV neurons had a variety of receptive fields, projected to both distal and proximal forelimb representations in the motor cortex, and nearly all group IV TCs were "slow." Two-thirds of group IV cells were located laterally in the VL.

In contrast to one-PEF neurons that only infrequently acquired a second PEF, the two-PEF cells often changed their discharge pattern ($P < 0.0001$, $\chi^2$-test). Nearly half of neurons (30/69, 43%) with two PEFs during simple locomotion had one PEF during walking on the ladder (Fig. 5.12E–H). We have named these group V neurons. In 18 of these neurons the reduction in PEF number occurred because one of the PEFs seen during simple locomotion was absent on the ladder (Fig. 5.12E and F). In the overwhelming majority of the neurons ($n = 14$) this was accompanied by an increase in the discharge rate within the remaining PEF. In 12 other cells the transition from a two-PEF to a one-PEF pattern was accomplished by an increase in the activity during one of the inter-PEF intervals, joining the previously distinct PEFs (Fig. 5.12G and H). There was no particular phase preference for which PEF tended to disappear in different neurons, and on the ladder the number of group V cells active in each phase of the cycle remained about the same as during simple locomotion, despite the fact that on the ladder they had only one PEF instead of two. The other major modification in the activity

of group V neurons was the change in the depth of modulation. It increased in one-third of the cells by an average 72 ± 56%. Group V neurons projected to both distal and proximal forelimb representations in the motor cortex, had a variety of conduction velocities, and were scattered across the nucleus, but almost all had receptive fields on the shoulder.

## Discussion

### VL Signals Conveyed during Simple Locomotion

The first goal of this study was to elucidate whether during locomotion VL neurons discharge in a manner that is suitable to contribute to the locomotion-related activity in the motor cortex. We found that they do. The activity of 92% of VL neurons was modulated in the rhythm of strides, and 67% of neurons, including 63% of TCs, discharged with a single period of elevated firing (PEF) per cycle. This discharge pattern is similar to that observed in the majority of neurons in the motor cortex (Armstrong and Drew 1984; Beloozerova and Sirota 1985a, 1993a, b; Drew 1993). Thus the one-PEF TCs can directly contribute to the activity in the motor cortex during locomotion. All phases of the step cycle are covered with a nearly equal neuronal representation (Fig. 5.6C, D, and F) and similar intensity (Fig. 5.6E). A substantial number of VL neurons discharge with two PEFs per stride, including 35% of TCs. Since a limb central pattern generator (CPG) discharges only one time per cycle (reviewed in McCrea and Rybak 2008), if a neuron discharges more than once, this means that it receives input from more than one limb CPG or that there are several peripheral inputs that significantly influence its discharge. The two-PEF pattern is less frequently observed in the motor cortex (Armstrong and Drew 1984; Beloozerova and Sirota 1985a, 1993a, 1993b; Drew 1993). However, in most two-PEF VL neurons one of the PEFs is typically

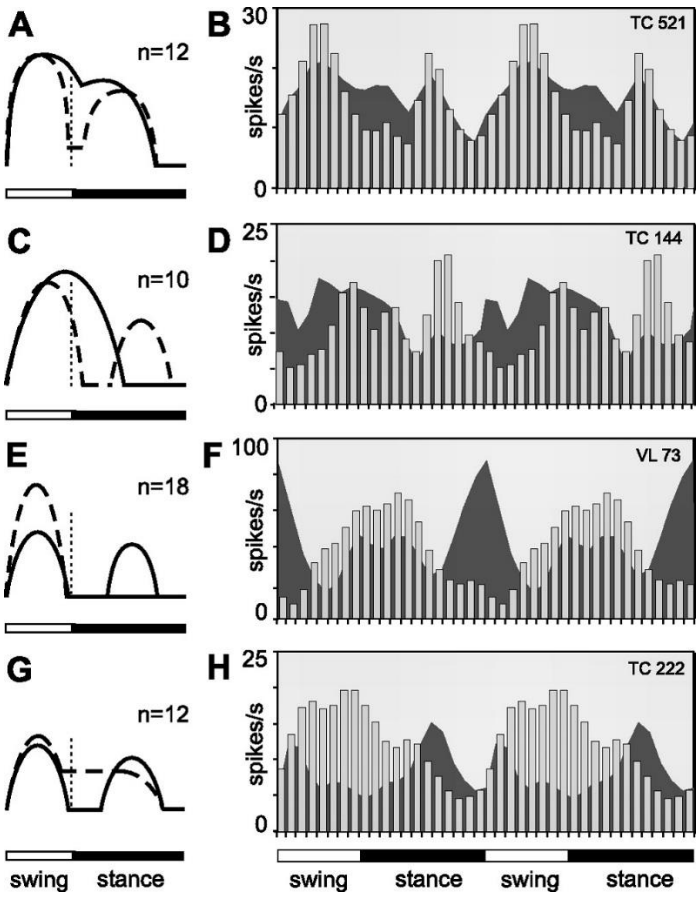


**Figure 5.12:** Changes in the activity characteristics of neurons that had different numbers of PEFs during different locomotion tasks (group IV and group V neurons). A: schematic presentation of the most frequently observed type of change in the discharge pattern of group IV neurons upon transition from simple to ladder locomotion. The change in the number of PEFs occurs because 2 small subpeaks in the PEF during simple locomotion are shaped into 2 full PEFs. This occurs most often by deepening the trough between the subpeaks, but also through a further increase in the discharge rate within these peaks. Solid line shows activity during simple locomotion, and interrupted line shows the activity during ladder task. Vertical dashed line shows transition between swing and stance. B: example of the activity of a typical group IV neuron. Area histogram shows the activity of the neuron during simple locomotion. Bar histogram shows its activity during the ladder task. To promote visualization of the difference in activities between two tasks, the cycle is repeated twice. C and D: same as A and B but showing the second most frequent type of activity change in group IV neurons. The change in the number of PEFs occurred because the PEF observed during simple locomotion asymmetrically changed in amplitude and became shorter, and a new PEF appeared in a former trough. E and F: same as A and B but showing the most frequent type of activity change in group V neurons. G and H: same as A and B but showing the second most frequent type of activity change in group V neurons.

shorter and weaker (Fig. 5.8E) and might lack a sufficient effect on the activity of the target cortical neurons.

We found two notable differences in the activity of VL neurons and efferent neurons of motor cortex layer V. The average depth of modulation (dM) is lower in the VL, 8 ± 0.2% vs. 10 ± 0.7% (Sirota et al. 2005; $P < 0.05$, *t*-test), and the discharge within the activity bursts is more variable. That is, stride-related responses of VL neurons are less phase specific compared with those of motor cortex layer V neurons. This agrees with previous findings of a weaker directional specificity of VL neuron discharges during arm and wrist movements compared with that of neurons in the motor cortex (Kurata 2005; Strick 1976) and is in line with an analogous observation in the visual system, where responses of neurons in the lateral geniculate nucleus are less specific to visual stimuli than those of cells in the visual cortex (see, e.g., Tsao and Livingstone 2008).

The locomotion-related modulation of activity in the VL during simple locomotion may arise from two main sources: the spino-thalamic projection (Craig 2008; Mackel et al. 1992; Yen et al. 1991) and the cerebello-thalamic projection (Evrard and Craig 2008; Ilinsky and Kultas-Ilinsky 1984; Nakano et al. 1980; Steriade 1995). In addition, influence from the motor cortex may partially contribute. In decerebrated cats, it was found that the cerebellum plays the pivotal role in driving locomotion-related discharges in neurons of subcortical motor centers, including neurons of the red and vestibular nuclei, and neurons of reticular formation giving rise to the reticulo-spinal tract (Orlovsky 1970; Orlovsky 1972a, b; reviewed in Arshavskii et al. 1986). It can be suggested that the VL, another subcortical motor nucleus receiving direct connections from the cerebellum, does not differ in this respect. Indeed, the cerebellum receives information both from the spinal locomotor CPG and from somatosensory receptors (Arshavsky et al. 1986; Lundberg and Oscarsson 1962; Oscarsson 1965) and can pass

both types of information to the VL. All deep cerebellar nuclei project to the area of VL that we have explored (see, e.g., Angaut 1979; Evrard and Craig 2008; Ilinsky and Kultas-Ilinsky 1984; Nakano et al. 1980; Rinvik and Grofová 1974; Rispal-Padel and Grangetto 1977), and it was shown that all these nuclei house neurons whose activity is strongly step related during locomotion (Armstrong and Edgley 1984; Nilaweera and Beloozerova 2009; Orlovsky 1972a, b). Interposed nucleus neurons more often (57% of cells) discharge one burst of spikes per stride, while 39% discharge two bursts (Armstrong and Edgley 1984). These proportions are similar to those that we found in the VL ($P > 0.05$, $\chi^2$-test). Also, similarly to VL neurons, periods of elevated firing in interposed nucleus neurons are widely distributed through the stride, and as a population they too have a slightly elevated activity during swing phase. Lateral nucleus neurons usually have a single PEF, and their preferred phases are also distributed across the step cycle (Beloozerova and Sirota 1998; Nilaweera and Beloozerova 2009).

VL Signals Conveyed During Complex Locomotion

The second goal of the present study was to explore whether VL neurons discharge in a manner that is suitable to contribute to the additional modulation of the activity in the motor cortex that occurs during locomotion over complex terrain. Do VL neurons transmit to the motor cortex signals that are needed to control the landing positions of feet during walking on a complex terrain? We found that the activity of VL neurons with one PEF was modulated more strongly on the ladder than during simple locomotion. The fast-conducting TCs and neurons with preferred phases in the first half of swing during simple locomotion increased their activity during swing, while the slow-conducting TCs and shoulder-related cells became more active during stance (Fig. 5.7). The overwhelming majority of individual one-PEF and two-PEF neurons changed their

discharges upon transition from simple to complex locomotion. The dominant change, similar to that in the motor cortex, was an increase in the depth and temporal precision of the modulation (Fig. 5.9B and C). In contrast to simple walking, during complex locomotion the depth of modulation in VL neurons was as high as in the motor cortex efferent population of layer V: 9.6 ± 0.3% vs. 11 ± 0.8% (Sirota et al. 2005; $P > 0.05$, *t*-test). The further increase in the similarity between VL and motor cortex discharges during complex locomotion suggests that the VL may have a significant contribution to the modulation of the motor cortex activity during this task.

The VL appears to be more than a simple relay for signals passing to the motor cortex during complex locomotion. Many of the VL neurons discharged in different phases of the cycle during simple and complex locomotion. This shows that the information related to the complex environment changes the basic locomotion-related discharge pattern of VL neurons. Five major modes of integration can be recognized.

The first two modes were represented in the activity of neurons of groups I and II, respectively (Fig. 5.6A, B, F, and G; Fig. 5.8A, B, F, and G; Fig. 5.13). These were adjustments of the modulation (with 1 PEF or 2 PEFs, respectively) with regard to magnitude only. In the activity of group I and II neurons the simple locomotion-related pattern was dominant, and the role of complex locomotion-related information was to increase the activity level and the efficacy of the stride-related modulation. These modes of integration could either occur in the cerebellum, with results transmitted to the VL, or take place in the VL itself. Having large numbers of neurons whose activity patterns retain their phase position within the step cycle might be beneficial for smooth performance of complex movements such as locomotion in challenging environments.

The third mode of integration was “fine-tuning” of timing of the activity, as seen in group III neurons (Figs. 11 and 13). The simple locomotion-related information also dominated the activity of these neurons, but on the ladder its timing was slightly adjusted by complex locomotion-related input. We hypothesize that this assisted in proper timing of foot placements on the crosspieces of the ladder.

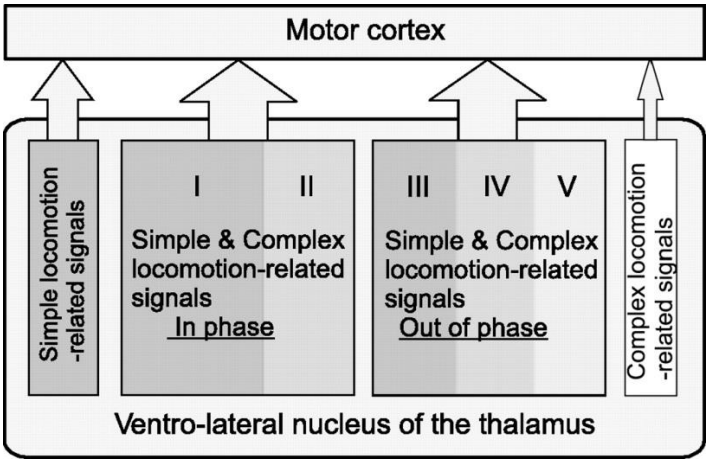


**Figure 5.13:** Signals conveyed from the VL to the motor cortex during locomotion. See DISCUSSION for details.

The fourth mode of integration was the conversion of two-PEF neurons into one-PEF neurons (Fig. 5.12E–H, and 5.13). In this mode, the simple and complex locomotion-related information had approximately equal roles, with the simple locomotion-related information still having a significant contribution to determining the discharge timing of the neuron and with the complex locomotion-related information substantially altering it. Conversion of two-PEF neurons into one-PEF neurons aligned the discharge pattern of VL neurons with that of cells in the motor cortex and presumably increased the efficacy of VL influences upon the motor cortex.

The fifth mode of integration was a summation of simple and complex locomotion-related inputs coming in different phases of the stride (Figs. 11A–D and 13). Here the

simple and complex locomotion-related information also had approximately equal roles, and the complex locomotion-based information simply supplemented the existing simple locomotion-based pattern. This changed phasing of neuron activity, and we hypothesize that it assisted in accurate timing of foot placements during complex locomotion.

## Nature of Signals Conveyed by the VL during Complex Locomotion

What is the content of the complex locomotion-related information conveyed by the VL to the motor cortex during complex locomotion? We have previously demonstrated that the mechanics of simple locomotion on the flat surface and complex locomotion along the horizontal ladder used in our studies are rather similar (Beloozerova et al. 2010). The crosspieces of the ladder are positioned at comfortable distances, and their tops are flat and wide enough to easily accommodate the foot. Under these conditions, in the forelimbs, there are only slight differences in the wrist movements and in the activity of some wrist-related muscles between simple and complex locomotion. One could hypothesize, however, that largely similar movements of simple and ladder locomotion still produce different proprioceptive afferentation, which is then transmitted to the motor cortex via the VL. It was shown that the level of fusimotor activity is often higher during difficult motor tasks, especially those that are novel, strenuous, or associated with a high degree of uncertainty (Hulliger et al. 1989; Prochazka et al. 1988). Our ladder locomotion task was well practiced, however, entirely predictable, and, judging from levels of EMG activity (Beloozerova et al. 2010), not at all strenuous. Thus it does not seem very likely that a potential difference in the proprioceptive afferentation between simple and ladder locomotion can be responsible for the entire volume and spectrum of discharge differences of VL neurons during these two tasks.

In addition to moving the wrist slightly differently during simple and ladder locomotion, on the ladder cats assume a more bent-forward posture by lowering the center of mass and rotating the neck down. These differences in the activity of very proximal as well as very distal limb muscles, albeit small, can be sensed by neurons in the VL and conveyed to the motor cortex. Indeed, we found that during ladder locomotion the neurons responsive to passive movements in the shoulder joint and/or palpation of back, chest, or neck muscles reorganize its activity compared with simple walking (Fig. 5.7A and D). It could then be expected that, similarly, the activity of the wrist-related population will also reflect the difference in kinematics in this joint during the two locomotion tasks. We did not see this, however. Although many individual wrist-related neurons changed their activity between the tasks, as a group the activity of this population was similar during simple and complex locomotion (Fig. 5.7C and F). This can be explained by the too small difference in the kinematics between the tasks. Alternatively, it can be suggested that the activity of some neurons in the VL is not directly related to mechanics of the movements and somatosensation.

While differences in mechanics of locomotion between simple and ladder tasks are on the small side, and the relationship of the activity of VL neurons to those differences is inconsistent, cats move their eyes and look at the walking pathway in a very different manner during simple and ladder locomotion (Beloozerova et al. 2010; Rivers et al. 2009). Cats only infrequently look at the surface of the pathway when it is flat, but they intensively inspect the surface on every single run when walking along the ladder. Moreover, on the ladder there is a correlation between timing of gaze shifts and stride phase (Rivers et al. 2010). Similar data have been reported for humans walking on stepping-stones (Hollands et al. 1995; Hollands and Marple-Horvat 2001). Considering the rather similar limb motor patterns in the two locomotion tasks but dramatically

different gaze behaviors, we want to suggest that at least a part of the differences in discharges of VL neurons during simple and ladder locomotion reflects differences in processing of visual information during these two tasks, as well as the changes in motor commands made on the basis of visual information. During locomotion in a complex environment visual information about the position of the stepping target is first processed through visual networks and then at some point is incorporated into the basic locomotion rhythm in order to guide the limb. From this point on it becomes integrated "visuo-motor" information that, in the afferent sense, is "[processed] visual information," while in the efferent sense it is a "limb control signal" reflecting preparation of the movement. It has been suggested that visual information about the environment is integrated with movement-related information in the cerebellum and then funneled to the motor cortex via the VL for control of limb movements (Glickstein 2000; Glickstein and Gibson 1976; Stein and Glickstein 1992). Martin and Ghez (1985) had found earlier that the onset of elbow movement-related activity in many motor cortical neurons, although indeed well time related to the movement, is even better time locked to the visual trigger stimulus. On the basis of anatomical evidence, the authors suggested that the most likely pathway to convey such processed visual information to the motor cortex is the VL.

## Relative Contribution of Signals from VL and Associative Cortex to Motor Cortex Activity during Visually Guided Locomotion

Two sources of signals that modify the stride-related modulation of neuronal activity in the motor cortex during locomotion over a complex terrain can be suggested: cortical and subcortical. While the VL appears to be well suited to supply the motor cortex throughout the entire step cycle with synthesized vision-related information thus

determining the motor cortex's overall activity outline, direct projections from associative cortical areas are likely to provide a more specific shaping.

Research in nonhuman primates has shown that neurons in the cortical area 6, the premotor cortex, discharge in close relationship with reaching movements to visual targets (see, e.g., Crammond and Kalaska 1994, Wise et al. 1992). Area 6 has substantial inputs from parietal visual areas as well as direct cortico-cortical projections to the motor cortex (Ghosh 1997; Jones et al. 1978; Marconi et al. 2001). So far only one study has examined the activity of area 6 during treadmill locomotion (Criado et al. 1997). It was found that 59% of neurons in area 6 change their discharge rate with the start of locomotion, typically by increasing it. The discharge of only ~30% of these cells, however, is stride related during locomotion. In addition, nearly all step-related area 6 cells discharge predominantly during the stance phase of the stride. Thus, although area 6 neurons may influence the motor cortex and VL during stance, when it is believed that planning of the next limb transfer takes place (Laurent and Thomson 1988; Hollands and Marple-Horvat 1996), they cannot be responsible for the majority of swing and transition phase-related activity in either the motor cortex or VL.

Regarding direct cortico-cortical projections from parietal area 5 to the motor cortex, although they are numerous (see, e.g., Andujar and Drew 2007; Babb et al. 1984; Caria et al. 1997; Mori et al. 1989), at least those that come from layer III of area 5b carry rather low activity during both simple and complex locomotion, both in terms of proportion of neurons involved (~50%) as well as their average discharge rates (~1 spikes/s; Beloozerova et al. 2011). The potential for the parietal area 5 to supply the motor cortex with vision-based information during complex locomotion has been considered in detail previously (Beloozerova and Sirota 2003). It was concluded that the

activity of this area is not well suited to directly determine the great majority of the locomotion-related responses in the motor cortex. On the basis of data obtained in experiments with cats overstepping a moving obstacle on the treadmill, a suggestion that area 5 is most likely to exert its influence on locomotion mechanism via subcortical motor centers such as basal ganglia or lateral cerebellum has been also made by Andujar and colleagues (2010). The role of the sparse direct projection from area 5 to the motor cortex during complex locomotion is yet to be determined.

Overall, available data suggest that subcortical routes that link visual and motor cortex areas participate very importantly in transmission of vision-based signals that modify stride-related modulation of neuronal activity in the motor cortex during complex locomotion. The pathway that runs via the pons, cerebellum, and VL is the largest of all subcortical links between visual and motor cortices. In this study we investigated signals that are conveyed from the VL to the motor cortex during visually guided locomotion. We suggest that they have significant contribution.

# 6. REFERENCES


Adkins, R. J., Cegnar, M. R., & Rafuse, D. D. (1971). Differential effects of lesions of the anterior and posterior sigmoid gyri in cats. *Brain Research*, *30*(2), 411-414.

Alink, A., Schwiedrzik, C. M., Kohler, A., Singer, W., & Muckli, L. (2010). Stimulus predictability reduces responses in primary visual cortex. *The Journal of Neuroscience*, *30*(8), 2960-2966.

Amos, A., Armstrong, D. M., & Marple-Horvat, D. E. (1990). Changes in the discharge patterns of motor cortical neurones associated with volitional changes in stepping in the cat. *Neuroscience Letters*, *109*(1), 107-112.

Andujar, J. É., & Drew, T. (2007). Organization of the projections from the posterior parietal cortex to the rostral and caudal regions of the motor cortex of the cat. *Journal of Comparative Neurology*, 504(1), 17-41.

Andujar, J. É., Lajoie, K., & Drew, T. (2010). A contribution of area 5 of the posterior parietal cortex to the planning of visually guided locomotion: limb-specific and limb-independent effects. *Journal of Neurophysiology*, *103*(2), 986-1006.

Angaut, P. (1979) The cerebello-thalamic projection in the cat. In: J. Massion & K. Sasaki, (eds). *Cerebro-Cerebellar Interactions* (pp. 19-43). Elsevier, North-Holland.

Armer, M. C., Nilaweera, W. U., Dasgupta, N. M., Rivers, T. J., Beloozerova, I. N. (2011). Walking in the light: Responses of motor cortical neurons. Program No. 802.04. *Society for Neuroscience*. Washington DC. Online.

Armer, M. C., Nilaweera, W. U., Rivers, T. J., Dasgupta, N. M., & Beloozerova, I. N. (2013). Effect of light on the activity of motor cortex neurons during locomotion. *Behavioural Brain Research, 250*, 238-250.

Armstrong, D. M., & Drew, T. (1984a). Discharges of pyramidal tract and other motor cortical neurons during locomotion in the cat. *The Journal of Physiology*, *346*(1), 471-495.

Armstrong, D. M., & Drew, T. (1984b). Locomotor-related neuronal discharges in cat motor cortex compared with peripheral receptive fields and evoked movements. *The Journal of Physiology*, *346*(1), 497-517.

Armstrong, D. M., & Drew, T. (1984c). Topographical localization in the motor cortex of the cat for somatic afferent responses and evoked movements. *The Journal of Physiology*, *350*(1), 33-54.

Armstrong, D. M., & Drew, T. (1985a). Forelimb electromyographic responses to motor cortex stimulation during locomotion in the cat. *The Journal of Physiology*, *367*(1), 327-351.

Armstrong, D. M., & Drew, T. (1985b). Electromyographic responses evoked in muscles of the forelimb by intracortical stimulation in the cat. *The Journal of Physiology*, *367*(1), 309-326.

Armstrong, D. M., & Edgley, S. A. (1984). Discharges of nucleus interpositus neurons during locomotion in the cat. *The Journal of Physiology*, *351*(1), 411-432.

Armstrong, D. M., & Marple-Horvat, D. E. (1996). Role of the cerebellum and motor cortex in the regulation of visually controlled locomotion. *Canadian Journal of Physiology and Pharmacology*, *74*(4), 443-455.

Arshavskii, I. I., Arshavsky, Y., Gel′fand, I. M., & Orlovskiĭ, G. N. (1986).*Cerebellum and Rhythmical Movements* (Vol. 13). Berlin, Heidelberg. Springer-Verlag.

Arshavsky, Y. I., Orlovsky, G. N., & Perret, C. (1988). Activity of rubrospinal neurons during locomotion and scratching in the cat. *Behavioural Brain Research*, *28*(1), 193-199.

Asanuma, C., Thach, W. T., & Jones, E. G. (1983). Cytoarchitectonic delineation of the ventral lateral thalamic region in the monkey. *Brain Research Reviews*, *5*(3), 219-235.

Asanuma, H., & Hunsperger, R. W. (1975). Functional significance of projection from the cerebellar nuclei to the motor cortex in the cat. *Brain Research*, *98*(1), 73-92.

Aumann, T. D., Rawson, J. A., Finkelstein, D. I., & Horne, M. K. (1994). Projections from the lateral and interposed cerebellar nuclei to the thalamus of the rat: a light and electron microscopic study using single and double anterograde labelling. *Journal of Comparative Neurology*, *349*(2), 165-181.

Avendano, C., Rausell, E., & Reinoso-Suarez, F. (1985). Thalamic projections to areas 5a and 5b of the parietal cortex in the cat: a retrograde horseradish peroxidase study. *The Journal of Neuroscience*, *5*(6), 1446-1470.

Avendano, C., Rausell, E., Perez-Aguilar, D., & Isorna, S. (1988). Organization of the association cortical afferent connections of area 5: a retrograde tracer study in the cat. *Journal of Comparative Neurology*, *278*(1), 1-33.

Babb, R. S., Waters, R. S., & Asanuma, H. (1984). Corticocortical connections to the motor cortex from the posterior parietal lobe (areas 5a, 5b, 7) in the cat demonstrated by the retrograde axonal transport of horseradish peroxidase. *Experimental Brain Research*, *54*(3), 476-484.

Babinski, J. (1901). *Définition de l'hystérie*. Masson et Cie, Éditeurs Libraires De L'Académie De Médecine 120, Boulevard Saint-Germain (6e).

Bastian, A. J., Zackowski, K. M., & Thach, W. T. (2000). Cerebellar ataxia: torque deficiency or torque mismatch between joints? *Journal of Neurophysiology*, *83*(5), 3019-3030.

Batshelet E. (1981) *Circular statistics in biology*. Academic Press, New York.

Bays, P. M., Flanagan, J. R., & Wolpert, D. M. (2006). Attenuation of self-generated tactile sensations is predictive, not postdictive. *PLoS Biology*,*4*(2), 281.

Beer, R. F., Dewald, J. P., & Rymer, W. Z. (2000). Deficits in the coordination of multijoint arm movements in patients with hemiparesis: evidence for disturbed control of limb dynamics. *Experimental Brain Research*, *131*(3), 305-319.

Beloozerova, I. N., & Sirota, M. G. (1984). Activity of neurons of the motosensory cortex during natural locomotion in the cat [in Russian]. *Neirofiziologiia=Neurophysiology*, 17(3), 406-408.

Beloozerova, I. N., & Sirota, M. G. (1985a). [Activity of neurons of the motor-sensory cortex of the cat during natural walking on the rungs of a horizontal ladder]. *Neirofiziologiia= Neurophysiology, 18*(4), 543-545.

Beloozerova, I. N., & Sirota, M. G. (1985b). [Activity of neurons of the motor-sensory cortex of the cat during natural locomotion while stepping over obstacles]. *Neirofiziologiia= Neurophysiology*, *18*(4), 546-549.

Beloozerova, I. N., & Sirota, M. G. (1988). The role of motor cortex in control of locomotion. In: Stance and Motion. Facts and Concepts. Eds.: Gurfincel, V.S., Ioffe, M.E., Massion, J., Roll, J.P., pp. 163-176. Plenum press, New York,.

Beloozerova, I. N., & Sirota, M. G. (1993a). The role of the motor cortex in the control of accuracy of locomotor movements in the cat. *The Journal of Physiology*, *461*(1), 1-25.

Beloozerova, I. N., & Sirota, M. G. (1993b). The role of the motor cortex in the control of vigor of locomotor movements in the cat. *The Journal of Physiology*, *461*(1), 27-46.

Beloozerova, I. N., & Sirota, M. G. (1998). Cortically controlled gait adjustments in the cat. *Annals of the New York Academy of Sciences*, 860(1), 550-553.

Beloozerova, I. N., & Sirota, M. G. (2002). Activity of ventrolateral thalamus (VL) during locomotion. Program No. 28: 62. *Society for Neuroscience*. Washington DC. Online.

Beloozerova, I. N., & Sirota, M. G. (2003). Integration of motor and visual information in the parietal area 5 during locomotion. *Journal of Neurophysiology*, *90*(2), 961-971.

Beloozerova, I. N., Farrell, B. J., Sirota, M. G., & Prilutsky, B. I. (2010). Differences in movement mechanics, electromyographic, and motor cortex activity between accurate and nonaccurate stepping. *Journal of Neurophysiology*, *103*(4), 2285-2300.

Beloozerova, I. N., Nilaweera, W. U., Viana di Prisco, G., Sirota, M. G., Marlinski, V. (2011). Sparse locomotion-related activity of cortico-cortical projection from parietal area 5 to motor cortex. Program No. 803.08. *Society for Neuroscience*. Washington DC. Online.

Beloozerova, I. N., Sirota, M. G., & Swadlow, H. A. (2003). Activity of different classes of neurons of the motor cortex during locomotion. *The Journal of Neuroscience*, *23*(3), 1087-1097.

Beloozerova, I. N., Sirota, M. G., Orlovsky, G. N., & Deliagina, T. G. (2005). Activity of pyramidal tract neurons in the cat during postural corrections. *Journal of Neurophysiology*, *93*(4), 1831-1844.

Beloozerova, I. N., Sirota, M. G., Orlovsky, G. N., & Deliagina, T. G. (2006). Comparison of activity of individual pyramidal tract neurons during balancing, locomotion, and scratching. *Behavioral Brain Research*, *169*(1), 98-110.

Biewener, A. A. (2003). *Animal locomotion*. Oxford University Press, New York.

Bishop, P. O., Burke, W., & Davis, R. (1962). The identification of single units in central visual pathways. *The Journal of Physiology*, *162*(3), 409-431.

Bishop, P. O., Jeremy, D., & Lance, J. W. (1953). Properties of pyramidal tract. *Journal of Neurophysiology*, *16*(5), 537-550.

Bloedel, J. R. (1992). Functional heterogeneity with structural homogeneity: how does the cerebellum operate? *Behavioral and Brain Sciences*, *15*(04), 666-678.

Brodal, P. (1979). The pontocerebellar projection in the rhesus monkey: an experimental study with retrograde axonal transport of horseradish peroxidase. *Neuroscience*, *4*(2), 193-208.

Brookhart, J. M., & Morris, R. E. (1948). Antidromic potential recordings from the bulbar pyramid of the cat. *Journal of Neurophysiology*, *11*(4), 387-398.

Brooks, D. J. (1995). The role of the basal ganglia in motor control: contributions from PET. *Journal of the Neurological Sciences*, *128*(1), 1-13.

Brown, T. G. (1911). The intrinsic factors in the act of progression in the mammal. *Proceedings of the Royal Society of London. Series B, 84*(572), 308-319.

Bubic, A., Von Cramon, D. Y., & Schubotz, R. I. (2010). Prediction, cognition and the brain. *Frontiers in Human Neuroscience*, *4*:25.

Buneo, C. A., & Andersen, R. A. (2006). The posterior parietal cortex: sensorimotor interface for the planning and online control of visually guided movements. *Neuropsychologia, 44*(13), 2594-2606.

Caria, M. A., Kaneko, T., Kimura, A., & Asanuma, H. (1997). Functional organization of the projection from area 2 to area 4γ in the cat. *Journal of Neurophysiology*, *77*(6), 3107-3114.

Carlsson, K., Petrovic, P., Skare, S., Petersson, K. M., & Ingvar, M. (2000). Tickling expectations: neural processing in anticipation of a sensory stimulus. *Journal of Cognitive Neuroscience*, *12*(4), 691-703.

Cerminara, N. L., Edge, A. L., Marple-Horvat, D. E., & Apps, R. (2005). The lateral cerebellum and visuomotor control. *Progress in Brain Research*, *148*, 213-226.

Chambers, W. W., & Liu, C. N. (1957). Cortico-spinal tract of the cat. An attempt to correlate the pattern of degeneration with deficits in reflex activity following neocortical lesions. *Journal of Comparative Neurology*, *108*(1), 23-55.

Chambers, W. W., & Sprague, J. M. (1955a). Functional localization in the cerebellum. I. Organization in longitudinal cortico-nuclear zones and their contribution to the control of posture, both extrapyramidal and pyramidal. *Journal of Comparative Neurology*, *103*(1), 105-129.

Chambers, W. W., & Sprague, J. M. (1955b). Functional localization in the cerebellum. II. Somatotopic organization in cortex and nuclei. *AMA Archives of Neurology & Psychiatry*, *74*(6), 653-680.

Chambers, W. W., Liu, C. N., McCouch, G. P., & d'Aquili, E. (1966). Descending tracts and spinal shock in the cat. *Brain*, *89*(2), 377-390.

Changizi, M. A., Hsieh, A., Nijhawan, R., Kanai, R., & Shimojo, S. (2008). Perceiving the present and a systematization of illusions. *Cognitive Science,32*(3), 459-503.

Chapin, J. K., & Woodward, D. J. (1981). Modulation of sensory responsiveness of single somatosensory cortical cells during movement and arousal behaviors. *Experimental Neurology*, *72*(1), 164-178.

Chapin, J. K., & Woodward, D. J. (1982a). Somatic sensory transmission to the cortex during movement: gating of single cell responses to touch. *Experimental Neurology*, *78*(3), 654-669.

Chapin, J. K., & Woodward, D. J. (1982b). Somatic sensory transmission to the cortex during movement: phasic modulation over the locomotor step cycle. *Experimental Neurology*, *78*(3), 670-684.

Chapman, C. E., Jiang, W., & Lamarre, Y. (1988). Modulation of lemniscal input during conditioned arm movements in the monkey. *Experimental Brain Research*, *72*(2), 316-334.

Chrachri, A., & Clarac, F. (1990). Fictive locomotion in the fourth thoracic ganglion of the crayfish, Procambarus clarkii. *The Journal of Neuroscience*,*10*(3), 707-719.

Clascá, F., Llamas, A., & Reinoso-Suárez, F. (2000). Cortical connections of the insular and adjacent parieto-temporal fields in the cat. *Cerebral Cortex*,*10*(4), 371-399.

Coquery, J. M. (1978). Role of active movement in control of afferent input from skin in cat and man. In *Active touch* (pp. 161-169). Pergamon Press New York.

Coulter, J. D. (1974). Sensory transmission through lemniscal pathway during voluntary movement in the cat. *Journal of Neurophysiology*, *37*(5), 831-845.

Courtine, G., Gerasimenko, Y., van den Brand, R., Yew, A., Musienko, P., Zhong, H., ... & Edgerton, V. R. (2009). Transformation of nonfunctional spinal circuits into functional states after the loss of brain input. *Nature Neuroscience*,*12*(10), 1333-1342.

Courtine, G., Roy, R. R., Raven, J., Hodgson, J., Mckay, H., Yang, H., ... and Edgerton, V. R. (2005). Performance of locomotion and foot grasping following a unilateral thoracic corticospinal tract lesion in monkeys (Macaca mulatta).*Brain*, *128*(10), 2338-2358.

Craig, A. D. (2008). Retrograde analyses of spinothalamic projections in the macaque monkey: input to the ventral lateral nucleus. *Journal of Comparative Neurology*, *508*(2), 315-328.

Crammond, D. J., & Kalaska, J. F. (1994). Modulation of preparatory neuronal activity in dorsal premotor cortex due to stimulus-response compatibility. *Journal of Neurophysiology*, *71*(3), 1281-1284.

Criado, J. M., Heredia, M., Riolobos, A. S., Yajeya, J., & de la Fuente, A. (1997). Electrophysiological study of prefrontal neurons of cats during a motor task. *Pflügers Archiv*, *434*(1), 91-96.

den Ouden, H. E., Daunizeau, J., Roiser, J., Friston, K. J., & Stephan, K. E. (2010). Striatal prediction error modulates cortical coupling. *The Journal of Neuroscience*, *30*(9), 3210-3219.

Diamond, M. E. (1989). *Organization of somatic sensory cortex: the detection of discrete topographic units and evidence for their integrative function*. PhD dissertation, University of North Carolina at Chapel Hill.

Dickinson, M. H., Farley, C. T., Full, R. J., Koehl, M. A. R., Kram, R., & Lehman, S. (2000). How animals move: an integrative view. *Science*, *288*(5463), 100-106.

Divac, I., LaVail, J. H., Rakic, P., & Winston, K. R. (1977). Heterogeneous afferents to the inferior parietal lobule of the rhesus monkey revealed by the retrograde transport method. *Brain Research*, *123*(2), 197-207.

Drew, P. J., & Abbott, L. F. (2006). Extending the effects of spike-timing-dependent plasticity to behavioral timescales. *Proceedings of the National Academy of Sciences*, *103*(23), 8876-8881.

Drew, T., & Doucet, S. (1991). Application of circular statistics to the study of neuronal discharge during locomotion. *Journal of Neuroscience Methods*,*38*(2), 171-181.

Drew, T., & Marigold, D. S. (2015). Taking the next step: cortical contributions to the control of locomotion. *Current Opinion in Neurobiology*,*33*, 25-33.

Drew, T., (1988). Motor cortical cell discharge during voluntary gait modification. *Brain research*, *457*(1), 181-187.

Drew, T., (1993). Motor cortical activity during voluntary gait modifications in the cat. I. Cells related to the forelimbs. *Journal of Neurophysiology, 70*(1):179-99.

Drew, T., Jiang, W., & Widajewicz, W. (2002). Contributions of the motor cortex to the control of the hindlimbs during locomotion in the cat. *Brain Research Reviews*, *40*(1), 178-191.

Drew, T., Jiang, W., Kably, B., & Lavoie, S. (1996). Role of the motor cortex in the control of visually triggered gait modifications. *Canadian Journal of Physiology and pharmacology, 74*(4), 426-442.

Dubuc, R., Brocard, F., Antri, M., Fénelon, K., Gariépy, J. F., Smetana, R., ... & Veilleux, D. (2008). Initiation of locomotion in lampreys. *Brain Research Reviews*, *57*(1), 172-182.

Duysens, J., & Van de Crommert, H. W. (1998). Neural control of locomotion; Part 1: The central pattern generator from cats to humans. *Gait and Posture*, *7*(2), 131-141.

Dykes, R. W., Rasmusson, D. D., & Hoeltzell, P. B. (1980). Organization of primary somatosensory cortex in the cat. *Journal of Neurophysiology*, *43*(6), 1527-1546.

Efron, B., Tibshirani, R.J. (1993). *An introduction to the bootstrap*. Chapman & Hall, New York.

Eidelberg, E., & Yu, J. (1981). Effects of corticospinal lesions upon treadmill locomotion by cats. *Experimental Brain Research*, *43*(1), 101-103.

Eidelberg, E., Walden, J. G., & Nguyen, L. H. (1981). Locomotor control in macaque monkeys. *Brain*, *104*(4), 647-663.

Evarts, E. V. (1971). Activity of thalamic and cortical neurons in relation to learned movement in the monkey. *International Journal of Neurology*, *8*(2), 321-326.

Evrard, H. C., & Craig, A. D. (2008). Retrograde analysis of the cerebellar projections to the posteroventral part of the ventral lateral thalamic nucleus in the macaque monkey. *Journal of Comparative Neurology*, *508*(2), 286-314.

Fabre-Thorpe, M., & Levesque, F. (1991). Visuomotor relearning after brain damage crucially depends on the integrity of the ventrolateral thalamic nucleus. *Behavioral Neuroscience*, *105*(1), 176.

Fang, P. C., Stepniewska, I., & Kaas, J. H. (2006). The thalamic connections of motor, premotor, and prefrontal areas of cortex in a prosimian primate (*Otolemur garnetti*). *Neuroscience*, *143*(4), 987-1020.

Favorov, O. V., Nilaweera, W. U., Miasnikov, A. A., & Beloozerova, I. N. (2010). Profound activity of somatosensory responsive neurons in high subdivisions of SI cortex during simple locomotion. *Society for Neuroscience,* 178.15

Favorov, O. V., Nilaweera, W. U., Miasnikov, A. A., & Beloozerova, I. N. (2015). Activity of somatosensory-responsive neurons in high subdivisions of SI cortex during locomotion. *The Journal of Neuroscience*, *35*(20), 7763-7776.

Fee, M. S., Mitra, P. P., & Kleinfeld, D. (1997). Central versus peripheral determinants of patterned spike activity in rat vibrissa cortex during whisking. *Journal of Neurophysiology*, *78*(2), 1144-1149.

Felleman, D. J., & Van Essen, D. C. (1991). Distributed hierarchical processing in the primate cerebral cortex. *Cerebral Cortex*, *1*(1), 1-47.

Fisher, N. I., (1993). *Statistical analysis of circular data*. Cambridge University Press. Cambridge, MA.

Fitzsimmons, N. A., Lebedev, M. A., Peikon, I. D., & Nicolelis, M. A. (2009). Extracting kinematic parameters for monkey bipedal walking from cortical neuronal ensemble activity. *Frontiers in Integrative Neuroscience*, *3*,3.

Forssberg, H., Grillner, S., & Halbertsma, J. (1980a). The locomotion of the low spinal cat I. Coordination within a hindlimb. *Acta Physiologica Scandinavica*, *108*(3), 269-281.

Forssberg, H., Grillner, S., Halbertsma, J., & Rossignol, S. (1980b). The locomotion of the low spinal cat. II. Interlimb coordination. *Acta Physiologica Scandinavica*, *108*(3), 283-295.

Friel, K. M., Drew, T., & Martin, J. H. (2007). Differential activity-dependent development of corticospinal control of movement and final limb position during visually guided locomotion. *Journal of Neurophysiology*, *97*(5), 3396-3406.

Fromm, C., & Evarts, E. V. (1982). Pyramidal tract neurons in somatosensory cortex: central and peripheral inputs during voluntary movement. *Brain Research*, *238*(1), 186-191.

Fuller, J. H., & Schlag, J. D. (1976). Determination of antidromic excitation by the collision test: problems of interpretation. *Brain Research*, *112*(2), 283-298.

Fulton, J. F., & Dow, R. S. (1937). The cerebellum: a summary of functional localization. *The Yale Journal of Biology and Medicine*, *10*(1), 89.

Garcia-Rill, E., & Dubrovsky, B. (1971). Organization of visual input in cat motor-sensory cortex. *Experimental Neurology*, *33*(3), 597-606.

Garcia-Rill, E., & Dubrovsky, B. (1974). Responses of motor cortex cells to visual stimuli. *Brain Research*, *82*(2), 185-194.

Gardner, E. P., Ro, J. Y., Babu, K. S., & Ghosh, S. (2007). Neurophysiology of prehension. II. Response diversity in primary somatosensory (SI) and motor (MI) cortices. *Journal of Neurophysiology*, *97*(2), 1656-1670.

Garraghty, P. E., Florence, S. L., & Kaas, J. H. (1990). Ablations of areas 3a and 3b of monkey somatosensory cortex abolish cutaneous responsivity in area 1. *Brain Research*, *528*(1), 165-169.

Garraghty, P. E., Pons, T. P., & Kaas, J. H. (1990). Ablations of areas 3b (SI proper) and 3a of somatosensory cortex in marmosets deactivate the second and parietal ventral somatosensory areas. *Somatosensory and Motor Research*,*7*(2), 125-135.

Georgopoulos, A. P., Kalaska, J. F., Caminiti, R., & Massey, J. T. (1982). On the relations between the direction of two-dimensional arm movements and cell discharge in primate motor cortex. *The Journal of Neuroscience*, *2*(11), 1527-1537.

Ghez, C., & Pisa, M. (1972). Inhibition of afferent transmission in cuneate nucleus during voluntary movement in the cat. *Brain Research*, *40*(1), 145-151.

Ghosh, S. (1997). Cytoarchitecture of sensorimotor areas in the cat cerebral cortex. *Journal of Comparative Neurology, 388*(3), 354-370.

Ghosh, S. (1997a). Identification of motor areas of the cat cerebral cortex based on studies of cortical stimulation and corticospinal connections. *Journal of Comparative Neurology*, *380*(2), 191-214.

Ghosh, S. (1997b). Comparison of the cortical connections of areas 4γ and 4δ in the cat cerebral cortex. *Journal of Comparative Neurology*, *388*(3), 371-396.

Gibson, A. R., Hansma, D. I., Houk, J. C., & Robinson, F. R. (1984). A sensitive low artifact TMB procedure for the demonstration of WGA-HRP in the CNS. *Brain Research*, *298*(2), 235-241.

Glickstein, M. (2000). How are visual areas of the brain connected to motor areas for the sensory guidance of movement? *Trends in Neurosciences*, *23*(12), 613-617.

Glickstein, M., & Gibson, A. R. (1976). Visual cells in the pons of the brain. *Scientific American*, *235*(5), 90.

Glickstein, M., Buchbinder, S., & Iii, J. L. M. (1998). Visual control of the arm, the wrist and the fingers: Pathways through the brain. *Neuropsychologia*, *36*(10), 981-1001.

Glickstein, M., Gerrits, N., Kralj-Hans, I., Mercier, B., Stein, J., & Voogd, J. (1994). Visual pontocerebellar projections in the macaque. *Journal of Comparative Neurology*, *349*(1), 51-72.

Grillner, S. (1973). Locomotion in the spinal cat. In *Control of Posture and Locomotion* (pp. 515-535). Springer US.

Grillner, S. (1975). Locomotion in vertebrates: central mechanisms and reflex interaction. *Physiol Rev*, *55*(2), 247-304.

Grillner, S. (2011). Control of locomotion in bipeds, tetrapods, and fish.*Comprehensive Physiology*.

Grillner, S., & Rossignol, S. (1978). On the initiation of the swing phase of locomotion in chronic spinal cats. *Brain Research*, *146*(2), 269-277.

Grillner, S., & Zangger, P. (1979). On the central generation of locomotion in the low spinal cat. *Experimental Brain Research*, *34*(2), 241-261.

Grillner, S., & Zangger, P. (1984). The effect of dorsal root transection on the efferent motor pattern in the cat's hindlimb during locomotion. *Acta Physiologica Scandinavica*, *120*(3), 393-405.

Grofova, I., & Rinvik, E. (1974). Cortical and pallidal projections to the nucleus ventralis lateralis thalami. *Anatomy and Embryology*, *146*(2), 113-132.

Guenther, E., & Zrenner, E. (1993). The spectral sensitivity of dark-and light-adapted cat retinal ganglion cells. *The Journal of Neuroscience*, *13*(4), 1543-1550.

Hassler, R., & Muhs-Clement, K. (1964). Architektonischer Aufbau des sensomotorischen und parietalen Cortex der Katze. *J. Hirnforsch, 6*, 377-420.

Hendry, S. H. C., Jones, E. G., & Graham, J. (1979). Thalamic relay nuclei for cerebellar and certain related fiber systems in the cat. *Journal of Comparative Neurology*, *185*(4), 679-713.

Hildebrand, M. (1965). Symmetrical gaits of horses. *Science*, *150*(3697), 701-708.

Hollands, M. A., & Marple-Horvat, D. E. (1996). Visually guided stepping under conditions of step cycle-related denial of visual information. *Experimental Brain Research*, *109*(2), 343-356.

Hollands, M. A., & Marple-Horvat, D. E. (2001). Coordination of eye and leg movements during visually guided stepping. *Journal of Motor Behavior*, *33*(2), 205-216.

Hollands, M. A., Marple-Horvat, D. E., Henkes, S., & Rowan, A. K. (1995). Human eye movements during visually guided stepping. *Journal of Motor Behavior*, *27*(2), 155-163.

Holmes, G. (1917). The symptoms of acute cerebellar injuries due to gunshot injuries. *Brain*, *40*(4), 461-535.

Holmes, G. (1939). The cerebellum of man. *Brain*, *62*(1), 1-30.

Hulliger, M., Dürmüller, N., Prochazka, A., & Trend, P. (1989). Flexible fusimotor control of muscle spindle feedback during a variety of natural movements. *Progress in Brain Research*, *80*, 87-101.

Hultborn, H. (2006). Spinal reflexes, mechanisms and concepts: from Eccles to Lundberg and beyond. *Progress in Neurobiology*, *78*(3), 215-232.

Hultborn, H., & Nielsen, J. B. (2007). Spinal control of locomotion–from cat to man. *Acta Physiologica*, *189*(2), 111-121.

Hyvarinen, J. (1982). *Parietal cortex of monkey and man*. Studies of brain function. V.8, Springer-Verlag, Berlin, Heidelberg, New York.

Ilinsky, I. A., & Kultas-Ilinsky, K. (1984). An autoradiographic study of topographical relationships between pallidal and cerebellar projections to the cat thalamus. *Experimental Brain Research*, *54*(1), 95-106.

Ito, S. I., Craig, A. D. (2003). Vagal input to lateral area 3a in cat cortex. *Journal of Neurophysiology*, *90*(1), 143-154.

Iwamura, Y. (1998). Hierarchical somatosensory processing. *Current Opinion in Neurobiology*, *8*(4), 522-528.

Iwamura, Y., Tanaka, M., Sakamoto, M., & Hikosaka, O. (1993). Rostrocaudal gradients in the neuronal receptive field complexity in the finger region of the alert monkey's postcentral gyrus. *Experimental Brain Research*,*92*(3), 360-368.

Jiang, W., Chapman, C. E., & Lamarre, Y. (1991). Modulation of the cutaneous responsiveness of neurones in the primary somatosensory cortex during conditioned arm movements in the monkey. *Experimental Brain Research*, *84*(2), 342-354.

Jones, E. G., Coulter, J. D., & Hendry, S. H. C. (1978). Intracortical connectivity of architectonic fields in the somatic sensory, motor and parietal cortex of monkeys. *Journal of Comparative Neurology*, *181*(2), 291-347.

Kaas, J. H., & Pons, T. P. (1988). The somatosensory system of primates. In Steklis H. P., (Ed.) *Comparative Primate Biology*. *4*. Neurosciences. Alan R. Liss Inc. New York. pp 421-468.

Kaas, J. H., (1990). The somatosensory system. In Praxinos (Ed.) *The Human Nervous System*. Academic Press, New York. pp 813-844.

Kaas, J. H., (1993). The functional organization of somatosensory cortex in primates. *Annals of Anatomy*. *175*(6), 509-518.

Kaas, J. H., Nelson, R. J., Sur, M., Dykes, R. W., & Merzenich, M. M. (1984). The somatotopic organization of the ventroposterior thalamus of the squirrel monkey, Saimiri sciureus. *Journal of Comparative Neurology*, *226*(1), 111-140.

Kakei, S., & Shinoda, Y. (1990). Parietal projection of thalamocortical fibers from the ventroanterior-ventrolateral complex of the cat thalamus. *Neuroscience Letters*, *117*(3), 280-284.

Kalaska, J. F. (1996). Parietal cortex area 5 and visuomotor behavior. *Canadian Journal of Physiology and pharmacology*, *74*(4), 483-498.

Kalaska, J. F. (2009). From intention to action: motor cortex and the control of reaching movements. In *Progress in Motor Control* (pp. 139-178). Springer US.

Kleinfeld, D., & Deschênes, M. (2011). Neuronal basis for object location in the vibrissa scanning sensorimotor system. *Neuron*, *72*(3), 455-468.

Klishko, A. N., Farrell, B. J., Beloozerova, I. N., Latash, M. L., & Prilutsky, B. I. (2014). Stabilization of cat paw trajectory during locomotion. *Journal of Neurophysiology, 112*(6), 1376-1391.

Koch, K. W., & Fuster, J. M. (1989). Unit activity in monkey parietal cortex related to haptic perception and temporary memory. *Experimental Brain Research*, *76*(2), 292-306.

Kotchabhakdi, N., & Walberg, F. (1978). Cerebellar afferent projections from the vestibular nuclei in the cat: an experimental study with the method of retrograde axonal transport of horseradish peroxidase. *Experimental Brain Research*, *31*(4), 591-604.

Kurata, K. (2005). Activity properties and location of neurons in the motor thalamus that project to the cortical motor areas in monkeys. *Journal of Neurophysiology*, *94*(1), 550-566.

Kurtzer, I., Herter, T. M., & Scott, S. H. (2005). Random change in cortical load representation suggests distinct control of posture and movement. *Nature Neuroscience*, *8*(4), 498-504.

Kuypers, H. G. J. M. (1964). The descending pathways to the spinal cord, their anatomy and function. *Progress in Brain Research*, *11*, 178-202.

Lajoie, K., & Drew, T. (2007). Lesions of area 5 of the posterior parietal cortex in the cat produce errors in the accuracy of paw placement during visually guided locomotion. *Journal of Neurophysiology*, *97*(3), 2339-2354.

Lajoie, K., Andujar, J. É., Pearson, K., & Drew, T. (2010). Neurons in area 5 of the posterior parietal cortex in the cat contribute to interlimb coordination during visually guided locomotion: a role in working memory. *Journal of Neurophysiology*, *103*(4), 2234-2254.

Larsen, K. D., & Asanuma, H. (1979). Thalamic projections to the feline motor cortex studied with horseradish peroxidase. *Brain Research*, *172*(2), 209-215.

Larsen, K. D., & McBride, R. L. (1979). The organization of feline entopeduncular nucleus projections: anatomical studies. *Journal of Comparative Neurology*, *184*(2), 293-308.

Laurent, M., & Thomson, J. A. (1988). The role of visual information in control of a constrained locomotor task. *Journal of Motor Behavior*, *20*(1), 17-37.

Lebedev, M. A., Denton, J. M., & Nelson, R. J. (1994). Vibration-entrained and premovement activity in monkey primary somatosensory cortex. *Journal of Neurophysiology*, *72*(4), 1654-1673.

Leclerc, S. S., Avendano, C., Dykes, R. W., Waters, R. S., & Salimi, I. (1994). Reevaluation of area 3b in the cat based on architectonic and electrophysiological studies: regional variability with functional and anatomical consistencies. *Journal of Comparative Neurology*, *341*(3), 357-374.

Liddell, E. G. T., & Phillips, C. G. (1944). Pyramidal section in the cat. *Brain*, *67*(1), 1-9.

London, B. M., & Miller, L. E. (2013). Responses of somatosensory area 2 neurons to actively and passively generated limb movements. *Journal of Neurophysiology*, *109*(6), 1505-1513.

Lundberg, A., & Oscarsson, O. (1962). Functional Organization of the Ventral Spino-Cerebellar Tract in the Cat. IV. Identification of Units by Antidromic Activation from the Cerebellar Cortex. *Acta Physiologica Scandinavica*, *54*(3-4), 252-269.

Mackel, R., Iriki, A., & Brink, E. E. (1992). Spinal input to thalamic VL neurons: evidence for direct spinothalamic effects. *Journal of Neurophysiology*, *67*(1), 132-144.

Marconi, B., Genovesio, A., Battaglia-Mayer, A., Ferraina, S., ... & Caminiti, R. (2001). Eye–hand coordination during reaching. I. Anatomical relationships between parietal and frontal cortex.*Cerebral Cortex*, *11*(6), 513-527.

Marigold, D. S., & Drew, T. (2011). Contribution of cells in the posterior parietal cortex to the planning of visually guided locomotion in the cat: effects of temporary visual interruption. *Journal of Neurophysiology*, *105*(5), 2457-2470.

Marigold, D. S., & Patla, A. E. (2008). Visual information from the lower visual field is important for walking across multi-surface terrain. *Experimental Brain Research*, *188*(1), 23-31.

Marigold, D. S., Andujar, J. E, Lajoie, K. Drew, T. (2011). Motor planning of locomotor adaptations on the basis of vision: the role of the posterior parietal cortex. *Progress in Brain Research, 188*, 83-100.

Marlinski, V., Nilaweera, W. U., Zelenin, P. V., Sirota, M. G., & Beloozerova, I. N. (2012). Signals from the ventrolateral thalamus to the motor cortex during locomotion. *Journal of Neurophysiology*, *107*(1), 455-472.

Marple-Horvat, D. E., & Criado, J. M. (1999). Rhythmic neuronal activity in the lateral cerebellum of the cat during visually guided stepping. *The Journal of Physiology*, *518*(2), 595-603.

Marple-Horvat, D. E., Amos, A. J., Armstrong, D. M., & Criado, J. M. (1993). Changes in the discharge patterns of cat motor cortex neurones during unexpected perturbations of on-going locomotion. *The Journal of Physiology*,*462*, 87.

Martin, J. H., & Ghez, C. (1985). Task-related coding of stimulus and response in cat motor cortex. *Experimental Brain Research*, *57*(3), 427-442.

Martin, J. H., & Ghez, C. (1993). Differential impairments in reaching and grasping produced by local inactivation within the forelimb representation of the motor cortex in the cat. *Experimental Brain Research*, *94*(3), 429-443.

Martin, J. H., Friel, K. M., Salimi, I., & Chakrabarty, S. (2007). Activity-and use-dependent plasticity of the developing corticospinal system. *Neuroscience and Biobehavioral Reviews*, *31*(8), 1125-1135.

Matsushita, M., & Ikeda, M. (1970). Olivary projections to the cerebellar nuclei in the cat. *Experimental Brain Research*, *10*(5), 488-500.

Mayer, N. H. (1997). Clinicophysiologic concepts of spasticity and motor dysfunction in adults with an upper motoneuron lesion. *Muscle and Nerve*, *20*(S6), 1-14.

Mayer, N. H., Esquenazi, A., & Childers, M. K. (1997). Common patterns of clinical motor dysfunction. *Muscle & Nerve*, *20*(S6), 21-35.

McCrea, D. A., & Rybak, I. A. (2008). Organization of mammalian locomotor rhythm and pattern generation. *Brain Research Reviews*, *57*(1), 134-146.

McKenna, T. M., Whitsel, B. L., Dreyer, D. A., & Metz, C. B. (1981). Organization of cat anterior parietal cortex: relations among cytoarchitecture, single neuron functional properties, and interhemispheric connectivity. *Journal of Neurophysiology*, *45*(4), 667-697.

Mesulam, M. M. (1982). Principles of horseradish peroxidase neurohistochemistry and their applications for tracing neural pathways-axonal transport, enzyme histochemistry and light microscopic analysis. In *tracing neural connections with horseradish peroxidase* (pp. 1-151). Wiley New York.

Metz, G. A., & Whishaw, I. Q. (2002). Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore-and hindlimb stepping, placing, and co-ordination. *Journal of Neuroscience Methods*, *115*(2), 169-179.

Mihaltchev, P., Archambault, P. S., Feldman, A. G., & Levin, M. F. (2005). Control of double-joint arm posture in adults with unilateral brain damage. *Experimental Brain Research*, *163*(4), 468-486.

Miles, Ö. B., Cerminara, N. L., & Marple-Horvat, D. E. (2006). Purkinje cells in the lateral cerebellum of the cat encode visual events and target motion during visually guided reaching. *The Journal of Physiology*, *571*(3), 619-637.

Miller, S., & Van der Meche, F. G. A. (1976). Coordinated stepping of all four limbs in the high spinal cat. *Brain Research*, *109*(2), 395-398.

Mori, A., Waters, R. S., & Asanuma, H. (1989). Physiological properties and patterns of projection in the cortico-cortical connections from the second somatosensory cortex to the motor cortex, area 4γ, in the cat. *Brain Research*, *504*(2), 206-210.

Mountcastle, V. B. (1995). The parietal system and some higher brain functions. *Cerebral Cortex, 5*(5), 377-390.

Murray, S. O., Schrater, P., & Kersten, D. (2004). Perceptual grouping and the interactions between visual cortical areas. *Neural Networks*, *17*(5), 695-705.

Mushiake, H., Inase, M., & Tanji, J. (1991). Neuronal activity in the primate premotor, supplementary, and precentral motor cortex during visually guided and internally determined sequential movements. *Journal of Neurophysiology*, 66(3), 705-718.

Na, J., Kakei, S., & Shinoda, Y. (1997). Cerebellar input to corticothalamic neurons in layers V and VI in the motor cortex. *Neuroscience Research*, *28*(1), 77-91.

Nakano, K., Kohno, M., Hasegawa, Y., & Tokushige, A. (1985). Cortical and brain stem afferents to the ventral thalamic nuclei of the cat demonstrated by retrograde axonal transport of horseradish peroxidase. *Journal of Comparative Neurology*, *231*(1), 102-120.

Nakano, K., Takimoto, T., Kayahara, T., Takeuchi, Y., & Kobayashi, Y. (1980). Distribution of cerebellothalamic neurons projecting to the ventral nuclei of the thalamus: an HRP study in the cat. *Journal of Comparative Neurology*, *194*(2), 427-439.

Neafsey, E. J., Hull, C. D., & Buchwald, N. A. (1978). Preparation for movement in the cat. II. Unit activity in the basal ganglia and thalamus. *Electroencephalography and Clinical Neurophysiology*, *44*(6), 714-723.

Nelson, R. J. (1987). Activity of monkey primary somatosensory cortical neurons changes prior to active movement. *Brain Research*, *406*(1), 402-407.

Nieoullon, A., & Rispal-Padel, L. (1976). Somatotopic localization in cat motor cortex. *Brain Research*, *105*(3), 405-422.

Niimi, K., Matsuoka, H., Yamazaki, Y., & Katayama, T. (1983). Thalamic afferents to the anterior and middle suprasylvian gyri in the cat traced with horseradish peroxidase. *Journal fur Hirnforschung*, *24*(2), 173-187.

Nilaweera, W. U., Beloozerova, I. N. (2009). Neurons of lateral nucleus of cerebellum change their step-related frequency modulation when visual control of stepping is required. Program No. 35: 367. *Society for Neuroscience*. Washington DC. Online.

Olson, C. R., & Lawler, K. (1987). Cortical and subcortical afferent connections of a posterior division of feline area 7 (area 7p). *Journal of Comparative Neurology*, *259*(1), 13-30.

Orlovskiĭ, G. N. (1970). Cerebellar influence on the reticulo-spinal neurons during locomotion. [In Russian]. *Biofizika*, *15*(5), 894-901.

Orlovskiĭ, G. N. (1972). Activity of cerebellar nuclei neurons during locomotion. [In Russian]. *Biofizika*, *17*(6), 1119-1126.

Orlovskiĭ, G. N., Deliagina, T. G., & Grillner, S. (1999). *Neuronal control of locomotion: from mollusc to man*. Oxford University Press.

Orlovsky, G. N. (1972a). Activity of vestibulospinal neurons during locomotion. *Brain Research*, *46*, 85-98.

Orlovsky, G. N. (1972b). Activity of rubrospinal neurons during locomotion. *Brain Research*, *46*, 99-112.

Orlovsky, G. N. (1972b). Activity of vestibulospinal neurons during locomotion. *Brain Research*, *46*, 85-98.

Oscarsson, O. (1965). Functional organization of the spino- and cuneocerebellar tracts. *Physiological Reviews*, *45*(3), 495-522.

Pearson, K. G. (1993). Common principles of motor control in vertebrates and invertebrates. *Annual Review of Neuroscience*, *16*(1), 265-297.

Phillips, C. G., & Porter, R. (1977). Corticospinal neurons. Their role in movement. *Monographs of the Physiological Society*, (34), v-xii, 1-450.

Pons, T. P., & Kaas, J. H. (1985). Connections of area 2 of somatosensory cortex with the anterior pulvinar and subdivisions of the ventroposterior complex in macaque monkeys. *Journal of Comparative Neurology*, *240*(1), 16-36.

Pons, T. P., Garraghty, P. E., Cusick, C. G., & Kaas, J. H. (1985). A sequential representation of the occiput, arm, forearm and hand across the rostrocaudal dimension of areas 1, 2 and 5 in macaque monkeys. *Brain Research*, *335*(2), 350-353.

Porter, R., & Lemon, R. (1993). *Corticospinal function and voluntary movement* (No. 45). Oxford University Press, USA.

Prilutsky, B. I., Sirota, M. G., Gregor, R. J., & Beloozerova, I. N. (2005). Quantification of motor cortex activity and full-body biomechanics during unconstrained locomotion. *Journal of Neurophysiology*, *94*(4), 2959-2969.

Prochazka, A., Hulliger, M., Trend, P., & Dürmüller, N. (1988). Dynamic and static fusimotor set in various behavioural contexts. In *Mechanoreceptors: Development, Structure and Function,* (pp. 417-430). New York: Plenum.

Pryor, K. (1975). *Lads before the wind: Adventures in porpoise training*. HarperCollins Publishers, New York.

Rao, R. P., & Ballard, D. H. (1999). Predictive coding in the visual cortex: a functional interpretation of some extra-classical receptive-field effects. *Nature Neuroscience*, *2*(1), 79-87.

Reinoso-Suárez, F., & Kornmüller, A. E. (1961). *Topographischer Hirnatlas der Katze: für experimental-physiologische Untersuchungen*. Darmstadt: Von E. Merck AG.

Reitboeck, H. J. (1983). Fiber microelectrodes for electrophysiological recordings. *Journal of Neuroscience Methods*, *8*(3), 249-262.

Renshaw, B. (1940). Activity in the simplest spinal reflex pathways. *Journal of Neurophysiology*, 3(5), 373-387.

Rinvik, E. (1968). The corticothalamic projection from the pericruciate and coronal gyri in the cat. An experimental study with silver-impregnation methods. *Brain Research*, *10*(2), 79-119.

Rispal-Padel, L., & Grangetto, A. (1977). The cerebello-thalamo-cortical pathway. Topographical investigation at the unitary level in the cat. *Experimental Brain Research*, *28*(1-2), 101-123.

Rivers, T. J., Shah, N. A., Sirota, M. G., Beloozerova, I. N. (2010). The relationship between vertical gaze shifts and stride cycle in freely walking cats. Program No. 36: 278.3. *Society for Neuroscience*. Washington DC. Online.

Rivers, T. J., Sirota, M. G., Guttentag, A. I., Ogorodnikov, D. A., Beloozerova, I. N. (2009). Gaze behaviors of freely walking cats. Program No. 35. 454.14. *Society for Neuroscience*. Washington DC. Online.

Rivers, T. J., Sirota, M. G., Guttentag, A. I., Ogorodnikov, D. A., Shah, N. A., & Beloozerova, I. N. (2014). Gaze shifts and fixations dominate gaze behavior of walking cats. *Neuroscience*, *275*, 477-499.

Robertson, R. T. (1977). Thalamic projections to parietal cortex. *Brain, Behavior and Evolution*, *14*(3), 161-184.

Robertson, R. T., & Cunningham, T. J. (1981). Organization of corticothalamic projections from parietal cortex in cat. *Journal of Comparative Neurology*, *199*(4), 569-585.

Sakai, S. T., Inase, M., & Tanji, J. (1996). Comparison of cerebellothalamic and pallidothalamic projections in the monkey (Macaca fuscata): a double anterograde labeling study. *Journal of Comparative Neurology*, *368*(2), 215-228.

Schmied, A., Benita, M., Conde, H., & Dorment, J. F. (1979). Activity of ventrolateral thalamic neurons in relation to a simple reaction time task in the cat. *Experimental Brain Research*, *36*(2), 285-300.

Scott, S. H. (2008). Inconvenient truths about neural processing in primary motor cortex. *The Journal of Physiology*, *586*(5), 1217-1224.

Seki, K., & Fetz, E. E. (2012). Gating of sensory input at spinal and cortical levels during preparation and execution of voluntary movement. *The Journal of Neuroscience*, *32*(3), 890-902.

Selemon, L. D., & Goldman-Rakic, P. S. (1988). Common cortical and subcortical targets of the dorsolateral prefrontal and posterior parietal cortices in the rhesus monkey: evidence for a distributed neural network subserving spatially guided behavior. *The Journal of Neuroscience*, *8*(11), 4049-4068.

Sherk, H., Fowler, G. A. (2001). Neural analysis of visual information during locomotion. *Progress in Brain Research*, *134*, 247-264.

Sherman, S. M., & Guillery, R. W. (2001). *Exploring the Thalamus* (Vol. 312). San Diego: Academic Press.

Sherrington, C. S. (1906). The Integrative Action of the Nervous System. Yale University Press. New Haven, CT

Sherrington, C. S. (1910a) Remarks on the reflex mechanism of the step. *Brain, 33*, 1-28.

Sherrington, C. S. (1910b). Flexor-reflex of the limb, crossed extension-reflex, and reflex stepping and standing. *The Journal of Physiology*, *40*(1-2), 28-121.

Sherrington, C. S., & Laslett, E. E. (1903). Observations on some spinal reflexes and the interconnection of spinal segments. *The Journal of Physiology*,29(1), 58-96.

Shik, M. L., & Orlovsky, G. N. (1976). Neurophysiology of locomotor automatism. *Physiol Rev*, *56*(3), 465-501.

Shik, M. L., Severin, F. V., & Orlovsky, G.N. (1966). Control of walking and running by means of electrical stimulation of mid-brain. *Biophysics-USSR*, *11*(4), 756.

Shin, H. C., Park, H. J., & Chapin, J. K. (1993). Differential phasic modulation of short and long latency afferent sensory transmission to single neurons in the ventroposterolateral thalamus in behaving rats. *Neuroscience Research*, *17*(2), 117-125.

Shin, H. C., Park, H. J., & Chapin, J. K. (1994). Differential phasic modulation of short and long latency afferent sensory transmission to single neurons in the primary somatosensory cortex in behaving rats. *Neuroscience Research*, *19*(4), 419-425.

Sirota, M. G., Di Prisco, G. V., & Dubuc, R. (2000). Stimulation of the mesencephalic locomotor region elicits controlled swimming in semi-intact lampreys. *European Journal of Neuroscience*, *12*(11), 4081-4092.

Sirota, M. G., Swadlow, H. A., & Beloozerova, I. N. (2005). Three channels of corticothalamic communication during locomotion. *The Journal of Neuroscience, 25*(25), 5915-5925.

Skinner, B. F. (1938). The behavior of organisms: An experimental analysis. Appleton-Century-Crofts Inc. New York.

Solomon, D. H., Barohn, R. J., Bazan, C., & Grissom, J. (1994). The thalamic ataxia syndrome. *Neurology*, *44*(5), 810-810.

Soso, M. J., & Fetz, E. E. (1980). Responses of identified cells in postcentral cortex of awake monkeys during comparable active and passive joint movements. *Journal of Neurophysiology*, *43*(4), 1090-1110.

Stein, J. F., & Glickstein, M. (1992). Role of the cerebellum in visual guidance of movement. *Physiological Reviews*, *72*(4), 967-1017.

Steriade, M. (1995). Two channels in the cerebellothalamocortical system. *Journal of Comparative Neurology*, *354*(1), 57-70.

Stout, E. E., & Beloozerova, I. N. (2012). Pyramidal tract neurons receptive to different forelimb joints act differently during locomotion. *Journal of Neurophysiology*, *107*(7), 1890-1903.

Stout, E. E., & Beloozerova, I. N. (2013). Differential responses of fast-and slow-conducting pyramidal tract neurons to changes in accuracy demands during locomotion. *The Journal of Physiology*, *591*(10), 2647-2666.

Strick, P. L. (1976). Activity of ventrolateral thalamic neurons during arm movement. *Journal of Neurophysiology*, *39*(5), 1032-1044.

Strick, P.L. (1973). Light microscopic analysis of the cortical projection of the thalamic ventrolateral nucleus in the cat. *Brain Research*, 55(1):1-24.

Stuart, D. G., & Hultborn, H. (2008). Thomas Graham Brown (1882–1965), Anders Lundberg (1920–), and the neural control of stepping. *Brain Research Reviews*, *59*(1), 74-95.

Symonds, L. L., & Rosenquist, A. C. (1984). Corticocortical connections among visual areas in the cat. *Journal of Comparative Neurology*, 229(1), 1-38.

Symonds, L. L., Rosenquist, A. C., Edwards, S. B., & Palmer, L. A. (1981). Projections of the pulvinar-lateral posterior complex to visual cortical areas in the cat. *Neuroscience*, *6*(10), 1995-2020.

Takahashi, K. (1965). Slow and fast groups of pyramidal tract cells and their respective membrane properties. *Journal of Neurophysiology*, *28*(5), 908-924.

Thach, W. T., Goodkin, H. P., & Keating, J. G. (1992). The cerebellum and the adaptive coordination of movement. *Annual Review of Neuroscience*, *15*(1), 403-442.

Trendelenburg, W. (1911). Untersuchungen über reizlose vorübergehende Ausschaltung am Zentralnervensystem. *Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere*, *137*(11-12), 515-544.

Tsao, D. Y., & Livingstone, M. S. (2008). Mechanisms of face perception. *Annual Review of Neuroscience*, *31*, 411.

Udo, M., Kamei, H., Matsukawa, K., & Tanaka, K. (1982). Interlimb coordination in cat locomotion investigated with perturbation. *Experimental Brain Research*, *46*(3), 438-447.

Van Donkelaar, P., Stein, J. F., Passingham, R. E., & Miall, R. C. (1999). Neuronal activity in the primate motor thalamus during visually triggered and internally generated limb movements. *Journal of Neurophysiology*, *82*(2), 934-945.

van Kan, P. L. E., Houk, J. C., & Gibson, A. R. (1993). Output organization of intermediate cerebellum of the monkey. *Journal of Neurophysiology, 69*(1), 57-73.

Van Kan, P. L., Gibson, A. R., & Houk, J. C. (1993). Movement-related inputs to intermediate cerebellum of the monkey. *Journal of Neurophysiology*, *69*(1), 74-94.

Vaziri, S., Diedrichsen, J., & Shadmehr, R. (2006). Why does the brain predict sensory consequences of oculomotor commands? Optimal integration of the predicted and the actual sensory feedback. *The Journal of Neuroscience*, *26*(16), 4188-4197.

Vicario, D. S., Martin, J. H., & Ghez, C. (1983). Specialized subregions in the cat motor cortex: a single unit analysis in the behaving animal. *Experimental Brain Research,* 51(3), 351-367.

Wallen, P., & Williams, T. L. (1984). Fictive locomotion in the lamprey spinal cord in vitro compared with swimming in the intact and spinal animal. *The Journal of Physiology*, *347*(1), 225-239.

Weber, D. J., London, B. M., Hokanson, J. A., Ayers, C. A., Gaunt, R. A., Torres, R. R., ... & Miller, L. E. (2011). Limb-state information encoded by peripheral and central somatosensory neurons: implications for an afferent interface. *Neural Systems and Rehabilitation Engineering, IEEE Transactions on*, *19*(5), 501-513.

Weyand, T. G., Updyke, B. V., & Gafka, A. C. (1999). Widespread distribution of visual responsiveness in frontal, prefrontal, and prelimbic cortical areas of the cat: an electrophysiologic investigation. *Journal of Comparative Neurology*,*405*(1), 99-127.

Widajewicz, W., Kably, B., & Drew, T. (1994). Motor cortical activity during voluntary gait modifications in the cat. II. Cells related to the hindlimbs. *Journal of Neurophysiology*, *72*(5), 2070-89.

Wise, S. P., Di Pellegrino, G., & Boussaoud, D. (1992). Primate premotor cortex: dissociation of visuomotor from sensory signals. *Journal of Neurophysiology*, *68*(3), 969-972.

Yakovenko, S., Krouchev, N., & Drew, T. (2011). Sequential activation of motor cortical neurons contributes to intralimb coordination during reaching in the cat by modulating muscle synergies. *Journal of Neurophysiology*, 105(1), 388-409.

Yen, C. T., Honda, C. N., & Jones, E. G. (1991). Electrophysiological study of spinothalamic inputs to ventrolateral and adjacent thalamic nuclei of the cat. *Journal of Neurophysiology*, *66*(3), 1033-1047.

Yumiya, H., & Ghez, C. (1984). Specialized subregions in the cat motor cortex: anatomical demonstration of differential projections to rostral and caudal sectors. *Experimental Brain Research*, *53*(2), 259-276.

Zelenin, P. V., Beloozerova, I. N., Sirota, M. G., Orlovsky, G. N., & Deliagina, T. G. (2010). Activity of red nucleus neurons in the cat during postural corrections. *The Journal of Neuroscience*, *30*(43), 14533-14542.

Zelenin, P. V., Deliagina, T. G., Orlovsky, G. N., Karayannidou, A., Dasgupta, N. M., Sirota, M. G., & Beloozerova, I. N. (2011). Contribution of different limb controllers to modulation of motor cortex neurons during locomotion. *The Journal of Neuroscience, 31*(12), 4636-4649.