The Role of Motor Cortical Neuron Subpopulations in the Adaptation of Locomotion Through Complex Environments

by

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ABSTRACT

Locomotion in natural environments requires coordinated movements from multiple body parts, and precise adaptations when changes in the environment occur. The contributions of the neurons of the motor cortex underlying these behaviors are poorly understood, and especially little is known about how such contributions may differ based on the anatomical and physiological characteristics of neurons. To elucidate the contributions of motor cortical subpopulations to movements, the activity of motor cortical neurons, muscle activity, and kinematics were studied in the cat during a variety of locomotion tasks requiring accurate foot placement, including some tasks involving both expected and unexpected perturbations of the movement environment. The roles of neurons with two types of neuronal characteristics were studied: the existence of somatosensory receptive fields located at the shoulder, elbow, or wrist of the contralateral forelimb; and the existence projections through the pyramidal tract, including fast- and slow-conducting subtypes.

Distinct neuronal adaptations between simple and complex locomotion tasks were observed for neurons with different receptive field properties and fast- and slow-conducting pyramidal tract neurons. Feedforward and feedback-driven kinematic control strategies were observed for adaptations to expected and unexpected perturbations, respectively, during complex locomotion tasks. These kinematic differences were reflected in the response characteristics of motor cortical neurons receptive to somatosensory information from different parts of the forelimb, elucidating roles for the various neuronal populations in accommodating disturbances in the environment during behaviors. The results show that anatomical and physiological characteristics of motor cortical neurons are important for determining if and how neurons are involved in precise control of locomotion during natural behaviors.

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TABLE OF CONTENTS

Page

LIST OF TABLES vi

LIST OF FIGURES vii

CHAPTER

1 INTRODUCTION 1

1.1 Historical and Current Perspectives 1

1.2 Pyramidal Tract Neurons (PTNs) 5

1.3 Fast and Slow PTNs 7

1.4 Somatosensory Receptivity Among Motor Cortical Neurons 9

1.5 Movement Corrections in the Motor Cortex 11

1.6 Overview of Dissertation Research 14

2 PYRAMIDAL TRACT NEURONS RECEPTIVE TO DIFFERENT SEGMENTS OF THE FORELIMB ACT DIFFERENTLY DURING LOCOMOTION 17

2.1 Abstract 17

2.2 Introduction 19

2.3 Methods 23

2.4 Results 32

2.5 Discussion 52

3 FAST- AND SLOW-CONDUCTING PYRAMIDAL TRACT NEURONS DURING SIMPLE AND COMPLEX LOCOMOTION 61

CHAPTER Page

3.1 Abstract 61

3.2 Introduction 63

3.3 Methods 66

3.4 Results 81

3.5 Discussion 99

4 NEURONAL, KINEMATIC, AND MUSCLE RESPONSES TO PERTURBATIONS OF THE ENVIRONMENT 109

4.1.1 Abstract 109

4.1.2 Introduction 111

4.1.3 Methods 112

4.1.4 Results 125

4.1.5 Discussion 136

4.2 Additional Investigation: Joint Kinetics 142

4.2.1 Abstract 142

4.2.2 Introduction 144

4.2.3 Methods 146

4.2.4 Results 154

4.2.5 Discussion 164

5 MOTOR CORTICAL SUBPOPULATIONAL RESPONSES TO PERTURBATION OF THE ENVIRONMENT 174

Abstract 174

CHAPTER Page

Introduction 176

Methods 178

Results 189

Discussion 201

6 CONCLUSION 208

REFERENCES 210

IACUC Approval for Animal Research 232

APPENDIX

A ENDPOINT VELOCITY CONTRIBUTIONS BY JOINT 233

LIST OF TABLES

Table		Page
2.1	PTNs Recorded in Different Subjects	35
3.1	PTNs Recorded in Different Subjects	84
3.2	Selected Parameters of Locomotion-Related Activity of Fast- and Slow-Conducting PTN Populations.	88
3.3	Fast- and Slow-Conducting PTNs with Different Numbers of PEFs During Simple and Ladder Locomotion.	98
4.2.1	Body Parameters for Cats 1 and 2	153
5.1	Number of Recorded Neurons of Each Subpopulation	189

LIST OF FIGURES

Figure		Page
1.1	Relationships Among Dissertation Sections	16
2.1	Locomotion Tasks	25
2.2	Location of PTNs and Their Identification	27
2.3	Example of the Typical Activity of a PTN	29
2.4	Activity of PTNs with Receptive Fields Involving Different Forelimb Joints During Simple Locomotion	38
2.5	Neuronal Changes Between Simple and Complex Locomotion	41
2.6	Typical Changes in the Depth of Modulation upon Transition from Simple to Ladder Locomotion	43
2.7	Activity of PTNs with Receptive Fields Involving Different Forelimb Joints During Ladder Locomotion	46
2.8	Comparison of Activities of Flexion- and Extension-Receptive PTNs	49
2.9	Proportions of PTNs Firing in Phase with Activation of their Receptive Field During Locomotion.	51
3.1	Locomotion Tasks	68
3.2	Identification of PTNs	70
3.3	Location of PTNs	75
3.4	Example Activity of Fast- and Slow-Conducting PTNs	79
3.5	Phase Distribution of PEFs, Preferred Phases, and Discharge Rates of Fast- and Slow-Conducting PTNs During Locomotion	86

Figure Page

3.6 Comparison of Activity Characteristics of Individual Fast-Conducting PTNs Between Simple and Ladder Locomotion 90

3.7 Comparison of Activity Characteristics of Individual Slow-Conducting PTNs Between Simple and Ladder Locomotion 93

3.8 Typical Changes in the Depth of Modulation upon Transition from Simple to Ladder Locomotion 96

4.1.1 Experimental Design 114

4.1.2 Area of Recording, Joint Definitions, and Example Muscle Activity 120

4.1.3 Support Vector Machine (SVM) Methods and Waveform Analysis..... 123

4.1.4 Kinematic Strategies for Making Perturbed Steps 127

4.1.5 Muscle Activity During Perturbed Steps 129

4.1.6 Neuronal Response Characteristics During Perturbed Steps 131

4.1.7 Characteristics Affecting Neuron Responsivity 133

4.1.8 Directional Sensitivity in Neuronal Responses 135

4.2.1 Experimental Design 148

4.2.2 Joint Angle Definition and Velocity Components 153

4.2.3 Joint Angle Movements During the Swing Phase of the Control Step..... 155

4.2.4 Joint Torques and Velocity Contributions During the Swing Phase of the Control Step..... 157

4.2.5 Changes in Velocity Contributions 160

4.2.6 Changes in Torques 162

Figure Page

5.1 Experimental Design 179

5.2 Area of Recording in the Motor Cortex and Test for Collision of Spikes 184

5.3 Support Vector Machine (SVM) Classification of Neuronal Activity .. 187

5.4 Visual Responses to Crosspiece Displacement 191

5.5 Motor Adaptation Responses Over the Disturbed Step 193

5.6 Populational Motor Adaptation Characteristics During the Disturbed Step 195

5.7 Populational Responses to Large and Small Steps 197

5.8 Time Sensitivity in Neuronal Responses 199

5.9 Relationship Between Discharge Rate and SVM Classification Accuracy 201

CHAPTER 1

INTRODUCTION

Most movements in natural environments require accuracy to be successful. Characteristics of the outside environment, such as obstacles, objects of varying textures, strength, and rigidity, gaps between support surfaces, angled surfaces, and movements of objects or other organisms can all pose complications to the successful completion of movements. The motor cortex plays a critical role in managing these and other complexities that emerge during a variety of movements. Damage to this structure imposes significant impairments to a variety of movements including reaching and grasping, locomotion, and facial movements (e.g. Martin and Ghez, 1993; Chambers and Liu, 1957; Kolb and Milner, 1981; Friel et al., 2007), across a wide variety of commonly studied mammalian species, such as monkeys, cats, and humans. As such, the role of the motor cortex in motor control has been the focus of intense investigation.

1.1 Historical and current perspectives

Early investigations, such as those of Leyton and Sherrington (1917), Boldrey and Penfield (1937), and Barnard and Woolsey (1956) demonstrated that surface stimulation of the motor cortex could produce muscle contractions, and the existence of a "motor homunculus", this is, an orderly representation of the body upon the surface area of the motor cortex. Investigations into the function of individual neurons of the motor cortex during reaching tasks in the monkey suggested that the discharge rates of neurons played a role in controlled muscle

force, as originally posed by Evarts (1967); this perspective was supported by microstimulation studies by Asanuma and colleagues (Asanuma and Sakata, 1967), among others. Investigations into the role of the activity of motor cortical neurons during locomotion tasks of varying complexity have yielded significant insights as well. Many motor cortical neurons discharge action potentials in a consistent pattern during locomotion (Armstrong and Drew, 1984). It has been found that neuronal activity changes substantially when complications such as obstacles or limited support surfaces are applied to the movement environment (e.g. Drew 1993; Beloozerova and Sirota 1993), and that these neurons are highly responsive to unexpected perturbations in the environment during locomotion (Marple-Horvat et al. 1993; Amos et al. 1989).

Investigations by Georgopoulos and colleagues (1982) showed that the motor cortex encodes high level, global parameters such as movement trajectory. They found the existence of motor cortical neuron "tuning curves" during reaching movements in the monkey, where a neuron would exhibit a preferred direction of movement that would elicit maximal activity, and would lower activity as the direction of movements shifted away from this preferred direction. Even more significantly, they found that populations of motor cortical neurons, when analyzed as a group and with appropriately weighted activity contribution, could approximate the direction of movements in three dimensional space (Georgopoulos et al. 1986, Schwartz et al. 1988). This suggested that, rather than simply controlling muscle contraction, the motor cortex encodes high-level task

information, including intended trajectory of movements, regardless of what specific muscular activity gives rise to the desired movement.

However, the question of whether motor cortex activity was primarily movement or muscle related was controversial, as was the choice of coordinate frame that the neural system used for sensorimotor transformations. Many researchers found that neuronal activity during reaching movements was related to an entire host of viable movement parameters, some movement related, and some muscle related, and that neuronal responses often depended strongly on limb and joint configuration. Supporting the population vector hypothesis, Moran and Schwartz (1999), for instance, found that neural coding during reaching movements was well-modeled by the direction of movement and speed, but inconsistent with the patterns of muscle activation occurring during that time period. Kakei and colleagues (1999), on the other hand, found neuronal coding that related to both abstract trajectory patterns and muscle activation, suggesting that both are represented. However, complicating the interpretation of these studies, it was also found that many neurons showed responses to motor tasks even when they showed no relationship to any tested kinematic parameter (Fetz 1992), suggesting that the observed trajectory coding of motor cortical neurons does not fully explain what activity in the motor cortex actually represents. It was also suggested that motor cortical activity could indeed code for muscle activity, but that this relationship is obscured by the motor periphery, including posture, multi-joint dynamics, and body configuration (Todorov, 2000), and that the observed neural coding for movement direction and other kinematic parameters

could simply be due to the correlation of these parameters with muscle force. The findings of Scott and Kalaska (1997) demonstrated that body configuration exerts an effect on neuron activity during reaching tasks, and that the relationship between neuronal activity and the direction of movements is altered when body or limb configuration is changed, becoming less related to the coded population vector. This is due to the fact that individual tuning curves are not uniformly distributed, and place stronger emphasis on movements away and to the left, or towards and to the right (Scott, 2003). Finally, Scott and colleagues (2001) demonstrated that visually guided reaching tasks towards a spatial target could be completed successfully in situations where the neuronal population vector was pointed in a different direction than the movement, directly contradicting the population vector hypothesis, and suggesting that this hypothesis does not fully describe motor cortical control of movement.

To overcome this issue, multiple hypotheses on whole brain control of movements have been proposed to incorporate aspects of body configuration and interjoint dynamics, and particularly to solve the "degrees of freedom" problem – that there are many ways to successfully complete a movement task, given the high degree of redundancy in joints and muscles (Bernstein, 1967). Many hypotheses posit the existence of an internal model of the body which is used to plan and execute voluntary movements (e.g. Buneo et al. 1995, Flanagan and Wing 1997). This model may be used to predict forward the consequences of a particular sequence of motor commands, or an inverse model which determines the commands needed to achieve a desired movement. The primary and premotor

cortices, as well as the cerebellum, have been implicated in the use of an inverse dynamics model (Schweighofer et al. 1998; Kawato 1999). However, how such a representation is expressed, the contributions of each structure, and what control strategies such a representation uses remain unclear.

One hypothesis suggests that a feedback control scheme corrects differences between the expected and occurring trajectory according to some optimality condition (e.g. Flash and Hogan, 1985; Todorov 2004; Diedrichsen et al. 2010; Scott 2004), which defines the optimal movement strategy. This strategy is then adapted to challenges that arise during the movement through use of fast feedback loops, and the primary motor cortex has a critical role in these adaptations (e.g. Scott 2008; Scott et al. 2011). The optimal feedback control hypothesis and the role of the motor cortex within this formulation has been able to successfully describe many behaviors (reviewed in Scott, 2012). Other hypotheses suggest that internal models of intersegmental and joint dynamics could also play a role in motor control strategies (e.g. Hollerbach 1982; Dounskaia 2005), and these hypotheses likewise have large bodies of experimental support (see Ambike and Schmiedeler 2013).

1.2 Pyramidal Tract Neurons (PTNs)

Layer V of the motor cortex contains a large population of neurons which directly synapse upon the spinal interneurons (Lloyd, 1941; Hoff & Hoff, 1934; Dyachkova et al. 1971; Antal, 1984; Lacroix et al. 2004; Rosenzweig et al. 2009), and synapse directly upon spinal motoneurons in higher primates and humans, but

not lower primates or other vertebrates (Bernhard and Widen 1953; Preston & Whitlock, 1961; Landgren et al. 1962; Clough et al. 1968; Fetz et al. 1976; Bortoff & Strick, 1993), directly influencing the spinal networks and the central pattern generator (CPG). The axons of these neurons project through the pyramidal tract (or corticospinal tract), and these neurons are called PTNs (pyramidal tract neurons). Due to this direct impact on spinal motor networks, PTNs have been studied in some detail, in terms of their anatomical, morphological, and functional characteristics. It has been found that sectioning of the pyramidal tract leads to acute deficits in general motor control, although the severity of these deficits can vary (Asanuma, 1989). While many of these deficits may recover partially or fully, some deficits, particularly in fine digit control, never recover (Liddell and Philips, 1944; Porter and Lemon, 1993). Likewise, this class of neurons has been found to have a significant role in accuracy during locomotion. The work by Beloozerova & Sirota in 1993 showed that PTNs demonstrate dramatic and marked changes to their activity between simple, non-accuracy demanding locomotion over a flat surface, and accurate visually-guided target stepping. These include changes to the level of activity and an increase in the frequency modulation of activity – a sharpening, so to speak, of the neuron's step-phase related activity towards a more precise firing profile. These changes in activity became only more drastic as the difficulty of the task increased. Drew, in 1993, showed that similar changes were observed in PTNs during locomotion when visually-guided changes to steps were made during overstepping of obstacles on the treadmill.

1.3 Fast and Slow PTNs

Motor cortical neurons projecting through the pyramidal tract can be subdivided into two groups, so-called "fast" and "slow" PTNs. The defining difference between fast- and slow-conducting PTNs is the speed of axonal conduction velocity. Slow-conducting PTNs are defined as possessing a conduction velocity below 21 m/s, while fast-conducting PTNs are defined as possessing a conduction velocity above this speed (Brookhart & Morris, 1948, Bishop et al. 1952; Takahashi, 1965). Slow-conducting PTNs are by far the more common type – it is estimated that as many as 90% of the neurons that project through the pyramidal tract are of the slow-conducting variety (Calvin and Sypert, 1976). In addition to this, the connectivity characteristics of these two subpopulations differ: fast-conducting PTNs are more likely to make disynaptic, inhibitory connections, and are more likely to influence distal muscle groups (Brookhart, 1952; Canedo, 1997). These anatomical and morphological differences imply that each subpopulation of PTNs may be better suited to different tasks, and the differences in physiological roles that are observed between these subpopulations may be reflected in their roles during training or sensorimotor adaptations to novel tasks.

The high conduction velocity of fast-conducting PTNs relative to slow-conducting PTNs makes them better suited to fast adaptations, as the motor commands of these neurons will reach the spinal cord more quickly. This characteristic makes fast-conducting PTNs more suited to short-notice, on-line

corrections of motor movements. On the other hand, the low conduction velocity of slow-conducting PTNs makes them poorly suited to low-latency error corrections. However, the large numbers of these neurons, and the lower levels of muscle facilitation produced by slow-conducting PTNs (Lemon et al. 1993), may enable finer-grained, "precision" control.

On the basis of these characteristics, it appears likely that slow-conducting PTNs are more significantly involved in the execution of an inversely modeled motor command towards a goal (feedforward control), while fast-conducting PTNs are more significantly involved in executing forward modeling, and error correction between the expected movement and the movement that actually occurs (feedback control). This perspective is supported by multiple lines of evidence. Fromm and Evarts (1977; 1981) found that during reaching movements, slow-conducting PTNs are maximally activated in small amplitude, "precision" tasks, while fast-conducting PTNs are increasingly active as the amplitude of the task increases. Thus, slow-conducting PTNs can be supposed to form the base set of motor commands that are generated, while fast-conducting PTNs modify these commands as the task is changed. Furthermore, while slow-conducting PTNs form primarily excitatory monosynaptic connections with fast-conducting PTNs, fast-conducting PTNs form inhibitory disynaptic connections to slow-conducting PTNs, suggesting that discharges of fast-conducting PTNs might replace, rather than supplement, the discharges of slow-conducting PTNs (Takahashi, 1965; Tsukahara et al. 1968; Ghosh & Porter, 1988).

1.4 Somatosensory responsivity among motor cortical neurons

Many neurons in the motor cortex, especially those projecting through the pyramidal tract, are receptive to somatosensory stimuli. Many motor cortical neurons will fire action potentials in response to tactile stimuli, palpation of muscles, and/or passive joint movements (e.g., Rosen and Asanuma, 1972; Stout and Beloozerova 2012). In addition, the motor cortex receives input from the visually receptive posterior parietal cortex and cerebellum through the ventrolateral thalamus (e.g. Asanuma et al. 1983, Andujar and Drew, 2007), and it has been found that the motor cortex is responsive to visual stimuli (e.g. Garcia-Rill and Dubrovsky, 1974; Martin and Ghez, 1985; Armer et al., 2013).

However, despite these responses, it is unlikely that proprioceptive activity plays a leading role in determining the motor commands generated by the motor cortex. Neurons with similar somatosensory receptive fields often discharge during quite different times of the locomotion cycle (Armstrong and Drew 1984b), and it has been shown that the locomotion-related responses of motor cortical neurons are only slightly affected by changes in the vigor of movements during up- and downslope walking, weight bearing, or alterations in speed (Armstrong and Drew 1984a; Beloozerova and Sirota 1993b)—changes that most certainly cause significant changes to proprioceptive afferentation. In regard to cutaneous input, Armstrong and Drew (1984b) have demonstrated that in motor cortex neurons with cutaneous receptive fields, including on the forefoot, the discharges during locomotion remained rhythmic and their phasing relative to the step cycle was unchanged when the response to mechanical stimulation in the

receptive field was temporarily much reduced or abolished by local anesthesia of the skin. Poor relationships between phasing of task-related discharges and directional specificity of PTN resting receptive fields were reported in previous studies (Armstrong and Drew 1984b; Prilutsky et al. 2005; Drew 1993). While it is true that somatosensory receptive fields during active movements may be somewhat different from those observed at rest (Chapman et al. 1988; Ghez and Pisa 1972), the above group of observations suggest that some factors other than stimulation of somatosensory receptive field drive PTN discharges during locomotion. It is quite likely that during locomotion the activity of PTNs of the motor cortex, rather than being driven by stimulation of somatosensory receptive fields, is significantly influenced by signals from the spinal locomotion CPG. At the same time, during complex locomotor tasks, dramatic changes to the activity of the neurons of the motor cortex have been observed versus simple locomotor tasks on a flat surface (e.g. Beloozerova and Sirota 1993; Drew 1993), despite the fact that the kinematic and EMG profiles of steps in either condition are quite similar, suggesting that similar proprioceptive information is received in both conditions (e.g. Beloozerva et al. 2010). These findings indicate that information about the locomotion environment, including information about constraints and complications, strongly influence stride-related activity in the motor cortex.

1.5 Motor corrections in the motor cortex

During natural behaviors, unexpected or emergent changes in the environment are commonplace, such as changing positions of support surfaces, or

alterations in the movements of predators or prey. To be successful, movements must be altered to compensate for the changing circumstances. The cerebellum and posterior parietal cortex, in particular, have been implicated in error-correction stemming from unexpected changes in the motor task (Desmurget and Grafton 2000, Desmurget et al. 1999) and the posterior parietal cortex plays a significant role in planning gait adaptation during visually guided stepping (Lajoie and Drew, 2007; Andujar et al. 2010). Both regions drive motor cortex activity: the cerebellum synapses upon the ventrolateral thalamus (Asanuma et al. 1983), whose neurons project to the motor cortex (e.g., Strick, 1976), and the parietal cortex extensively innervates the motor cortex through transcortical fibers (e.g., Andujar and Drew 2007; Petrides and Pandya 1984).

However, while the motor cortex has been shown to be necessary for modifying movement trajectories, little is known about how the motor cortex functionally compensates for emergent or unexpected changes in the movement environment during locomotion, with the exception of the study by Marple-Horvat and colleagues (1993), which found significant responses in the motor cortex when support surfaces would unexpectedly depress upon foot placement. While previous studies suggest that the motor cortex is involved in execution, rather than planning, of gait adaptations (Drew 1993), it is unknown whether this activity is dependent on the amount of time used to plan a gait adaptation.

During planned gait adaptations, kinematic adjustments will often be made in preparation of the adaption (Mohagheghi et al. 2004). During unexpected or emergent disturbances, preparatory movements are impossible, however, and

strategy selection is constrained (Patla 1999); often the smallest kinematic adjustments that meet the adaptive constraint are preferred (Patla et al. 2004). For an unexpected or emergent obstacle avoidance during walking, it was also found that the latency of such obstacle avoidance is shorter than the typical latency of a planned voluntary gait modification (Weerdesteyn et al 2004), suggesting that distinct neuronal processes are taking place in these situations. Indeed, during reaching, the motor cortex is known to encode multiple potential movement strategies (Genovesio 2005; Carmena et al. 2005), any of which may be followed based on the context of the individual movement. Therefore, it appears likely that the neuronal strategies employed to overcome emergent changes in the environment may be dependent on the latency between when the disturbance is perceived and the motor adaptation is made.

It is likely that the motor cortex is critical for the execution of motor adaptions to changes in the environment. The motor cortex has been strongly implicated in the control of movements, and substantial literature suggests that the motor cortex directly codes for on-going movement commands (e.g. Evarts, 1967; Georgopoulos, 1986), and is involved in the accuracy of movements during reaching (e.g. Scott, 2008; 2011) as well as locomotion (e.g. Beloozerova and Sirota, 1993; Drew 1993). During unexpected depression of a ladder rung during walking, motor cortical neurons rapidly respond to the depression, and are suggested to be involved in accommodating this environmental change (Marple-Horvat et al. 1993). During reaching tasks, unexpected changes in load, such as those investigated by Evarts (1973) and Porter and Rack (1976) likewise caused

rapid responses in motor cortical neurons. Similarly, unexpected application of a force-field leads to rapid adaptations in the motor cortex activity (e.g. Gandolfo 2000; Paz et al. 2005; Cherian et al. 2013), although whether these adaptations represent changes to an inverse model instantiated in the motor cortex, or an upstream adaptation whose effects are reflected in the activity motor cortex is a matter of contention. Therefore, it appears that the motor cortex plays a principal role in executing motor commands in response to complexities in the movement environment.

1.6 Overview of dissertation research

The central role of the motor cortex in modification of motor behaviors has been established, but the mode and mechanism of contribution of its various neuronal groups with different anatomical and physiological characteristics remains far less clear. In the chapters that follow, the activity and contributions of individual neurons belonging to distinct subpopulations within the motor cortex are described and discussed for a variety of locomotor tasks. This includes determination and characterization of the roles for subpopulations of motor cortical neurons in control of locomotion over simple and complex surfaces (Chapters 2 and 3), and, additionally, determination of whether and how the roles of these subpopulations differ between adaptations to known and unexpected challenges that arise in the environment (Chapters 4 and 5). The work presented here had two interlocking objectives (see Fig. 1.1 for the relationship between different chapters):

Aim 1: To characterize the roles of fast- and slow-conducting pyramidal tract neurons (PTNs), as well as somatosensory receptive or non- receptive neurons of the motor cortex between simple and complex locomotion.

Chapter 2: Comparison of the responses of shoulder-, elbow-, wrist-, and non-receptive PTNs during simple and complex locomotion tasks.

Chapter 3: Comparison of the responses of fast- and slow-conducting PTNs during simple and complex locomotion tasks.

Aim 2: To characterize the response of individual motor cortical neurons and subpopulations of neurons to known and unexpected changes in the locomotion environment.

Chapter 4: Comparison of the responses of motor cortex neurons between environmental complications that are known and planned for, and complications that unexpectedly arise and cannot be planned for.

Chapter 5: Characterization of responses of shoulder-, elbow-, wrist-, and non-receptive motor cortical neurons, as well as PTNs and Non-PTNs, between locomotion through known and unexpected environmental complications.

The work contained in Chapters 2 and 3 has been published in the *Journal of Neurophysiology* and *Journal of Physiology*, respectively. The work contained in Chapter 4 is under review at the *Journal of Neuroscience*, and the work

contained in Chapter 5 is in final preparations for submission. Note that the roles of fast- and slow-conducting PTNs were not discussed in Chapter 5; this is because the collected sample of slow-conducting PTNs was too small to permit confidence in the conclusions (see Chapter 3 for a discussion of the challenges in identifying and recording from slow-conducting PTNs).

The work contained in this dissertation involves the contributions of many individuals in the Motor Systems Research Laboratory (see *Acknowledgements*), especially where data collection is concerned. Successful experimental sessions often required the coordinated efforts of multiple individuals, often involving many members of the laboratory. The specific contributions of the author for these investigations include: shared responsibility for design and conception of dissertation research goals with co-chair Irina Beloozerova; shared responsibility for data collection from 4/8 cats in Chapters 2 and 3 and 2/2 cats in Chapters 4 and 5 with other members of the lab; primary responsibility for kinematic, muscle, and neuronal data analysis for all chapters; shared responsibility for drafting publications forming Chapters 2 and 3 with co-chair Irina Beloozerova; and primary responsibility for drafting publications forming Chapters 4 and 5.

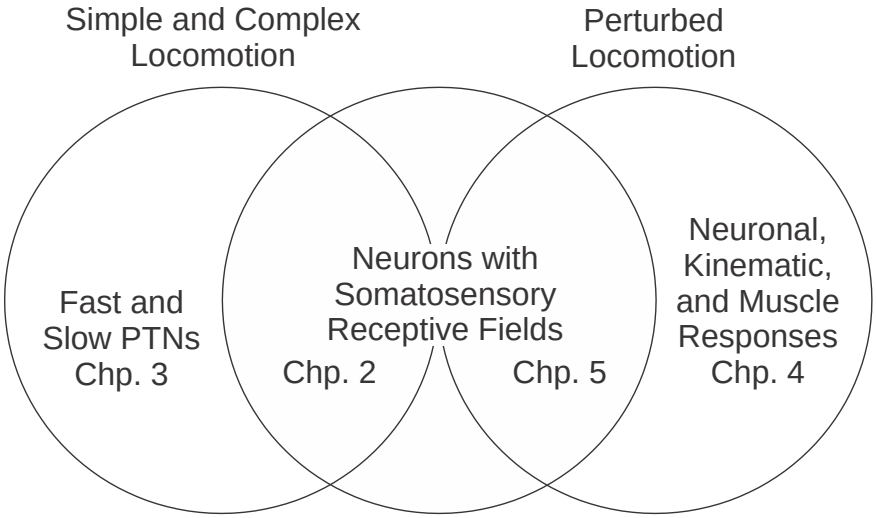

Figure 1.1: Relationships among dissertation sections.

CHAPTER 2

SOMATOSENSORY-RECEPTIVE NEURONS DURING SIMPLE AND COMPLEX LOCOMOTION

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2.1 ABSTRACT

During locomotion, motor cortical neurons projecting to the pyramidal tract (PTNs) discharge in close relation to strides. How their discharges vary based on the part of the body they influence is not well understood. We addressed this question with regard to segments of the forelimb in the cat. During simple and ladder locomotion, we compared the activity of four groups of PTNs with somatosensory receptive fields involving different forelimb segments: (1) 45 PTNs receptive to movements of shoulder, (2) 30 PTNs receptive to movements of elbow, (3) 40 PTNs receptive to movements of wrist, and (4) 30 non-responsive PTNs. In the motor cortex, a relationship exists between the location of the source of afferent input and the target for motor output. Based on this relationship, we inferred the forelimb segment that a PTN influences from its somatosensory receptive field. We found that different PTNs tended to discharge differently during locomotion. During simple locomotion, shoulder-related PTNs were most active during late stance/early swing, and upon transition from simple to ladder locomotion, often increased activity and step-related modulation while reducing discharge duration. Elbow-related PTNs were most active during late swing/early stance, and typically did not change activity, modulation, or discharge duration on the ladder. Wrist-related PTNs were most active during swing, and

upon transition to the ladder often decreased activity and increased modulation while reducing discharge duration. These data suggest that during locomotion the motor cortex uses distinct mechanisms to control the shoulder, elbow, and wrist.

2.2 INTRODUCTION

During locomotion, nearly all neurons that project to the pyramidal tract (pyramid tract neurons, PTNs) discharge in close relation to strides (Armstrong and Drew 1984a,b, 1985; Beloozerova and Sirota 1985). This stride-related modulation of activity is substantially enhanced when locomotion requires accurate stepping, e.g. while negotiating barriers or walking along a horizontal ladder (Beloozerova and Sirota 1993a; Drew 1993; Widajewicz et al. 1994; Marple-Horvat and Armstrong 1999; Sirota et al. 2005; Beloozerova et al. 2010). While lesions to the motor cortex or its short-lasting inactivation do not disturb simple locomotion over flat surface, they have devastating effect on complex locomotion tasks involving accurate paw positioning (Trendelenburg 1911; Chambers and Liu 1957; Liddell and Phillips 1944; Beloozerova and Sirota 1988, 1993a; Drew et al. 1996). Thus, it appears that the enhancement of PTN activity during complex locomotion composes cortical commands for accurate foot placement. PTNs, however, exhibit diverse locomotion-related activity patterns, and the differences in their activity between simple and complex locomotion vary in magnitude, depth of modulation, duration, and, occasionally, preferred phase. The commands that PTNs transmit during locomotion are not uniform. Whether and how the different commands are channeled to spinal cord networks remains poorly understood. In a few previous studies, the activity of forelimb and hindlimb-related PTNs were compared and it was found that while some quantitative differences exist, qualitatively, commands sent by PTNs to forelimbs and hindlimbs are quite similar (Karayannidou et al. 2009, Widajewicz et al.

1994, Zelenin et al. 2011). In this study we hypothesized that those are different spinal targets within each girdle's neuronal network that receive different signals from the motor cortex during locomotion. Specifically, we hypothesized that spinal networks related to different segments of the limb receive different commands from the motor cortex.

Indeed, segments of the limb differ in mechanical characteristics, such as dimensions and weight, and differ in their role during movements. Whereas displacement of a proximal segment greatly affects the kinematics and kinetics of more distal segments, the influence of a distal segment movement on the mechanical characteristics of proximal segments is much smaller. Many observations suggest that during movements, different segments have different functions and are likely to be controlled in different manners. For example, during a reach and prehension task, motor cortex PTN postspike effects are both more numerous and more prominent on distal as compared to proximal muscles (McKiernan et al. 1998). It has long been known that lesions to the pyramidal tract in primates destruct fine movements of the fingers and wrist, while the disturbances to movements in the proximal segments are much less severe (e.g. Lawrence and Kuypers 1968). In contrast, a poor control over the shoulder joint appears to be one of signature deficits of cerebellar patients (Bastian et al. 2000). During locomotion, the angle of the hip is an important factor in determining initiation of the swing phase of the stride, while the positions of distal joints have no effect (Grillner and Rossignol 1978). In a recent study we found that when stepping has to be accurate during walking along a horizontal ladder, movements

in different joints adapt differently to the accuracy demands (Beloozerova et al. 2010). In a study of postnatal development of the forelimb representation in the motor cortex in the cat, Chakrabarty and Martin (2000) found that the motor map develops in a proximal-to-distal sequence, with shoulder and elbow controls developing earlier than wrist and digit controls. Developmental differences in the controls for different forelimb joints have been reported in humans as well (e.g. Konczak and Dichgans 1997). Different controls for different forelimb segments have also been suggested based on the results of biomechanical analyses. For example, Galloway and Koshland (2002) studied point-to-point whole arm movements in humans and found that movement dynamics differed greatly between the joints. Based on this and other biomechanics evidence, a "leading joint hypotheses" has been advanced (Dounskaia 2005), proposing that the joints of a limb play different roles in movement production according to their mechanical subordination in the joint linkage. It is not known, however, whether the motor cortex conveys differential controls to the spinal networks associated with different segments of a limb.

In this study, we addressed this question with regard to the forelimb. We took advantage of the fact that in the spinal cord most PTNs influence the same part of the limb that they receive somatosensory information from (Asanuma et al. 1968; Sakata and Miyamoto 1968; Rosen and Asanuma 1972; Murphy et al. 1975). Moreover, even though axons of individual PTNs from the forelimb representation of the motor cortex branch along cervical and thoracic segments of the spinal cord (Shinoda et al. 1986), physiological experiments have shown that

micro-stimulation in about half of sites within the forelimb motor cortex at 15 μA produce effects in only one or two muscles (Armstrong and Drew 1985a). Spike-triggered averaging of EMGs in primates showed that about half of PTNs influence motoneuron pools that innervate muscles on a single segment of the limb (Buys et al. 1986; McKiernan et al. 1998). Thus, using the correspondence between the locations of the source of afferent input and the target of motor output, we inferred which part of the limb a PTN influences based on its somatosensory receptive field. We recorded the activity of individual PTNs from the motor cortex in chronically instrumented cats. We selected only PTNs that receive somatosensory input from only shoulder, only elbow, only wrist, and asked whether these PTNs act differently during locomotion. We tested two locomotion tasks: simple locomotion over a flat surface, a task that does not require participation of the motor cortex, and a complex locomotion task over the crosspieces of a horizontal ladder, a task that requires the activity of the motor cortex to be successful (Trendelenburg 1911; Chambers and Liu 1957; Liddell and Phillips 1944; Beloozerova and Sirota 1988, 1993a; Drew et al. 1996). We found that PTNs receptive to different forelimb segments - and thus likely influencing those different segments - tended to discharge differently during locomotion of both types, and often adjusted their activity patterns between the two tasks in unique, stereotyped manners. We suggest that during locomotion the motor cortex, via subpopulations of PTNs with precisely targeted connections, uses distinct mechanisms to control the shoulder, elbow, and wrist.

A brief account of this study was published in abstract form (Stout and Beloozerova 2009).

2.3 METHODS

Recordings were obtained from 8 adult cats, 5 males and 3 females (Table 1). Some data on the activity of the motor cortex in several of these cats have been included in previous publications (Sirota et al. 2005, Beloozerova et al. 2010), however, the selection of neurons for this study is unique. Methods of data collection and spike train analysis have been described (Beloozerova and Sirota 1993a, Prilutsky et al. 2005, Beloozerova et al. 2010) and will be briefly reported below. All experiments were conducted in accordance with NIH guidelines and with the approval of the Barrow Neurological Institute Animal Care and Use Committee.

Locomotion tasks

Two locomotion tasks were used: 1) simple locomotion on a flat surface, and 2) complex locomotion over the crosspieces of a horizontal ladder (Fig. 1A). It has been demonstrated in several studies that simple locomotion does not require participation of the motor cortex, while complex locomotion does (Trendelenburg 1911; Chambers and Liu 1957; Liddell and Phillips 1944; Beloozerova and Sirota 1993a).

Positive reinforcement (food) was used to adapt cats to the experimental situation and to engage them in locomotion (Skinner 1938; Pryor 1975). A box

2.5 m long and 0.5 m wide served as the experimental chamber. A longitudinal wall divided the box into two corridors that cats passed through sequentially and repeatedly. In one of the corridors, the floor was flat, while the other corridor contained a horizontal ladder (Fig. 1A). The cross-pieces of the horizontal ladder were flat and 5 cm wide. The width of the cross-pieces was chosen to slightly exceed the cat's mean foot length (3 cm), so that cats had full foot support on the crosspieces. Crosspieces were spaced 25 cm apart, that is, at half of the mean stride length observed in the chamber during locomotion on flat floor at a self-selected pace (Beloozerova and Sirota 1993a; Beloozerova et al. 2010). After each round, food was dispensed into a feeding dish in one of the corners. Cats were trained, upon arrival, to stand in front of the feeding dish quietly on all four feet during a delay period of 4 sec. During data analysis, one second in the middle of this period was considered as "standing".

Cats were accustomed to wearing a cotton jacket, a light backpack with connectors, and an electro-mechanical sensor on the paw for recording of swing and stance phases of stride. The floor in the chamber and the crosspieces of the ladder were covered with an electro-conductive rubberized material. During locomotion the duration of the swing and stance phases of the forelimb contralateral to the side of recording in the motor cortex was monitored by measuring the electrical resistance between the electromechanical sensor and the floor (Sw/St trace in Fig. 3A) (e.g. Beloozerova and Sirota 1993a; Beloozerova et al. 2010).

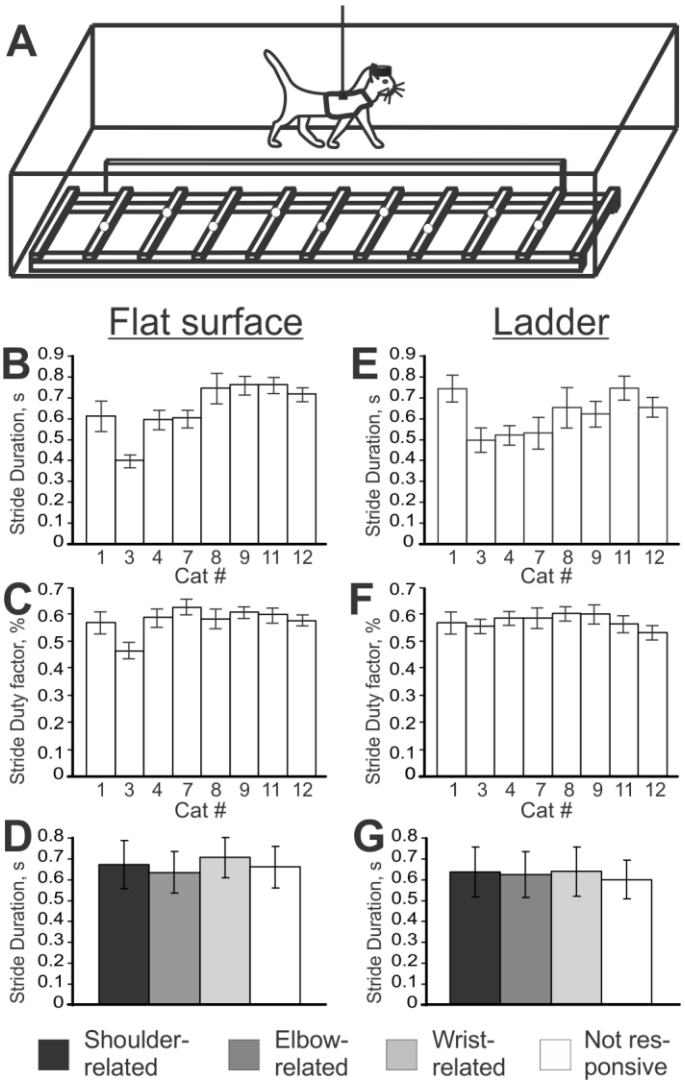

Fig. 1. Locomotion tasks. A: the experimental box was divided into 2 corridors. In 1 of the corridors the floor was flat, while the other corridor contained a horizontal ladder. White circles on the crosspieces of the ladders schematically show placements of cat forelimb paws. B and E: average durations of the step cycles in different cats during simple (B) and ladder (E) locomotion. C and F: average step duty factors (ratios of stance duration to cycle duration) in different cats during simple (C) and ladder (F) locomotion. D and G: average durations of the step cycles taken for the analysis of activity of different pyramidal tract neuron (PTN) groups during simple (D) and ladder (G) locomotion. In B–G vertical bars are SDs.

Surgical procedures

After cats were trained, surgery was performed under Isoflourane anesthesia using aseptic procedures. A portion of the skull and dura above the left motor cortex were removed. The area of the motor cortex was identified by the surface features and photographed (Fig. 2A). The aperture was then covered by a 1 mm thick acrylic plate. The plate was pre-perforated with holes of 0.36 mm in diameter spaced 0.5 mm, and wholes were filled with bone wax. 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; Figure 2B). These electrodes were used for identification of pyramidal tract neurons (PTNs) in the awake animal. A ring-shaped base was formed around all implants and a plastic cap was used to protect them.

Cell recording and identification

Experiments were initiated after several days of recovery. Extracellular recordings were obtained using conventional tungsten varnish-insulated microelectrodes (120 μm OD, Frederick Haer & Co) or platinum-tungsten quartz insulated microelectrodes (40 μm OD, Reitboeck 1983). The impedance of both types of electrodes was 1-3 MΩ at 1000 Hz. A custom made light-weight (2.5g) manual single-axis micro-manipulator chronically mounted to animal's skull was

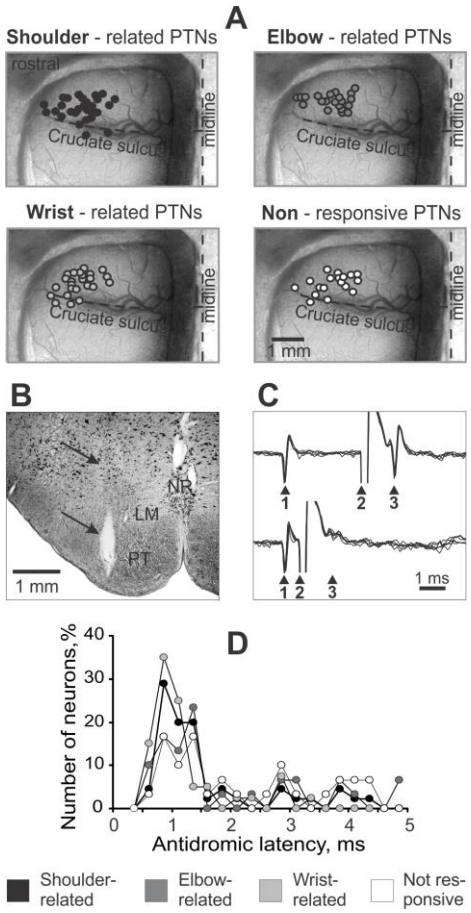

Fig. 2. Location of PTNs and their identification. A: area of recording in the forelimb representation of the left motor cortex. Microelectrode entry points into the cortex are combined from all cats and shown by circles on the photograph of cat 9 cortex. Tracks where PTNs with shoulder-related, elbow-related, and wrist-related receptive fields and nonresponsive PTNs were recorded are shown by black, dark gray, light gray, and white circles, respectively. B: reference electrolytic lesion in the left pyramidal tract made with the stimulation electrode in cat 8. Gliosis surrounding the electrode track and the reference lesion are indicated by arrows. The electrode was positioned approximately at the Horsley-Clarke rostro-caudal coordinate of P7.5. LM, lemniscus medialis; NR, nucleus raphes; PT, pyramidal tract. Frontal 50-um thick section, cresyl violet stain. C: collision test determines whether PTN response is antidromic. Top: the PTN spontaneously discharges (arrowhead 1), and the pyramidal tract is stimulated 3 ms later (arrowhead 2). The PTN responds with latency of 1 ms (arrowhead 3). Bottom: the PTN spontaneously discharges (arrowhead 1), and the pyramidal tract is stimulated 0.7 ms later (arrowhead 2). The PTN does not respond (arrowhead 3) because in 0.7 ms its spontaneous spike was still en route to the site of stimulation in the pyramidal tract, and thus collision/nullification of spontaneous and evoked spikes occurred. D: distribution of latencies of antidromic responses to stimulation of the pyramidal tract of PTNs of different groups. Shoulder-related, elbow-related, wrist-related, and nonresponsive PTNs are denoted by black, dark gray, light gray, and white circles, respectively.

used to advance the microelectrode. Signals from the microelectrode were

pre-amplified with a miniature custom made 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 hard- and software package (Power-1401/Spike-2 System, Cambridge Electronic Design, Cambridge, UK). An example recording from a pyramidal tract neuron during locomotion is shown in Figure 3.

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 in the medullary pyramidal tract. The criterion for identification of antidromic responses was the test for collision of spikes (Bishop et al. 1962; Fuller and Schlag 1976). It is illustrated in Figure 2C. Neurons were checked for antidromic activation before, during, and after testing during locomotion.

Receptive field classification

The somatic receptive fields of the PTNs were examined in the animals sitting on a comport pad with their head restrained. Stimulation was produced by palpation of muscle bellies, tendons, and by passive movements of joints. For any region found to consistently elicit action potentials, the extent of the receptive field was determined by listening to the audio monitor and determining the entire

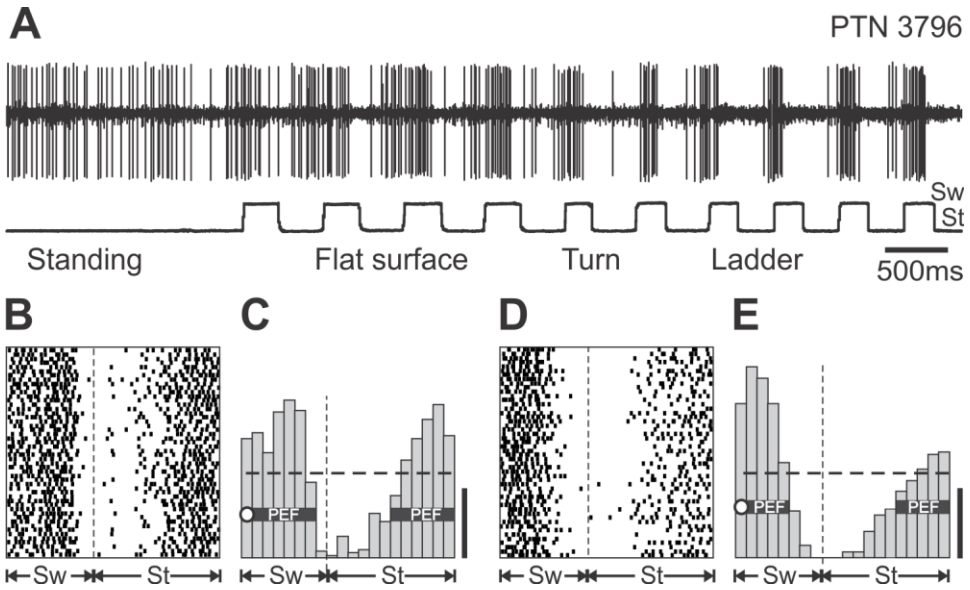

Fig. 3. Example of the typical activity of a PTN. A: activity of the PTN neuron during standing and simple and ladder locomotion. Bottom: swing (Sw) and stance (St) phases of the step cycle of the right forelimb that is contralateral to the recording site in the cortex. B and C: activity of the same neuron during simple locomotion presented as a raster of 50 step cycles (B) and as a histogram (C). In the raster, the duration of step cycles is normalized to 100%. In the histogram, the interrupted line shows the level of activity during standing. The horizontal black bar shows the period of elevated firing (PEF), and the circle indicates the preferred phase (see definition in METHODS). D and E: activity of the same neuron during ladder locomotion presented as a raster (D) and as a histogram (E). In C and E, the vertical scale bar equals 20 imp/s.

expanse that the cell was responsive to. PTNs responsive to passive movements of joints were assessed for directional preference. For this study, only neurons with the following somatosensory receptive fields were included in the analysis. (1) The shoulder-related group included PTNs responsive only to passive movements in the shoulder joint, and/or palpation of upper back, chest, or lower neck muscles. (2) The elbow-related group included PTNs responsive only to passive movements in the elbow joint and/or palpation of upper arm muscles. (3) The wrist-related group included PTNs responsive only to passive movements in the wrist joint, and/or palpation of the lower arm muscles, and/or to stimulation of the palm or back of the paw. (4) The non-responsive group included neighboring PTNs that showed no somatosensory responses. PTNs that had receptive field spanning more than one forelimb segment, for example those responsive to movements in both wrist and elbow joints, were not included in the analysis. Neurons responsive to movements of toes or claws were not included.

Processing of neuronal activity

From each run down a corridor, two or three strides made in the middle of the walkway were selected for the analysis. The onset of swing phase was taken as the beginning of step cycle. The duration of each step cycle was divided into 20 equal bins, and a phase histogram of spike activity of the neuron in the cycle was generated and averaged over all selected cycles. The Rayleigh test for directionality was used to determine whether the activity of a neuron was modulated in relation to the step cycle (Batshelet 1981; Fisher 1993). If the

activity of a neuron was judged to be step cycle-related, the “depth” of modulation, dM, was calculated using the histogram. It was defined as dM = (N_{max} - N_{min})/N x 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. In addition, the portion of the cycle in which the activity level exceeded 25% of the difference between the maximal and minimal frequencies in the histogram was defined as a "period of elevated firing” or “PEF" (as illustrated in Fig. 3 C,E). The "preferred phase" of discharge of each neuron with a single PEF was assessed using circular statistics (Batshelet 1981; Fisher 1993; see also Beloozerova et al. 2003a; Sirota et al. 2005).

To determine what natural fluctuations exist in the locomotion-related discharge of individual neurons, we performed a comparison of neuronal activity between randomly selected sets of steps from the same locomotion task. For 75 PTNs, at least two sets of 25-40 steps for each task were selected, and over one hundred comparisons were made. For each neuron, mean discharge frequency, dM, preferred phase, and duration of PEF were calculated for each set of steps and compared. For each parameter, a 95% confidence interval for the difference was determined. It was, respectively: ±20%, ±20%, ±10% of the step cycle, and ±10% of the step cycle. Thus, when comparing different tasks, changes within this interval were considered to be due to natural fluctuations in neuronal locomotion-related activity, while changes outside of this interval were considered, with 95% confidence, to be caused by differences in the locomotion tasks.

Parametric tests were used when possible to compare between groups. Unless noted otherwise, for all mean values, the standard error of the mean (SEM) is given. The discharge frequency and modulation of neurons during different tasks was compared using a paired samples *t*-test, and comparisons across different groups of neurons were assessed using ANOVA. When data were categorical, a nonparametric Chi-Square test was used.

Histological procedures

At the termination of experiments, cats were deeply anaesthetized with pentobarbital sodium. Several reference lesions were made in the region of the motor cortex from which neurons were sampled. Cats were then perfused with isotonic saline followed by a 3% formalin solution. Frozen brain sections of 50 μm thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet. The positions of recording tracks in the motor cortex were estimated in relation to the reference lesions. The position of stimulation electrodes in the medullar pyramids was verified (Fig. 2B).

2.4 RESULTS

Characteristics of locomotion tasks

Cats ran between 10 and 100 (typically 20-40) times down each of the chamber's corridors during the recording of each individual PTN. From these runs, 25–150 strides (70±30) taken in the middle of each corridor (during walking

on the flat surface or along the horizontal ladder) were selected for analysis. Four of the cats ran relatively quickly during simple locomotion (cats 1, 3, 4, and 7) and four were relatively slow (cat 8, 9, 11, and 12). Their average step durations were around 600 ms and 750 ms, respectively (Fig. 1B). This corresponded to a walking speed of 0.7-0.8 m/s. The ratio of the stance duration to the cycle duration (the stride duty factor) varied only slightly between cats, however (Fig. 1C), and was 0.59±0.05 (mean±SD) on average. Because cats contributed fairly equally to each of the databases on PTNs with different receptive fields (Table 1), the average duration of steps chosen for PTNs of different groups was very close ($p>0.05$, ANOVA, Fig. 1D), as was the duty factor ($p>0.05$, ANOVA).

When walking along the ladder, four cats walked with nearly same speed as on the flat surface, three were somewhat faster, and one was slower (Fig. 1E). The stride duty factor was 0.58±0.04 (mean±SD) on average, similar to simple locomotion, and was consistent across cats (Fig. 1F). Again, because cats contributed rather equally to the different PTN groups (Table 1), the average duration of steps included in the analyses of the activity of different groups was similar (Fig. 1G). The average duration of selected simple and ladder locomotion strides for shoulder-related, elbow-related, and non-responsive PTNs was similar (Fig. 1D,G). Strides selected for wrist-related PTNs were on average just slightly faster on the ladder than during simple locomotion.

The gait that cats used during locomotion both on flat surface and along the ladder was a walk with the support formula of 2-3-2-3-2-3-2-3, indicating the number of limbs supporting the body during different phases of the step cycle

(Hildebrand 1965). Details of the biomechanics and muscle activities of cats during walking on the flat surface and along the horizontal ladder in a similar experimental setup have been recently reported elsewhere (Beloozerova et al. 2010). Ladder locomotion is similar to simple locomotion in nearly all kinematic and EMG parameters; the few forelimb-related differences include a somewhat more bent-forward posture, a lower wrist flexion moment during stance, and a slightly enhanced activity of selected distal muscles during ladder locomotion.

Characteristics of neurons

The activity of 145 PTNs was included in the analysis. Of these, 45 responded exclusively to passive movements in the shoulder joint and/or palpation of upper back, chest, or lower neck muscles (Shoulder-related group, Table 1). Thirty PTNs responded exclusively to passive movements in the elbow joint or palpation of upper arm muscles (Elbow-related group, Table 1). Forty PTNs responded to passive movements in the wrist joint, palpation of the lower arm muscles, or to stimulation of the palm or back of the paw (Wrist-related group, Table 1). Finally, 30 PTNs had no receptive field (Non-responsive group, Table 1).

Of the 115 PTNs with receptive fields, most had some directional preference. Among shoulder-related PTNs, 33% (15/45) were preferentially receptive to flexion, while 20% (11/45) were preferentially receptive to extension. The remaining 43% (19/45) were receptive to abduction or adduction of the joint, or to palpation of the muscles on the back or chest. Among elbow-receptive

Table 1. PTNs recorded in different subjects.

Cat #	Gender	Mass, (kg)	Shoulder-related	Elbow-related	Wrist-related	Non res-ponsive	Total
1	male	3.9	7	4	1	4	**16**
3	female	3.0	2	1	1	2	**6**
4	male	3.8	3	5	2	6	**16**
7	female	2.7	13	5	10	6	**34**
8	male	4.5	8	2	9	4	**23**
9	male	3.9	5	8	5	4	**22**
11	female	3.7	5	1	8	4	**18**
12	male	4.0	2	4	4	0	**10**
Total 8			**45**	**30**	**40**	**30**	**145**

PTNs, 37% (11/30) were preferentially receptive to flexion, and 60% (18/30) were preferentially receptive to extension. Finally, among wrist-receptive PTNs, 42.5% (17/40) were receptive to ventral flexion of the wrist, while 32.5% (13/40) were receptive to its dorsal flexion. The remaining 25% (10/40) of the wrist-related PTNs were receptive to palpation of muscles on the forearm or paw, including two cells that additionally responded to cutaneous stimulation.

The vast majority of PTNs were recorded from the region of the motor cortex rostral to the cruciate sulcus. In Figure 2A, color-coded dots overlaying the cortex schematically show microelectrode entry point into the cortex for tracks, in which PTNs of different groups were recorded during locomotion. There was extensive overlap between PTN groups.

The latencies of antidromic responses of different PTNs to pyramidal tract stimulation varied in the range of 0.4-5.0 ms (Fig. 2D). Estimated conduction velocities were between 5 and 80 m/s. Approximately three fourths of neurons (107/145) responded at 2.0 ms or faster, conducting at 25 m/s or faster, and thus were "fast conducting" PTNs (Brookhart 1952, Bishop et al. 1953). In shoulder-, elbow-, wrist-related, and non-responsive PTN groups, the proportions of fast and slow conducting neurons were similar (Fig. 2D).

An example of typical activity of a PTN during standing, as well as simple and ladder locomotion is shown in Figure 3. This PTN was non-responsive to somatosensory stimulation. The PTN was steadily active during standing. Once locomotion began, the PTN's activity became modulated with respect to the step cycle. The neuron was highly active during most of the swing and second half of

the stance phase and less active during the end of the swing and the early stance phase. Upon transition from simple to ladder locomotion, the neuron's activity became even more strongly modulated. The neuron became even more active during the swing phase while its activity during the stance phase decreased. The rasters in Figure 3B,D show the activity of the neuron across 50 individual strides during simple (B) and ladder (D) locomotion. The pattern of activity was very consistent across strides of each locomotion task. The activity is summed in Figures 3C,E showing a histogram of PTN firing rate across the step cycle during simple (C) and ladder (E) locomotion. 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 simple and ladder locomotion, and was 15% of the cycle shorter during ladder locomotion. The preferred phase (indicated by a circle in Figures 3C and 3E) was in the very beginning of the swing phase during both locomotion tasks.

Activity during locomotion on the flat surface

While the cat was standing, all PTNs were active. The average discharge rate was 13.0±0.7 imp/s. The discharge rates of different PTN groups were similar (Fig. 4A). Upon transition from standing to walking, the average discharge rate of PTNs increased to 17.4±0.9 imp/s ($p<0.05$, t-test). Elbow-related PTNs were now, however, less active than either shoulder- or wrist-related PTNs ($p<0.05$, ANOVA; Fig. 4B).

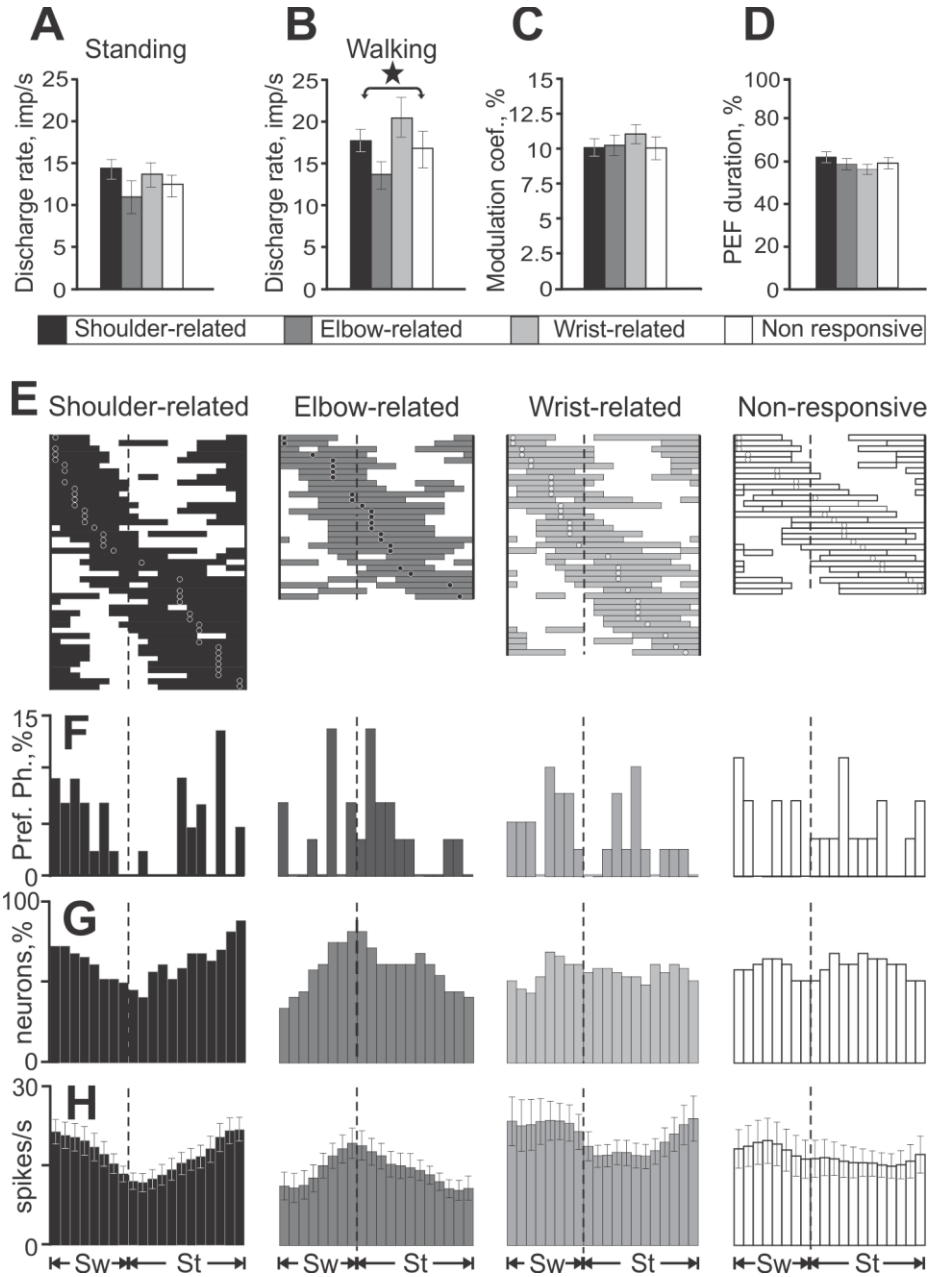

Fig. 4. Activity of PTNs with receptive fields involving different forelimb joints during simple locomotion. A: discharge rate during standing in different PTN groups. B: discharge rate during walking. C: depth of modulation. D: duration of the PEF. In A–D, error bars are SE and the star indicates significant differences in discharge rates during walking (P 0.05, ANOVA). E: distribution of PEFs of individual PTNs in the step cycle. Each trace represents PEF of 1 PTN. Circles indicate preferred phase of each neuron. Neurons are rank ordered so that those whose preferred phase is earlier in the cycle are plotted at top of graph. F: distribution of preferred phases of neurons across the step cycle. G: proportion of cells active during the step cycle. The traces from E were summed into a histogram and normalized. H: phase histogram of the average firing rate of PTNs across the step cycle. Error bars are SE. E–H: Sw, swing phase; St, stance phase.

During locomotion, the discharge of 97% (141/145) of PTNs was modulated with respect to the stride: it was greater in one phase of the stride and smaller in another phase. A period of higher activity was denoted as a period of elevated firing (PEF). Most PTNs (79%, 115/145) had one PEF, while 21% (29/141) had two PEFs per step cycle. The proportion of two-PEF cells was similar between groups of PTNs with different somatosensory receptive fields. The depth of modulation was also similar between the groups and was 10.2±0.4% on average (Fig. 4C; one-PEF and two-PEF neurons were considered jointly). The duration of the PEF was similar as well and lasted between 55 and 60% of the cycle on average (Fig. 4D). PEFs and preferred phases of individual PTNs of all groups were distributed across the step cycle. However, this distribution was uneven, and different between PTN groups. Shoulder-related PTNs were most often active during the late stance and early swing and elbow-related PTNs were most often active during the late swing and early stance, while the periods of elevated activity of both wrist-related and non-responsive neurons were distributed fairly equally throughout the step cycle (Fig. 4E,F,G). In accordance with the phase distribution of PEFs and preferred phases, the mean discharge rate of the shoulder-related group was highest during the stance-to-swing transition, at 21.8±2.0 imp/s, while the firing rate during the opposite phase was 13.4±1.4 imp/s ($p<0.05$, t-test; 8.4 imp/s difference) (Fig. 4H). The mean discharge rate of the elbow-related group was higher during the swing-to-stance transition period and was 17.4±2.4 imp/s and 10.6±2.1 imp/s during the stance-to-swing transition ($p<0.05$, t-test; 6.8 imp/s difference, Fig. 4H). In contrast, the average discharge

rate of wrist-related and non-responsive PTNs overall was around 20 and 17 imp/s, respectively, with only slight fluctuations (Fig. 4H).

Activity during locomotion on the ladder

The ladder added accuracy requirements to the locomotion task. The cat was forced to constrain its paw placement during locomotion to the raised crosspieces of the ladder. It has been shown that the activity of the motor cortex is required to successfully perform this task (Trendelenburg 1911; Liddell and Phillips 1944; Chambers and Liu 1957; Beloozerova and Sirota 1988, 1993a). All PTNs that were tested during walking on the flat surface were also tested during complex locomotion along the ladder. Upon transition from simple to ladder locomotion, high proportions of PTNs in all groups, 27-42% depending on the group, increased their discharge on average by 99±74%, while somewhat smaller proportions (15-40%) decreased it, on average by 43±16% (Fig. 5A). Thus, the average rate of discharge across all PTN groups during complex locomotion was slightly higher than during simple locomotion (19.1±1.0 *vs.* 17.4±0.9 imp/s, $p<0.05$, t-test). In addition, disproportional changes in the activity of different groups (relatively more neurons increased activity in shoulder and elbow-related groups, and more neurons decreased in the wrist-related group (Fig. 5A) led to more homogeneous discharge rates between groups during ladder as compared to simple locomotion (Figs. 7A and 4A). There were now no significant differences in the mean discharge rates of different groups of PTNs.

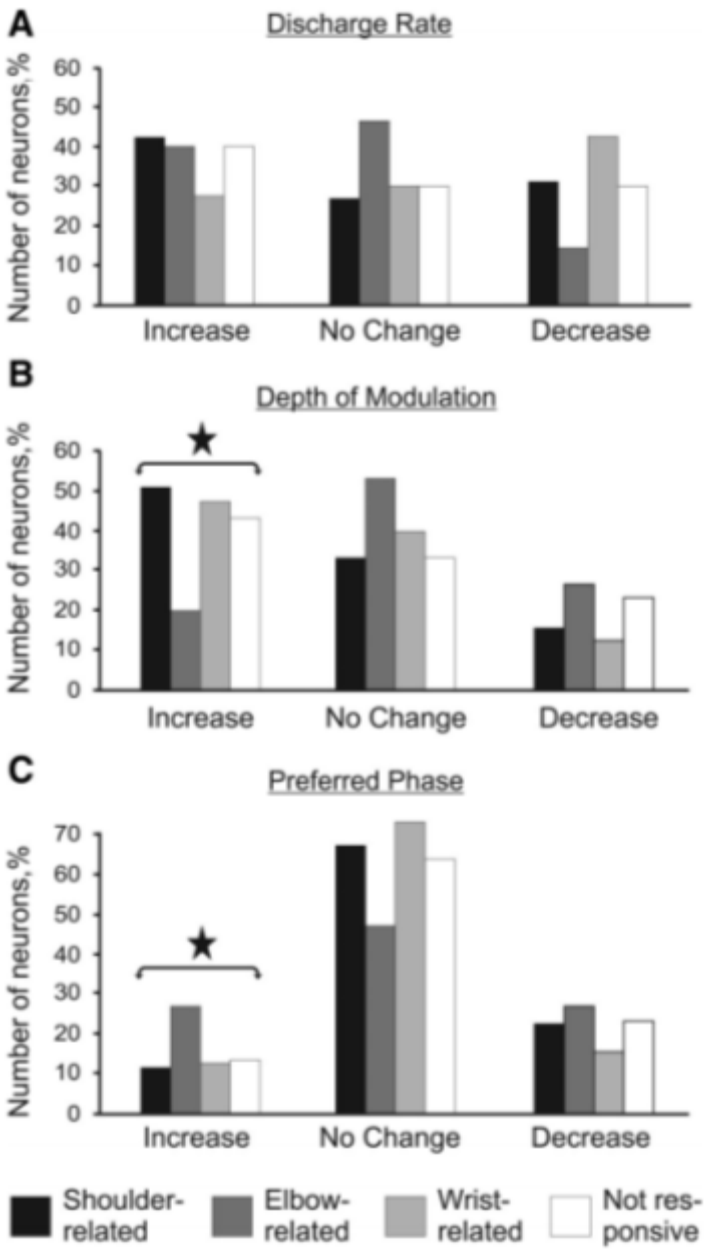

Fig. 5. Neuronal changes between simple and complex locomotion. Changes in mean discharge rate (A), depth of modulation (B), and preferred phase (C) of PTN populations observed upon transition from simple to ladder locomotion. Star indicates significant differences (P 0.05, ANOVA).

Substantial changes were also observed in the magnitude of frequency modulation (Fig. 5B). Half (51%) of shoulder-related PTNs and 40-45% of wrist-related and non-responsive cells showed increases in the depth of modulation on the ladder, on average by 62±44%). Decreases of modulation were also observed, but only half as frequently. Relatively less elbow-related PTNs changed the depth of modulation upon transition from simple to complex locomotion as compared to shoulder- and wrist-related groups (Fig. 5B). These trends caused groups of PTNs with different somatosensory receptive fields to produce activity with more heterogeneous modulation depth during ladder locomotion as compared to simple locomotion, with shoulder- and wrist-related PTNs having higher depths of modulation on average than elbow- and non-receptive neurons ($p<0.05$, ANOVA; Fig. 7B).

The observed increases in the depth of modulation upon transition from simple and complex locomotion could be achieved by a variety of changes to neuronal activity patterns: (a) an increase in firing rate during the PEF (additive increase in modulation), (b) a decrease in the firing rate during the inter-PEF interval (subtractive increase in modulation), or (c) by a combination of both mechanisms. Purely additive or subtractive mechanisms accounted for the vast majority of changes to the depth of modulation, and only ~15% of changes were achieved by both mechanisms. PTNs of different groups tended to exhibit different mechanisms (Fig. 6). Only shoulder-related PTNs would often use a purely additive mechanism to increase modulation depth (Fig. 6A), while the purely subtractive mechanism, although seen in shoulder-related and non-

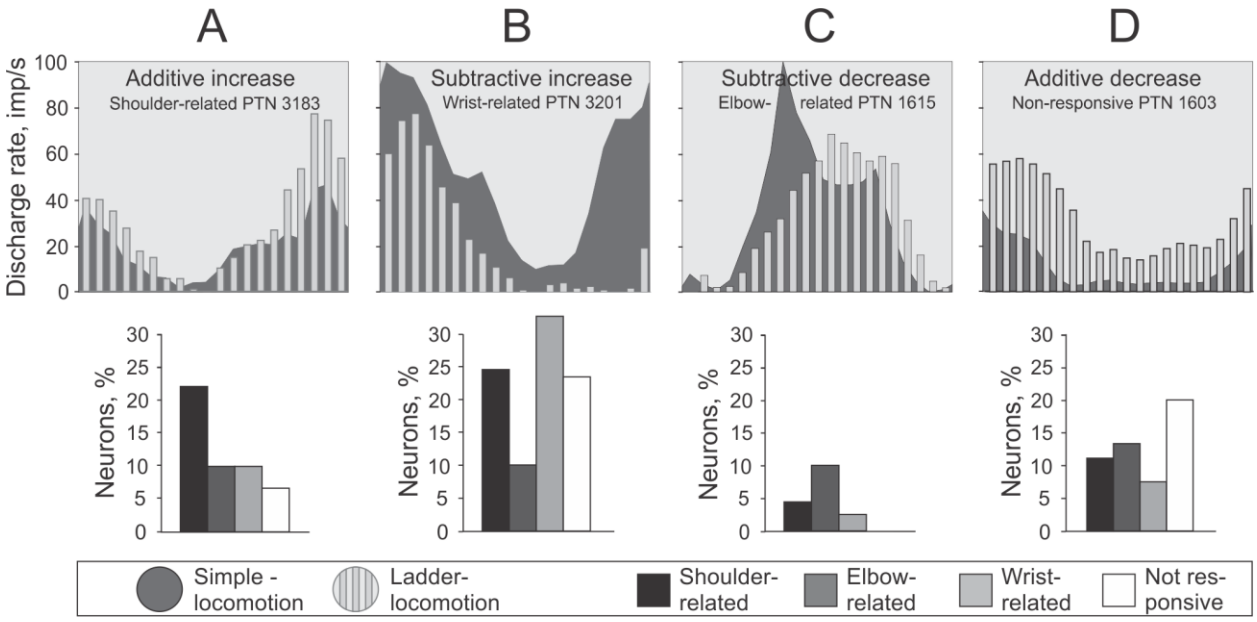

Fig. 6. Typical changes in the depth of modulation upon transition from simple to ladder locomotion. Area histograms show the activity of a typical PTN during simple locomotion. Bar histograms show activity of the same PTN during ladder locomotion. Bar graphs beneath the histograms show the proportion of neurons from each group exhibiting that type of modulation change. A: increase in depth of modulation by additive mechanism. B: increase in depth of modulation by subtractive mechanism. C: decrease in depth of modulation by subtractive mechanism. D: decrease in depth of modulation by additive mechanism

receptive PTNs, was most common for wrist-related PTNs (Fig. 6B). Additive modulation increase accounted for 33% (19/57) of all modulation increases, while subtractive modulation increase accounted for 54% (30/57).

Decreases in the depth of modulation were overall much less common. Elbow-related PTNs were the only group to decrease modulation in a purely subtractive manner in any significant numbers (Fig. 6C). However, additive decreases in modulation, achieved by a discharge rate increase during the inter-PEF interval, were comparatively common, and non-receptive PTNs most often exhibited this change to their discharge patterns (Fig. 6D). Overall, subtractive modulation decrease accounted for 21% (6/28) of all decreases in modulation, while the additive mechanism accounted for 64% (18/28).

In addition to the activity and depth of modulation changes, modifications to the duration of the PEF were also observed upon transition from simple to complex locomotion. About one third (31%) of shoulder-related PTNs and 33% of wrist-related PTNs decreased the duration of their PEF, on average by 43±9% and 36±9% (SDs), respectively. In contrast, elbow-related and non-responsive PTNs tended not to change the duration of their PEF. As a result, during ladder locomotion, shoulder- and wrist-related PTNs had average PEF durations of 55±2% and 51±3% of the cycle, respectively, shorter than the averaged PEF duration of elbow-related PTNs, which was 63±2% of the step cycle (t-test, $p<0.05$).

The preferred phases of most PTNs were similar during simple and complex locomotion, with the exception of elbow-related PTN group (Fig. 5C).

Only 11 to 23% of shoulder-related, wrist-related, and unresponsive PTNs had preferred phase either earlier or later in the cycle during ladder locomotion as compared to simple walking. The preferred phases moved from the stance to swing phase slightly more often than from swing to stance. In contrast, in the elbow-related PTN group the preferred phases of a half of neurons were different between the tasks.

Despite some changes in the preferred phases in a number of individual PTNs, the phasing preferences of PTN groups were largely similar during both tasks (compare Fig. 7D-G for ladder locomotion and Fig. 4E-H for simple locomotion). The strength of the phasing preference of shoulder-related PTNs remained unchanged: their mean discharge rate during stance-to-swing transition slightly rose to 24.4 ± 2.9 imp/s, however, the activity during the opposite phase also rose, reaching 16.1 ± 2.4 imp/s (Figs. 7G and 4H). Elbow-related PTNs still had a tendency to discharge more intensively during swing-to-stance transition, and the activity of non-responsive PTNs was still distributed evenly throughout the cycle. In stark contrast to those groups, wrist-related PTNs developed a strong phase preference. Although during simple locomotion this group showed a slight tendency (not statistically significant) to discharge more intensively during swing, during ladder locomotion this preference became pronounced. The discharge during swing was now slightly higher and, in addition, the discharge rate during stance substantially decreased (to 12.5±1.7 imp/s *vs.* 17.5±2.1 imp/s during simple locomotion; t-test, $p<0.05$). So, the difference in the discharge rate

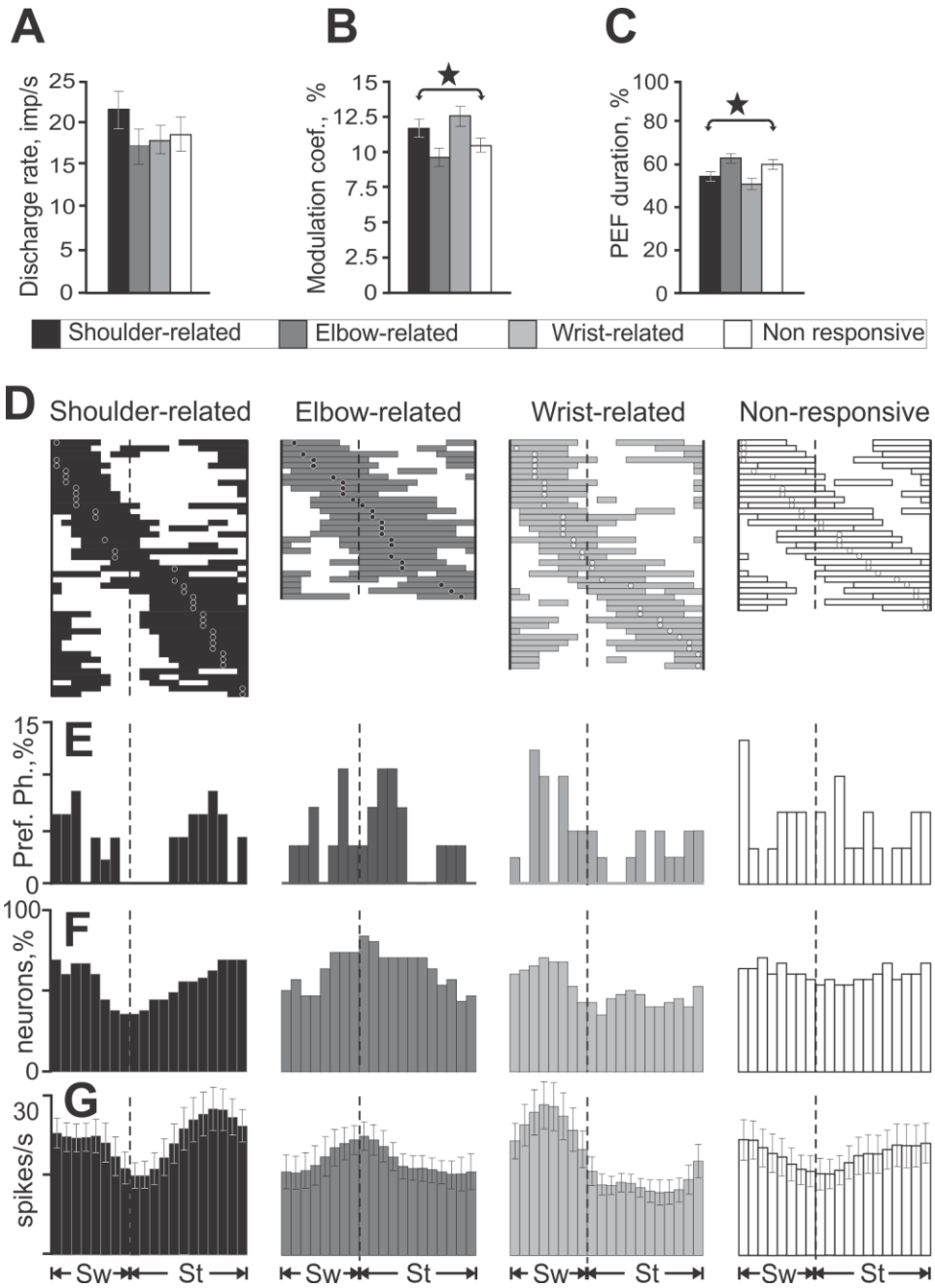

Fig. 7. Activity of PTNs with receptive fields involving different forelimb joints during ladder locomotion. A: discharge rate during walking. B: depth of modulation. C: duration of the PEF. In A–C, error bars are SE and stars indicate significant differences in values (P 0.05, ANOVA). D: distribution of PEFs of individual PTNs in the step cycle. Each trace represents PEF of 1 PTN. Neurons are rank ordered so that those whose preferred phase is earlier in the cycle are plotted at top of graph. E: distribution of preferred phases of neurons across the step cycle. F: proportion of cells active during the step cycle. G: phase histogram of the average firing rate of PTNs across the step cycle. E–G: Sw, swing phase; St, stance phase.

between swing and stance of the wrist-related PTNs was 14.6 imp/s during ladder locomotion.

To summarize, upon transition from simple to complex locomotion, different PTN groups changed their activity in distinct manners. Shoulder-related PTNs often increased their activity and depth of modulation while reducing their discharge duration, and typically did not alter their preferred phase. As a group, they became slightly more active during stance-to-swing transition. Wrist-related PTNs often decreased their activity, increased depth of modulation while also reducing discharge duration, and typically did not change their preferred phase. As a group, they became more active during swing phase. Elbow-related PTNs most often did not change their activity, depth of modulation, or discharge duration but relatively often changed their preferred phase. Their group activity was distributed more evenly throughout the cycle during complex locomotion. Non-responsive PTNs had mixed responses and had no preferred phase as a population.

Comparison of the activity of PTNs responsive to flexion or extension of the same joint

When we separated PTNs into groups that responded preferentially to either flexion or extension, we found that many of these groups exhibited distinct activity during simple locomotion (Fig. 8A, light colored bars). Wrist-related PTNs responsive to wrist dorsal (n=13) or ventral (n=17) flexion were dissimilar in all characteristics. PTNs responsive to the wrist ventral flexion were

substantially more active than their counterparts. However, PTNs responsive to the wrist dorsal flexion were more strongly modulated and their PEFs were shorter. Elbow-related PTNs that were responsive to extension (n=18) had longer PEFs, but were otherwise similar to elbow flexion-related PTNs (n=11). Only shoulder-related PTNs that were responsive to flexion (n=11) and extension (n=15) were similar in all characteristics tested.

Many PTNs changed their discharge characteristics upon transition from simple to ladder locomotion (Figs. 5-7). PTNs responsive to flexion or extension of the same joint often altered activity in distinct manners (Fig. 8B). Shoulder-related PTNs that were responsive to extension of the shoulder changed both their average discharge rate and the depth of step-related modulation, and had a tendency to have a shorter PEF in comparison with simple locomotion. Their counterparts (those responsive to shoulder flexion) discharged similarly in both tasks. Elbow extension-related PTNs substantially increased their average activity while elbow-flexion related PTNs did not; in contrast, only elbow flexion-related cells increased their average PEF duration. Wrist-related PTNs that were responsive to ventral flexion of the wrist decreased their average discharge rate and increased depth of step-related modulation in comparison to simple locomotion while their counterparts showed no significant changes.

As a result, during complex locomotion there were fewer differences to the activity of PTNs responsive to flexion or extension of the same joint as compared to simple locomotion (Fig. 8A, heavy colored bars). Wrist dorsal and ventral flexion-receptive PTNs became similar in all parameters, and the average

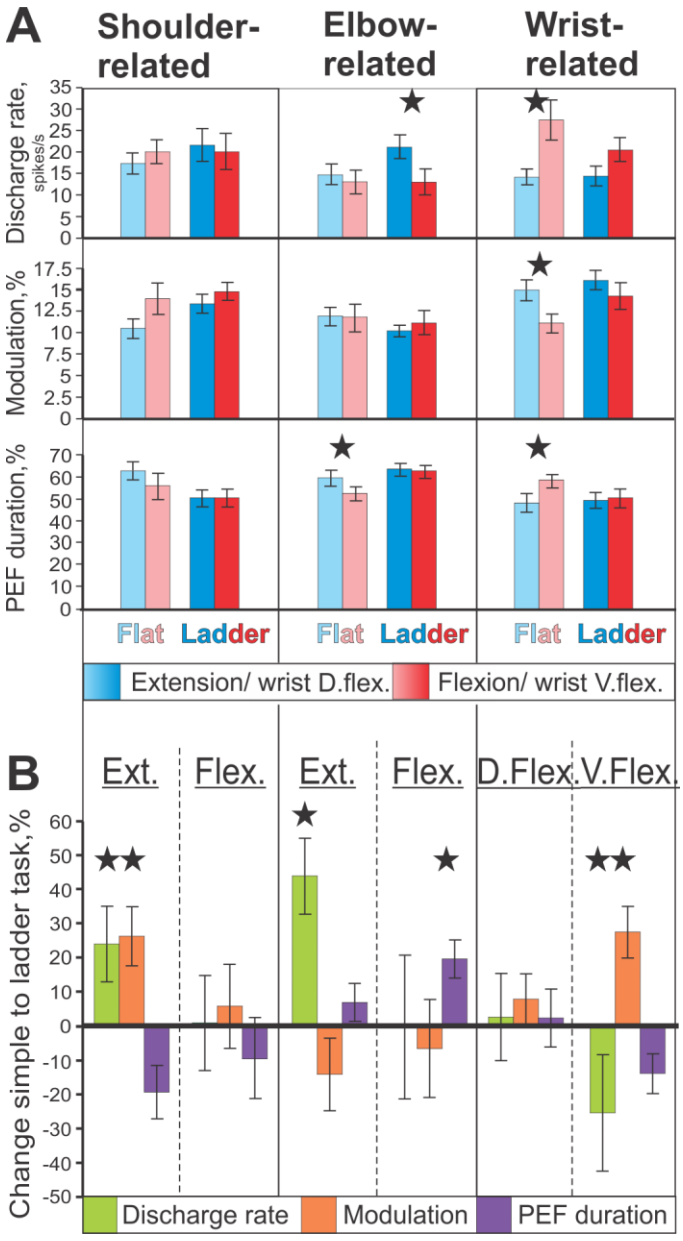

Fig. 8. Comparison of activities of flexion- and extension-receptive PTNs. A: discharge rate, modulation depth, and PEF duration are compared for flexion-extension pairs of each PTN group during simple (Flat) and complex (Ladder) locomotion. Extension-receptive cells, including wrist dorsal flexion related, are colored a lighter color. B: % change in activity parameters for each group. A and B: significant changes are denoted with a star (t-test, P 0.05); error bars are SE.

duration of PEFs in the groups of elbow-receptive PTNs became similar. Although elbow extension-receptive PTNs became more active than elbow-flexion PTNs, this was the only observed group difference between any flexion and extension-receptive pairing during complex locomotion. Shoulder-receptive PTNs remained similar in all parameters tested.

Relation of the activity phasing and kinematics

During locomotion, each joint undergoes repeating phases of flexion and extension throughout the step cycle. We tested whether PTNs that respond to the movement of a joint in a single direction at rest would discharge in-phase with that joint movement during locomotion or out of phase.

Figure 9A shows the distribution of PEFs of those neurons that were responsive exclusively to flexion of shoulder (left panel), elbow (middle panel), or ventral flexion of wrist (right panel). Angle movements of these joints, modeled after Prilutsky and colleagues (2005), are shown in Figure 9C. We found that shoulder flexion-responsive PTNs typically discharged in-phase with flexion of the shoulder in both locomotion tasks (Figs 9B). However, elbow-related PTNs most often discharged out of phase, and wrist ventral flexion-related PTNs had no preference (Figs 9B). The same analysis applied to extension-receptive PTNs showed that shoulder and wrist-related PTNs had no preference to discharge in or out of phase with their respective joint extension, while elbow-related PTNs preferred to discharge out of phase (Figs. 9D-F).

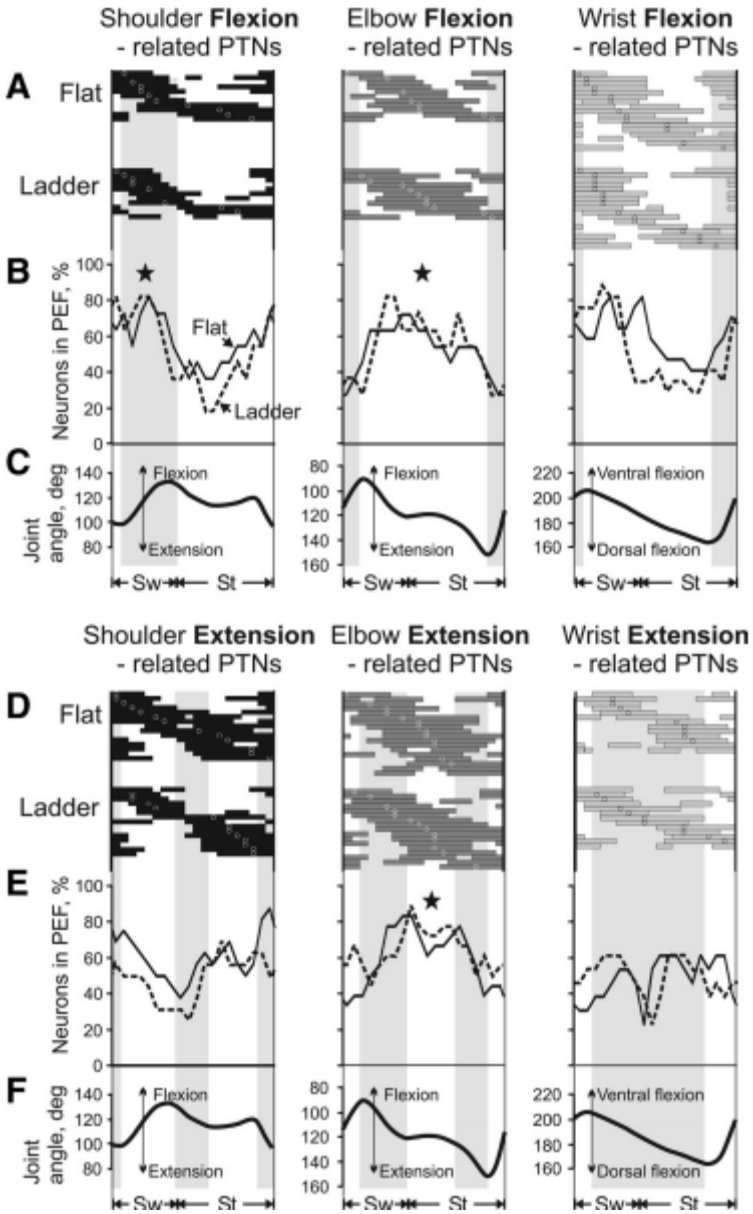

Fig. 9. Proportions of PTNs firing in phase with activation of their receptive field during locomotion. A and D: distribution of PEFs of individual flexion-related (A) and extension-related (D) PTNs in the step cycle. Each trace represents the PEF of 1 PTN. PEFs during simple (Flat) and ladder locomotion (Ladder) are individually rank ordered. B and E: proportion of flexion-related (B) and extension-related (E) PTNs active during the step cycle during simple (solid line) and ladder (dashed line) locomotion. C and F: movements in forelimb joints during the step cycle (Prilutsky et al. 2005). In A–C, periods of the step cycle when the joint flexes are highlighted in gray. In D–F, periods when the joint extends are highlighted in grey. Sw, swing phase; St, stance phase. Stars indicate significant difference between the average number of PTNs that were in their PEF when the associated joint movement was occurring (in-phase firing) and when it was not (out-of-phase firing) (t-test, P 0.05)

2.5 DISCUSSION

The main finding of this study is that PTNs responsive to stimulation of different forelimb segments have different activity characteristics during locomotion, both simple and complex. While it might be tempting to suggest that these differences are due to differences in the PTNs somatosensory receptive field characteristics, in fact, somatosensory information seems not to play a leading role in determining the locomotion-related discharges of most PTNs during either simple or complex locomotion. Indeed, neurons with similar receptive fields often discharge during quite different times of locomotion cycle (Fig. 9, Armstrong and Drew 1984b). It has been shown that the locomotion-related responses of motor cortical neurons are only slightly affected by changes in the vigour of movements during up and down slope walking, weight bearing, or alterations in speed, (Armstrong and Drew 1984a; Beloozerova and Sirota 1993b) - changes that most certainly cause significant changes to proprioceptive afferentation. In regard to cutaneous input, Armstrong and Drew (1984b) have demonstrated that in motor cortex neurons with cutaneous receptive fields, including on the forefoot, the discharges during locomotion remained rhythmic and their phasing relative to the step cycle was unchanged when the response to mechanical stimulation in the receptive field was temporarily much reduced or abolished by local anesthesia of the skin. In the present study we found that the great majority of PTNs with *direction*-specific receptive fields did not show any particular preference to discharge in phase with stimulation of their receptive field during locomotion, and elbow-related PTNs even preferred to discharge out of phase (Fig. 9). Similarly

poor relationships between phasing of task-related discharges and directional specificity of PTN resting receptive fields were reported in previous studies from this and other laboratories (Armstrong and Drew 1984b; Drew 1993; Beloozerova et al. 2003, 2005, Karayannidou et al. 2008). While it is true that somatosensory receptive fields during active movements may be somewhat different from those observed at rest (Ghez and Pisa 1972, Chapman et al. 1988), the group of above observations suggests that some factor other than stimulation of somatosensory receptive field drives PTN discharges during locomotion. In fact, in decerebrated cats neurons of both reticulospinal and rubrospinal tracts display locomotion-related modulation of their activity even during fictive locomotion when the subject is motionless and thus no rhythmic afferentation is present (Arshavsky et al. 1988, Perret 1976), suggesting that the spinal cord locomotion central pattern generator (CPG) plays a significant role in modulating their discharges. It is quite likely that during simple locomotion the activity of PTNs of the motor cortex also, rather than being driven by stimulation of somatosensory receptive fields, is significantly influenced by signals from the spinal locomotion CPG. If so, then the influence appears to be somewhat different for PTNs associated with different segments of the forelimb (Fig. 4), as we found that PTNs with receptive fields involving different joints – PTNs with receptive fields in different *locations* on the limb – tend to discharge differently during simple locomotion. Shoulder-related PTNs are most active during the late stance and early swing, elbow-related PTNs are most active during the late swing and early stance, and the activity of wrist-related PTNs is roughly even throughout the step cycle.

The motor cortex does not appear, however, to exert decisive control over simple locomotion, because a lesion or even short reversible inactivation of it has no effect on performance (Trendelenburg 1911; Chambers and Liu 1957; Liddell and Phillips 1944; Beloozerova and Sirota 1988, 1993a; Drew et al. 1996). We have previously suggested that the stride-related modulation of activity that the motor cortex exhibits during simple locomotion has an informational character, allowing the motor cortex to influence the spinal locomotor mechanism during correction of movements without disturbing the overall stepping rhythm (Beloozerova and Sirota 1993a).

Locomotion on the ladder adds accuracy constraints to the locomotion task, as cats are required to step precisely on the crosspieces. It was previously demonstrated that this task requires the activity of the motor cortex to be successful (Trendelenburg 1911; Chambers and Liu 1957; Liddell and Phillips 1944; Beloozerova and Sirota 1988, 1993a; Drew et al. 1996). On the ladder, most PTNs changed their activity as compared to simple locomotion (Figs. 5-7). Again, this change does not appear to be caused by a difference in somatosensory afferentation between the two tasks. Indeed, we have shown that mechanical parameters of simple and ladder locomotion differ only very slightly, making it likely that only small dissimilarities exist in the afferent signals that arrive to the motor cortex during these two tasks (Beloozerova et al. 2010). For the forelimbs, we found that on the ladder as compared to simple locomotion cats only rotate their neck down, increase flexion in the metacarpophalangeal joint, and reduce the wrist flexion moment during stance. Other mechanical variables, out of over one

hundred tested, are similar during two tasks. Based on this evidence, we feel that the small differences in joint kinematics are insufficient to cause the very pronounced differences observed in neuronal discharges. On the other hand, we found that 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, 2010, 2011) and that ladder locomotion is not possible in complete darkness (Beloozerova and Sirota 2003). Considering rather similar motor patterns in the two locomotion tasks but dramatically different gaze behaviors and the need for vision, we have previously suggested that during locomotion on the ladder, which requires visual guidance of stepping, motor cortex PTNs transmit processed visual information by modulating their simple locomotion-related discharges (Beloozerova et al. 2010). These integrated visuo-motor signals appear to control accurate placing of feet on crosspieces of the ladder. The purpose of the present study was to investigate whether and how these PTN signals vary depending on the part of the forelimb they control.

In this study we took an advantage of the fact that in small loci in the forelimb representation of the motor cortex, a relationship exists between afferent input and motor output. This relationship makes it is possible to infer the forelimb joint that an individual PTN influences from the somatosensory receptive field that it has. Indeed, although axons of individual PTN from the forelimb representation of the motor cortex give off several branches along cervical and thoracic segments of the spinal cord most often synapsing upon interneurons of laminae IV-VII (Chamber and Liu 1957; Shinoda et al. 1986), and there is a rich

spinal interneuron network that mediates signals from PTNs to motoneurons, earlier reports have shown that micro-stimulation in the forelimb region of the motor cortex typically produces contraction in single muscles or in small groups of muscles in the area that composes the receptive field at the stimulation site (Armstrong and Drew 1985a, Asanuma et al. 1968; Sakata and Miyamoto 1968; Rosen and Asanuma 1972; Murphy et al. 1975) and affects the monosynaptic reflexes of only one or two muscles (Asanuma and Sakata 1967). Even when series of pulses of 20 μA were used in locomoting subjects, micro-stimulation of a quarter of sites within forelimb motor cortex still affected only one or two muscles (Fig. 3 in Armstrong and Drew 1985b). Experiments that used spike-triggered averaging of EMGs in primates showed that although many PTNs excite several motoneuron pools including those related to muscles on two different segments of the limb or occasionally even across the entire forelimb, about half of PTNs influence motoneuron pools that only innervate muscles on one segment of the limb (Buys et al. 1986; McKiernan et al. 1998).

For this study we selected only PTNs with a receptive field constrained to a single forelimb segment, and we found that these PTNs - PTNs with receptive fields in different localized *locations* - tend to discharge differently during complex locomotion. Based on the information above, and also taking into account limitations of those experiments, which have been carefully reviewed by Schieber (2001), we believe that our main result can be restated as: PTNs assumedly influencing different joints of the forelimb have different activity

characteristics during visually guided locomotion. These PTNs exert influence in distinct manners, and therefore have different roles in control of locomotion.

On the ladder, most PTNs changed their activity as compared to simple locomotion; however, each group changed it in a specific and unique way (Figs. 5-7). Shoulder-related PTNs often increased their discharge rate and depth of modulation while reducing discharge duration. They typically did not change their preferred phase, but as a group became more active at the end of stance. Such activity modifications are consistent with the hypothesis that during precise stepping shoulder-related PTNs have a significant role in planning of limb transfer, which is hypothesized to occur at the end of stance phase (Laurent and Thomson 1988; Hollands and Marple-Horvat 1996), as well as in the initial phases of limb transfer when adjustment of the foot trajectory is still possible (Reynolds and Day 2005; Marigold et al. 2006). Also, during the second half of stance, accurate paw placement of the opposing limb is taking place, and precise posture maintenance from the supporting limb is important to maintain balance. This could be another reason that shoulder-related PTNs, specifically those related to shoulder extension, increase their activity and modulation during stance (Figs. 5, 8).

Wrist-related PTN activity was fairly evenly distributed throughout the cycle during simple locomotion, but during complex locomotion, wrist-related PTNs became strongly modulated as a group, exhibiting a prominent activity peak during swing (Fig. 7G). In contrast to shoulder-related PTNs, individual wrist-related PTNs often decreased discharge rate while also increasing depth of

modulation and reducing their discharge duration. Such activity modifications are consistent with the hypothesis that wrist-related PTNs, specifically those related to the wrist plantar (ventral) flexion, are involved in distal limb transfer during challenging tasks. This view is further supported by the fact that wrist ventral flexion-related PTNs increased their depth of modulation more than wrist dorsal flexion-related PTNs (Fig. 8), and indeed in a previous study we found that during locomotion on the ladder, wrist is more flexed in the plantar (ventral) direction as compared to simple locomotion (Beloozerova et al. 2010).

Although both shoulder- and wrist-related PTNs often increased modulation during complex locomotion as compared to simple walking, they generally did so using different mechanisms (Fig. 6). Shoulder-related PTNs commonly achieved an increase in modulation by increasing their peak discharge rate. This would result in a more intensive signal to the spinal network, often along with a more specific timing of the discharge. Wrist-related PTNs, achieved increases in the modulation almost exclusively by decreasing the firing outside of PEF, increasing the salience of the signal without making it more intense. This modification could specifically improve the temporal precision of the controls for limb transfer during a precision stepping task.

In contrast to shoulder and wrist-related PTN, elbow-related PTNs did not often change activity, modulation depth, or discharge duration upon transition from simple to complex locomotion, but often changed their preferred phase. Their group activity became evenly distributed throughout the cycle during complex locomotion (Fig. 7G). Their generally elevated activity might improve

overall limb control during locomotion tasks that require accurate foot placement. Non-receptive PTNs showed no changes to discharge rate, modulation depth, discharge duration, or preferred phase between tasks. The functions of these cells as well as their spinal targets remain to be determined. The diversity of responses between different PTN groups suggests that each group exerts influence within a different domain of the movement control.

An effective way for PTNs to differentially influence different segments of the forelimb during locomotion would be to individually influence the respective locomotion pattern formation networks in the spinal cord (McCrea and Rybak, 2008), modulating the amplitude and potentially the timing of their output. Indeed, Asante and Martin (2010) recently found that in the mouse that spinal projections from shoulder-, elbow-, and wrist-related areas in the motor cortex primarily contact those spinal premotor circuits, which connect to shoulder-, elbow-, and wrist-related motoneuron pools, respectively. Based on results of experiments with micro-stimulation in the motor cortex, analogous mechanisms for control of limb segments have been previously suggested by Drew (1991) for the forelimb and by Bretzner and Drew (2005) for the hind limb of the cat. However, these authors now stress the likelihood that the motor cortex controls locomotion movements based on muscles synergies that appear to be formed during stepping (Krouchev et al. 2006; Drew et al., 2008). While the concept of synergies is indeed very helpful for understanding the organization and neuronal control of movements (e.g., rev. in Bizzi et al. 2008 and Latash 2008) it does not exclude a possibility that, within the entire limb or even the entire body locomotor

synergy, individual elements of the synergetic network may receive individual commands when conditions of the task warrant it. The inability of the cat to continue on the ladder for even a single step after lights are taken off (Beloozerova and Sirota 2003), and the persistent visual sampling of every single crosspiece of the ladder on every run (Rivers et al. 2009, 2010, 2011) strongly suggest that, despite significant training, our cats did not establish a "ladder locomotion" synergy, but controlled foot landing on each crosspiece, step-by-step. Our data suggest that within the basic locomotion synergy, spinal mechanisms related to different segments of the forelimb receive different commands from the motor cortex during ladder locomotion.

Although the neuronal mechanisms underlying the differences in motor cortex controls for different forelimb segments have been never directly studied, there exists evidence suggesting that the mechanisms for their controls during tasks other than locomotion might be different. For example, it has been found that nearly all neurons in the shoulder/elbow area of the motor cortex modulate their activity during reaching in accordance with the posture of the arm (Scott and Kalaska 1997), while the activity of only a fraction of neurons in the hand area is wrist posture-related (Kakei et al. 2003).

While in our study the inference about the area of the forelimb that is controlled by individual PTNs may be imprecise, the data nevertheless suggest that there is likely to be a significant distinction in the commands that are sent from the motor cortex to different segments of the forelimb during complex locomotion.

CHAPTER 3

DIFFERENTIAL RESPONSES OF FAST- AND SLOW-CONDUCTING PYRAMIDAL TRACT NEURONS TO CHANGES IN ACCURACY DEMANDS DURING LOCOMOTION

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3.1 ABSTRACT

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

accurate stepping on the ladder slow-conducting PTNs more profoundly increase the magnitude of their stride-related frequency modulation compared with fast-conducting PTNs. We suggest that slow-conducting PTNs are involved in control of accuracy of locomotor movements to a greater degree than fast-conducting PTNs.

3.2 INTRODUCTION

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

Locomotion is one of the most essential and common motor behaviors. Locomotion often requires precise stepping, as humans and animals have to navigate through complex natural environments filled with obstacles and variable support surfaces. It was shown that lesions to the motor cortex or even its short lasting inactivation deprive subjects of the ability to step accurately (Trendelenburg, 1911; Liddell & Phillips, 1944; Chambers & Liu, 1957; Beloozerova & Sirota, 1988, 1993a; Drew et al. 1996; Metz & Whishaw, 2002;

Friel et al., 2007). It was also shown that when stepping has to be accurate during negotiation of obstacles or walking on crosspieces of a horizontal ladder, the activity of neurons in the motor cortex differs dramatically from that during simple locomotion over flat terrain (Beloozerova & Sirota, 1993a; Drew, 1993; Marple-Horvat et al. 1993; Widajewicz et al. 1994; Sirota et al. 2005). Moreover, we recently found that, as accuracy demand during stepping progressively increases, 30% of neurons in the motor cortex progressively refine their discharge timing, producing activity more precisely in specific phases of the stride (Beloozerova et al. 2010). Thus, it appears that during accurate stepping the discharges of neurons in the motor cortex contain cortical commands for accurate foot placement.

The motor cortex is connected to the spinal cord via pyramidal tract neurons (PTNs), large pyramid shaped cells located in the layer V of the cortex. In the spinal cord PTNs synapse mostly on interneurons (Lloyd, 1941; Hoff & Hoff, 1934; Dyachkova et al. 1971; Antal, 1984; Lacroix et al. 2004; Rosenzweig et al. 2009). Based on their axonal conduction velocity, PTNs can be subdivided into two distinct groups: "fast" PTNs, conducting with velocities of 21-80+ m/s, and "slow" PTNs, conducting with velocities below 21 m/s (Lassek & Rasmussen, 1940; Brookhart & Morris, 1948, Bishop et al. 1953; Takahashi, 1965). Fast-conducting PTNs have larger somas but account for only 10-20% of the PTN population, while slow-conducting neurons represent the smaller-bodied majority of PTNs (Calvin & Sypert, 1976; Humphrey & Corrie, 1978). In addition to axonal conduction velocities, a number of other biophysical properties such as

the duration of the spike, membrane resistance, amplitude of after-hyperpolarization, and others distinguish fast- and slow-conducting PTNs (Takahashi, 1965; Baranyi et al. 1993). Fast- and slow-conducting PTNs are also distinct in the manner by which they contact neurons within the cortex and subcortically (e.g. Towe et al. 1968; Takahashi, 1965; Ghosh & Porter, 1988; Lemon et al. 1993; Canedo, 1997). For example, in the spinal cord, fast-conducting PTNs preferentially influence distal muscle-related networks, while slow-conducting PTNs influence both proximal and distal muscle-related networks (Brookhart, 1952; Wiesendanger, 1981; Canedo, 1997). The activity of fast- and slow-conducting PTNs was compared in primates during movements of the forelimb (Evarts, 1965; Fromm & Evarts, 1977, 1981; Fromm et al. 1984). It was found that slow-conducting PTN are more readily activated by small movements, whereas many of fast- conducting PTNs only engage during large movements. Based on this observation and considering the nature of axonal projections of slow-conducting PTN, Fromm and Evarts (1977) suggested that, slow-conducting PTN may have a special role in control of accuracy of movements. No experiments so far, however, were actually designed to provide direct data on whether fast- and slow-conducting PTNs transmit differing cortical commands regarding accuracy during movements. It remains unclear whether the efficient activation of slow-conducting PTNs during small movements is truly due to the accuracy requirements of small tasks, or merely due to a low activation threshold for these PTNs.

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

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

3.3 METHODS

Recordings were obtained from 8 adult cats, 5 males and 3 females (Table 1). Some data on the activity of the motor cortex in several of these cats have been included in previous publications (Sirota et al. 2005, Beloozerova et al.

2010; Stout & Beloozerova, 2012), however, the selection of neurons for this study is unique. Methods of data collection and spike trains analysis have been described (Beloozerova & Sirota, 1993a, Prilutsky et al. 2005, Beloozerova et al. 2010) and will be briefly reported below. All experiments were conducted in accordance with NIH guidelines and with the approval of the Barrow Neurological Institute Animal Care and Use Committee.

Locomotion tasks

Two locomotion tasks were used: 1) simple locomotion on a flat surface, and 2) accurate stepping on the crosspieces of a horizontal ladder (Fig. 1*A*). A box 2.5 m long and 0.6 m wide served as the experimental chamber. A longitudinal wall divided the box into two corridors that cats passed through sequentially and repeatedly. In one of the corridors, the floor was flat, while the other corridor contained a horizontal ladder. The crosspieces of the horizontal ladder were flat and 5 cm wide. The width of the crosspieces was chosen to exceed the cat's mean foot length (3 cm), so that cats had full foot support on the crosspieces. Crosspieces were spaced 25 cm apart, that is, at half of the mean stride length observed in the chamber during locomotion on flat floor at a self-selected pace (Beloozerova & Sirota 1993; Beloozerova et al. 2010). Crosspieces were elevated 6 cm above the floor of the chamber.

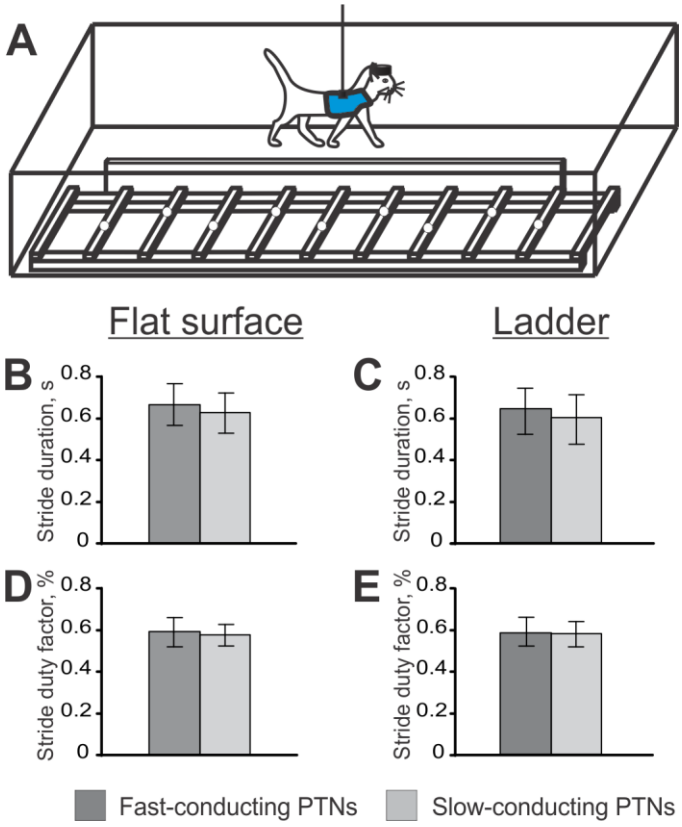

Figure 1. Locomotion tasks. A, the experimental box was divided into two corridors. In one of the corridors, the floor was flat, while the other corridor contained a horizontal ladder. White circles on the crosspieces of the ladder schematically show placements of cat forelimb paws. B, C, average durations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

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

Cats were accustomed to wearing a cotton jacket, a light backpack with connectors, and an electro-mechanical sensor on the paw for recording of swing

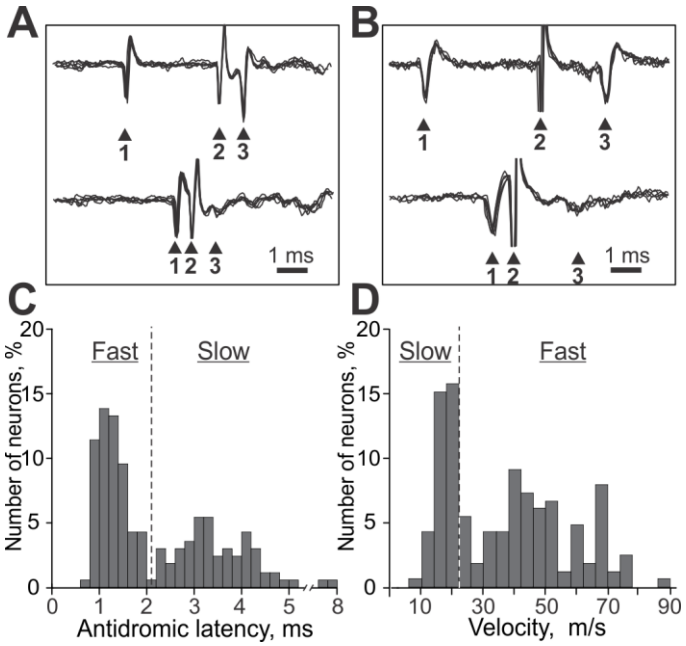

Figure 2. Identification of PTNs A, B, a collision test determines whether a neuron's response is antidromic for fast- (A) and slow-conducting (B) PTNs. A, top trace, the PTN spontaneously discharges (arrowhead 1), and the pyramidal tract is stimulated 3 ms later (arrowhead 2). The PTN responds with latency of 1 ms (arrowhead 3). A, bottom trace, the PTN spontaneously discharges (arrowhead 1) and the pyramidal tract is stimulated 0.7 ms later (arrowhead 2). PTN does not respond (arrowhead 3) because in 0.7 ms its spontaneous spike was still en route to the site of stimulation in the pyramidal tract, and thus collision/nullification of spontaneous and evoked spikes occurred. B, results are analogous for the slow-conducting PTN, with a latency of 2.5 ms. C, distribution of latencies of PTN responses to stimulation of the pyramidal tract. The dashed line denotes the division between fast- and slow-conducting PTNs. D, axonal conduction velocities of PTNs. The dashed line denotes the division (21 m s−1) between fast and slow-conducting PTNs.

and stance phases of stride. The floor in the chamber and the crosspieces of the ladder were covered with an electro-conductive rubberized material. During locomotion the duration of the swing and stance phases of the right forelimb (contralateral to the side of recording in the motor cortex) was monitored by measuring the electrical resistance between the electromechanical sensor and the floor (Sw/St trace in Fig. 4*A,F*) (Beloozerova & Sirota, 1993a,b; Beloozerova et al. 2010). The passage of a cat through the beginning or the end of each corridor was monitored using infrared photodiodes. While walking in the chamber, cats occasionally changed direction from clockwise to counterclockwise. After each round, food was dispensed into a feeding dish in one of the corners (Skinner, 1938; Pryor, 1975). Cats were trained, upon arrival, to stand in front of the feeding dish quietly on all four feet during a delay period of four sec. During data analysis, one second in the middle of this period was considered as "standing".

Cats walked in the experimental chamber on the flat floor and horizontal ladder one to two hours per day 5–6 days a week for at least one month before recordings were made. Thereafter, experiments proceed 6 days a week for 5-10 weeks. On a particular day, experiments lasted for as long as the cat was interested to walk for food reward.

Surgical procedures

After cats were trained, surgery was performed. Anesthesia was induced using ketamine (8 mg/kg), which was followed by 2–5% isofluorane mixed with oxygen (flow rate 0.8 l/min) administered by inhalation for the length of the

surgical procedure. The skin and fascia were removed from the dorsal surface of the skull. At ten points around the circumference of the head, stainless steel screws were screwed into the skull and connected together with a wire; the screw heads and the wire were then inserted into a plastic cast to form a circular base. Later, while searching for neurons before locomotion tests, cats were held rigidly by this base. The base was also used to fixate connectors, a miniature micro-drive, a pre-amplifier, contacts for stimulating electrodes, and a protective cap. A portion of the skull and dura above the left motor cortex (approximately 0.6 cm^2) were removed. The approximate area of the motor cortex was identified by the surface features and photographed (Fig. 3*A*-*H*). The aperture was then covered by a 1 mm thick acrylic plate. The plate was pre-perforated with holes of 0.36 mm in diameter spaced 0.5 mm, and holes were filled with bone wax. The plate was fastened to the surrounding bone by orthodontic resin (Densply Caulk). Two 26 gauge hypodermic guide tubes were implanted vertically above the medullary pyramids with tips approximately at the Horsley-Clarke coordinates (P7.5, L0.5) and (P7.5, L1.5), and 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 PTNs in the awake animal. Immediately after the surgery and then 12 hours thereafter an analgesic buprenorphine was administered intramuscularly.

Cell recording and identification

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

Extracellular recordings were obtained using conventional tungsten varnish-insulated microelectrodes (120 μm OD, Frederick Haer & Co; 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. 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 miniature custom made 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 hard- and software package (Power-

1401/Spike-2 System, Cambridge Electronic Design, Cambridge, UK). Example recordings from pyramidal tract neurons during locomotion are shown in Figure 3*A,F*.

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

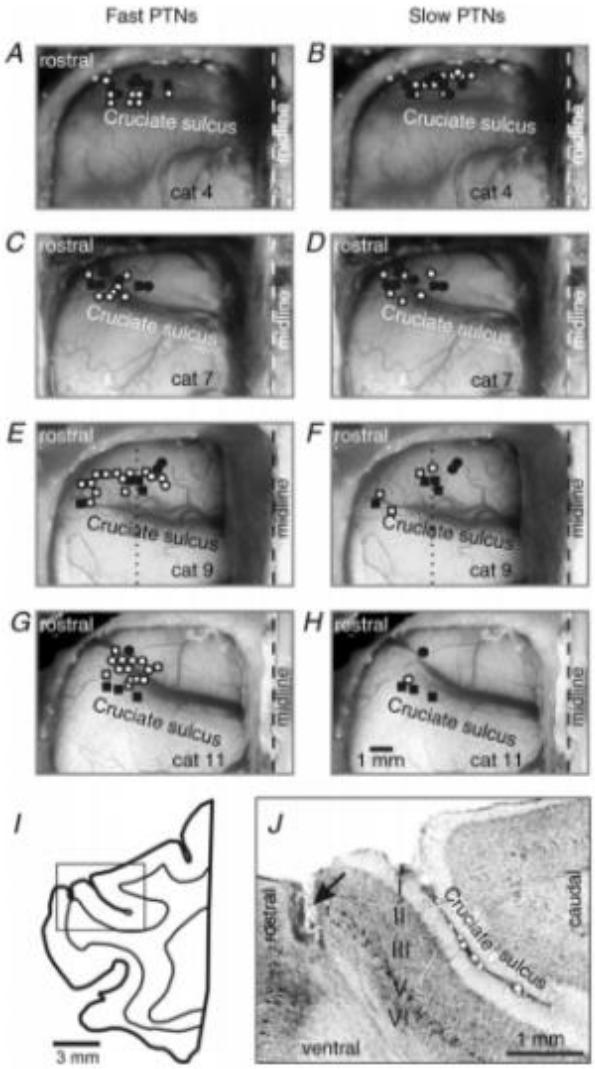

Figure 3. Location of PTNs. A–H, areas of recording in the forelimb representation of the left motor cortex. Microelectrode entry points into the cortex were combined from pairs of cats whose cortices were most similar and are shown as shapes on photographs of one of the cat's cortex for fast- (A, C,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

Determination of axonal conduction velocity

All encountered neurons were tested for antidromic activation using pulses of graded intensity (0.2 ms duration, up to 0.5 mA) delivered through a bipolar stimulating electrode in the medullary pyramidal tract. The stimulating electrode consisted from two platinum-iridium wires 200 µm in outer diameter, insulated with Teflon to within 0.4 mm of the tip. Animals gave no sign of discomfort or notice of the stimulation, suggesting that current did not spread to afferent pathways. The criterion for identification of antidromic responses was the test for collision of spikes (Bishop et al. 1962; Fuller & Schlag, 1976). It is illustrated in Figures 2*A,B*. The distance between electrodes in the medullary pyramidal tract and at recording sites in the pre-cruciate cortex was estimated at 51.5 mm, which includes the curvature of the pathway, as well as the spread of current and the refractory period at the site of stimulation. Neurons were classified as fast- or slow-conducting based on the criteria of Takahashi (1965): neurons with conduction velocity of 21 m/s or higher were considered to be fast-conducting, while those with conduction velocities below this were considered to be slow-conducting. A bimodal distribution of PTN conduction velocities had been documented in a number of previous studies (e.g., Towe et al. 1963; Takahashi 1965; Calvin & Sypert 1976, Humphrey & Corrie 1978, Armstrong & Drew 1984; Vigneswaran et al. 2011; see Fig. 2C,D). Neurons were checked for antidromic activation before, during, and after testing during locomotion. In addition, waveform analysis was employed to identify and isolate the spikes of a single neuron using the Power-1401/Spike-2 system waveform-matching

algorithm. Only the neurons with a stable response latency and spike shape that consistently satisfied the collision test were used for analysis.

Processing of neuronal activity

From each run down a corridor, two or three strides made in the middle of the walkway were selected for the analysis. It was previously shown that the strides in the middle of the corridor are normally made at a nearly constant speed with no acceleration or deceleration, and that their average length during flat surface and ladder locomotion is identical (Beloozerova et al. 2010). For the comparison of discharges of individual neurons between two locomotion tasks we selected strides from different tasks of as close duration as possible. The onset of swing phase was taken as the beginning of step cycle. The duration of each step cycle was divided into 20 equal bins, and a phase histogram of spike activity of the neuron in the cycle was generated and averaged over all selected cycles (Fig. 4*C,E,H,J*). The discharge frequency in a bin was derived according to the method of Udo et al. (1982) that averages the instantaneous frequency of inter-spike intervals that fall within the bin and also accounts for those intervals that overlap with bin's beginning and end. The phase histogram was smoothed by recalculating the value of each bin as follows: $F_n' = 0.25*F_{n-1} + 0.5*F_n + 0.25*F_{n+1}$, where F_n is the original value of a bin. The first bin was considered to follow the last one; the last bin was considered to precede the first one. The coefficient of stride-related frequency modulation, *M*, was calculated using the histogram. It was defined as $M = (1 - Fmin/Fmax) * 100\%$, where Fmin and Fmax are the

minimal and the maximal frequencies of discharge in the histogram. In addition, the "depth" of modulation, *dM,* characterizing fluctuation in probability of the discharge, was calculated as $dM = (N_{max} - N_{min})/N * 100\%$, 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. The two measures for the modulation, *M* and *dM,* enabled characterization of fluctuation of PTN discharge rate both in terms of variation in frequency (*M*) and probability (*dM*) of discharge. Neurons with *dM*>4% and *M*>50% were judged to be stride-related. This was based on an analysis of fluctuation in the activity of neurons in the resting animal. For this analysis, the activities of 100 neurons recorded while the cat was sitting in the head-restraining device were processed as if the cat was walking (Marlinski et al. 2012). The timing of steps made by the same cat during the preceding walking test was used to construct the histogram. This analysis showed that at rest, the values of *dM* exceeded 4% in only five cells. Therefore, when the *dM* of activity of a neuron was greater than 4% during locomotion, we could conclude with 95% confidence that it was due to stride-related modulation. In stride-related neurons, the portion of the cycle in which the activity level exceeded 25% of the difference between the maximal and minimal frequencies in the histogram was defined as a "period of elevated firing, PEF" (illustrated in Figure *4C,E,H,J*). The "preferred phase" of discharge of each neuron with a single PEF was assessed using circular statistics (Batshelet, 1981; Drew & Doucet, 1991; Fisher, 1993; see also Beloozerova et al. 2003; Sirota et al. 2005); while neurons exhibiting two or more PEFs were excluded from this analysis. The coefficient of variability of discharge

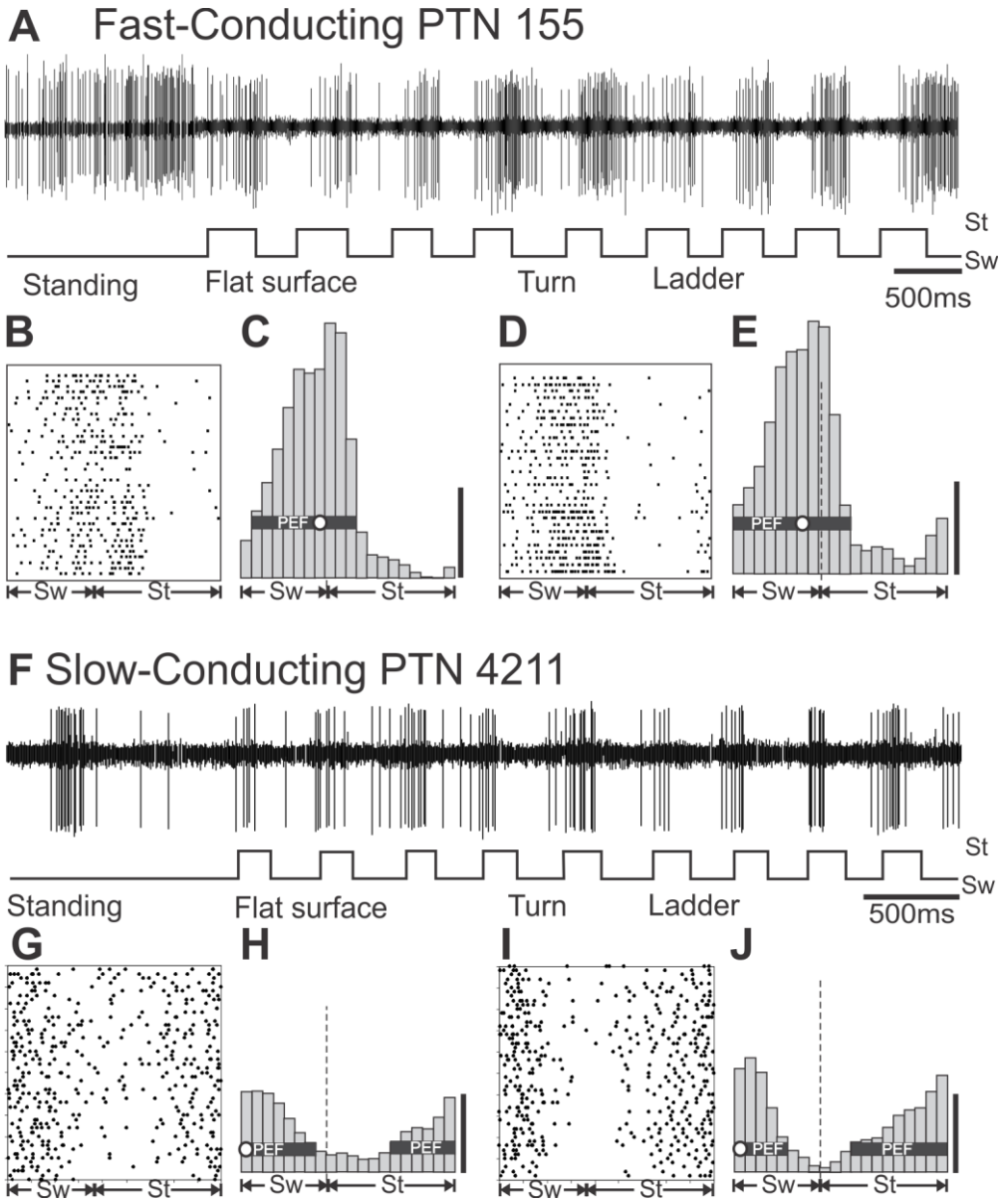

Figure 4. Example activity of fast- and slow-conducting PTNs. A, F, activity of individual fast- (A) and slow-conducting (F) PTNs during standing, simple, and ladder locomotion. The bottom trace shows the swing (Sw) and stance (St) phases of the step cycle of the right forelimb that is contralateral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

rate, *CV*, was defined as $CV = \sigma^2/m$, where σ is standard deviation and *m* is mean firing rate. The activity during standing was assessed by averaging discharges during all one second periods occurring a second after the right forelimb (contralateral to the recorded cortex) was placed on ground when cat stopped for food reward at the end of each walking round.

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

Histological procedures

At the termination of experiments, cats were deeply anaesthetized with pentobarbital sodium. Several reference lesions were made in the region of the

motor cortex from which neurons were sampled. Cats were then perfused with isotonic saline followed by a 3% paraformaldehyde solution. Frozen brain sections of 50 μm thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet. Zoning of the cortex was performed according to criteria established by Hassler and Muhs-Clement (1964). The positions of recording tracks in the cortex were estimated in relation to the reference lesions (Fig. 3*I,J*). The position of stimulation electrodes in the medullar pyramids was verified.

3.4 RESULTS

Characteristics of locomotion tasks

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

PTN populations, the average duration of the chosen strides was similar during simple and ladder locomotion (Fig. 1*B*,*C*), as was the ratio of the stance duration to the cycle duration, the stride duty factor (Fig. 1*D*,*E*).

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

Characteristics of neurons

The activity of 165 PTNs was included in the analysis (Table 1). Of these PTNs, 95 were fast-conducting (21-80 m/s), and 70 were slow-conducting (5-20 m/s; Fig. 2*C*,*D*). Cells were collected from a total of 87 microelectrode tracks, with an average 10±4 (mean ± SD) tracks used per cat (Fig. 3 *A*-*H*). In Figures 3*A*-*H*, shapes overlaying the cortex schematically show microelectrode entry

points into the cortex for tracks, in which PTNs of different groups were recorded during locomotion. Filled shapes indicate the 23 tracks where both fast- (n=33) and slow-conducting (n=39) PTNs were recorded, typically 1-2 of each type per track (Table 1). This included five pairs of fast- and slow-conducting PTNs recorded simultaneously by the same electrode. Histological inspection confirmed that all neurons were collected from the motor cortex area 4□. A drawing of a parasagittal section through the frontal cortex, whose approximate position is indicated by a dotted line in Figures 3 *E,F*, is given in Figure 3*I*. Figure 3*J* shows a photomicrograph of a portion of the cortex that is outlined by a square in *I*. Numerous giant pyramidal neurons characteristic of area 4□ can be seen in layer V throughout the pre-cruciate cortex.

Responses of 83 fast- and 53 slow-conducting PTNs to somatosensory stimulation were tested. A somatosensory receptive field was found in 87% (72/83) of fast-conducting PTNs, but in only 68% (36/53) of slow-conducting PTNs, a significantly lower proportion (χ^2test, p=0.037). In both PTN groups, all receptive fields were located on the contralateral (right) side of the body and all but two were excitatory. Both PTNs responding with inhibition were slow-conducting. Among slow-conducting PTNs, approximately equal number of neurons had receptive fields on the shoulder, elbow, and wrist/paw. In the fast-conducting group, however, there were more neurons that responded to movements of the shoulder than to either elbow or wrist/paw (χ^2 test, p<0.03). This bias is due to the fact that slow-conducting PTNs were often found in the medial regions of the motor cortex, and many fast-conducting PTNs in the same

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

Cat #	Gender	Mass, kg	Fast-conducting PTN tracks	Fast-conducting PTNs	Slow-conducting PTN tracks	Slow-conducting PTNs	F & S common tracks	PTNs in common tracks (F/S)	Total PTNs
1	male	3.9	7	14	4	10	3	6/9	**24**
3	female	3.0	7	12	5	7	3	4/4	**19**
4	male	3.8	9	13	13	22	4	4/10 (2/2)	**35**
7	female	2.7	6	12	6	11	3	8/5 (2/2)	**23**
8	male	4.5	10	12	2	4	1	2/1	**16**
9	male	3.9	13	13	7	9	4	4/4 (1/1)	**22**
11	female	3.7	8	11	3	4	3	3/4 (1/1)	**15**
12	male	4.0	7	8	3	3	2	3/2	**11**
Total 8			**67**	**95**	**43**	**70**	**23**	**34/39**	**165**

tracks were also recorded (Fig. 3); these regions are more likely to contain neurons with proximal receptive fields. In both fast- and slow-conducting populations several neurons were activated by a movement in both shoulder and elbow. Neurons activated by a joint movement often had a preferred direction. Fast-conducting PTNs with receptive fields on the shoulder were more often excited by the shoulder extension or abduction than by flexion or adduction (χ^2test, p=0.018). At the same time, elbow- and wrist/paw-related fast-conducting PTNs, as well as any slow-conducting neurons were as likely to respond to flexion as to extension.

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

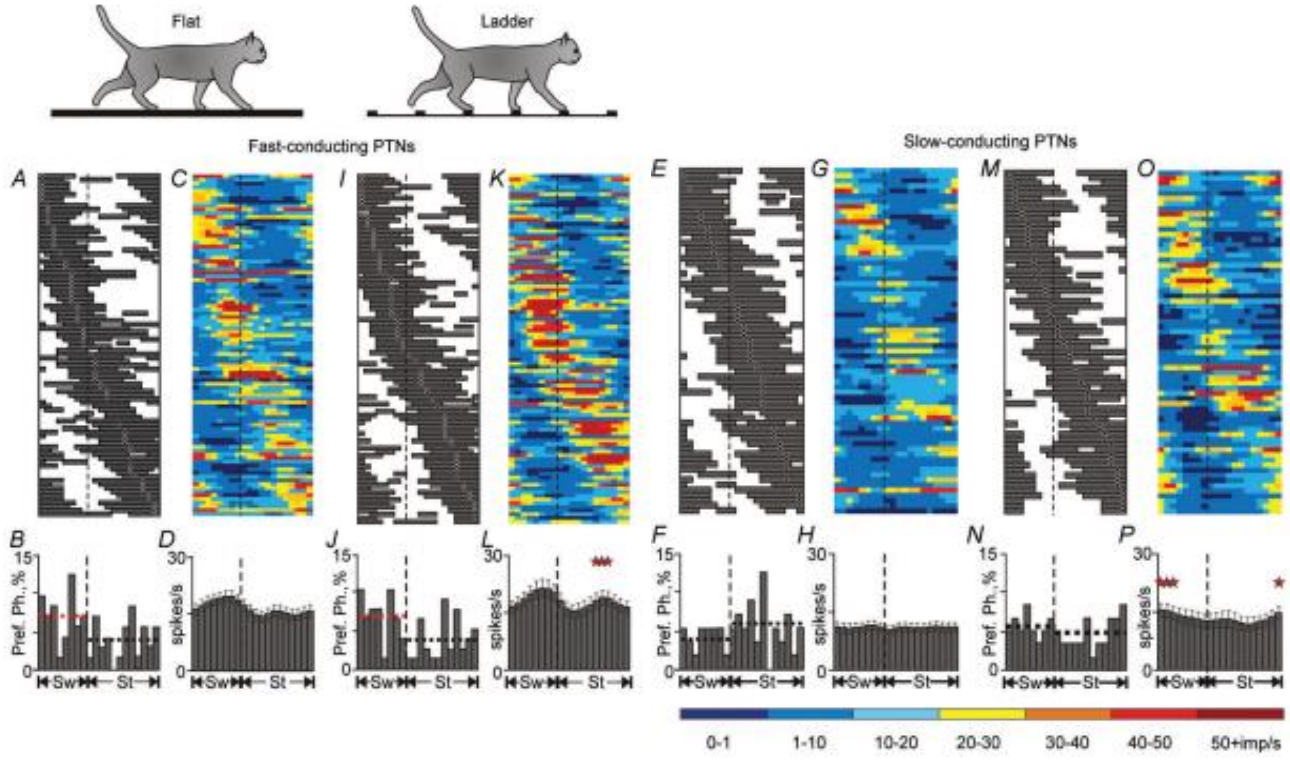

Figure 5. Phase distribution of PEFs, preferred phases, and discharge rates of fast- and slow-conducting PTNs during locomotion A, E, I, M, distribution of PEFs of individual fast- (A, I) and slow-conducting (E, M) PTNs in the step cycle of simple (A, E) and ladder (I, M) locomotion. Each trace represents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

magnitude of frequency modulation for both PTNs was larger during ladder locomotion, but to a greater extent for slow-conducting PTNs.

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

Activity during standing and simple locomotion

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

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

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

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

	Parameters of PTN activity	Fast-conducting, all n=95	Fast-conducting, in same tracks with slow PTNs n=33	Slow-conducting, all n=70	Slow-conducting, in same tracks with fast PTNs n=39
	Prop. of cells with receptive fields,%	**87**	86	**68**	71
Standing	Proportion of active cells, %	**100**	100	**94**	98
	Average activity, spikes/s	**16.0 ± 1.0**	17.6 ± 1.9	**9.4 ± 0.8**	8.9 ± 0.9
	Discharge variability, CV	**2.2 ± 0.36**	2.4 ± 0.6	**1.08 ± 0.11**	1.0 ± 0.1
Simple locomotion	Average activity, spikes/s	**16.6 ± 1.1**	17.1 ± 1.6	**11.4 ± 0.9**	9.9 ± 0.8
	Discharge variability, *CV*	**1.85 ± 0.12**	1.8 ± 0.2	**1.79 ± 0.13** *	1.7 ± 0.1 *
	Proportion modulated,%	**98**	97	**96**	97
	Proportion with 0 sp/s in any bin,%	**8.4**	5.4	**17.1**	22
	Mean peak rate, spikes/s	**35.2 ± 2.1**	37.4 ± 3.7	**23.0 ± 1.8**	21.5 ± 2.1
	Depth of modulation, *dM*, %	**10.6 ± 0.5**	11.1 ± 0.9	**9.6 ± 0.5**	9.8 ± 0.6
	Coefficient of modulation, *M*, %	**87.3 ± 1.4**	89.2 ± 2.2	**86.1 ± 1.8**	86.7 ± 2.2
	Duration of PEF, % of cycle	**56.5 ± 2.0**	56.5 ± 2.5	**60.5 ± 14**	60.5 ± 2.0
	Proportion with single PEF, %	**76**	84	**82**	83
Ladder locomotion	Average activity, spikes/s	**18.1 ± 1.2**	20.3 ± 2.0	**13.5 ± 1.2** ★	11.4 ± 1.2 ★
	Discharge variability, *CV*	**1.72 ± 0.08**	1.6 ± 0.1	**1.49 ± 0.9** ★	1.6 ± 0.1 ★
	Proportion modulated,%	**100**	100	**96**	97
	Proportion with 0 sp/s in any bin,%	**14.7**	16	**18**	21
	Mean peak rate, spikes/s	**41 ± 2.7**	47.2 ± 4.5	**29.1 ± 2.6**	25.8 ± 2.7
	Depth of modulation, *dM*, %	**11.0 ± 0.4**	12.9 ± 0.6	**11.2 ± 0.5** ★	11.1 ± 0.6 ★
	Coefficient of modulation, *M*, %	**91.0 ± 1.1** ★	92.6 ± 1.6 ★	**91.7 ± 1.2** ★	91.4 ± 1.5 ★
	Duration of PEF, % of cycle	**56.5 ± 2.5**	56.5 ± 2.5	**60.5 ± 2.0**	60.5 ± 2.5
	Proportion with single PEF, %	**77**	81	**90**	93

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

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

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

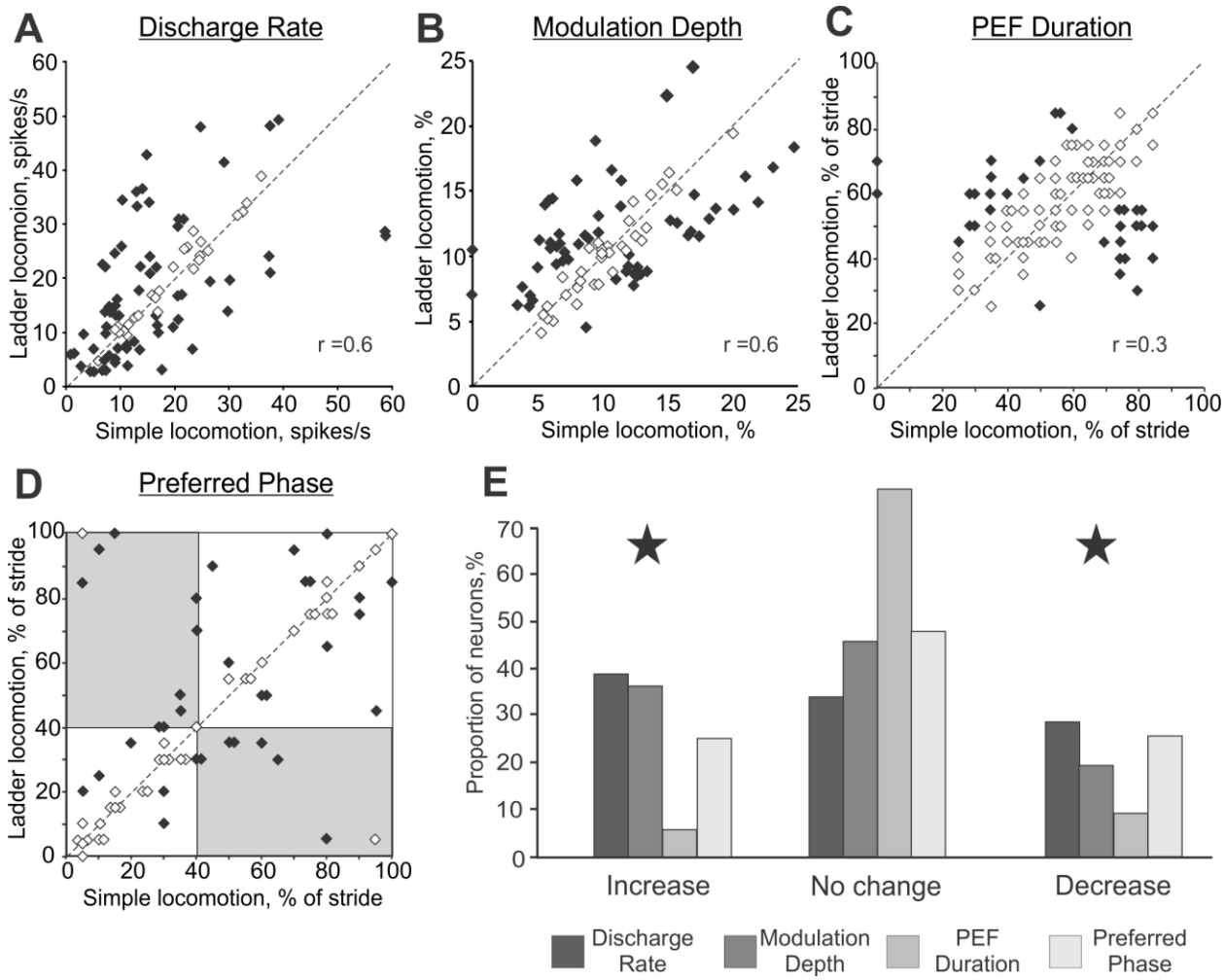

Figure 6. Comparison of activity characteristics of individual fast-conducting PTNs between simple and ladder locomotion. A–D, the abscissa and ordinate of each point show the values of a characteristic of a neuron during simple and ladder locomotion, respectively. Neurons whose characteristics were statistically significantly different during the two tasks (according to criteria established using a bootstrapping analysis, see Methods) are shown as filled diamonds; others are shown as open diamonds. In D, areas that correspond to the swing phase during one task but stance phase during the other task are shaded. E: percentage of neurons significantly changing a parameter upon transition from simple to ladder locomotion. Stars indicate that significantly more neurons increased than decreased the depth of modulation ($\chi 2$ test, $P < 0.05$).

discharge rate averaged over one histogram bin was more than 10 spikes/s less than in the fast-conducting population (t^2 test, $p<0.05$; Table 2).

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

Activity during ladder locomotion

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

Upon transition from simple to ladder locomotion 90 of 95 fast-conducting and 66 of 70 slow-conducting PTNs experienced significant changes to their activity characteristics (Figs. 6*E*, 7*E*). To facilitate comparison between the characteristics of individual neurons during two tasks, we used scatter diagrams. In Figures 6*A* and 7*A* the mean discharge rate of individual neurons during ladder locomotion is plotted against that during simple walking for fast- and slow-conducting PTNs, respectively. The great majority of both fast- and slow-

conducting PTNs changed discharge rate upon transition from simple to ladder locomotion: 39% and 40%, respectively, increased it, by two folds on average, while 33% and 27%, respectively, decreased, on average by one half. In result, slow-conducting PTN population average activity rose to 13.5 spikes/s, and was now greater than during both standing and simple locomotion (*t* test, $p<0.05$; Table 2); highest during the beginning of stance and end of swing (Fig. 5*P*). In addition, the discharge variability of slow-conducting PTNs during ladder locomotion diminished as compared to simple walking (*t* test, $p<0.05$; Table 2). At the same time, for the fast-conducting population, neither the mean discharge rate nor the discharge variability changed (*t* test, $p>0.05$; Table 2).

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

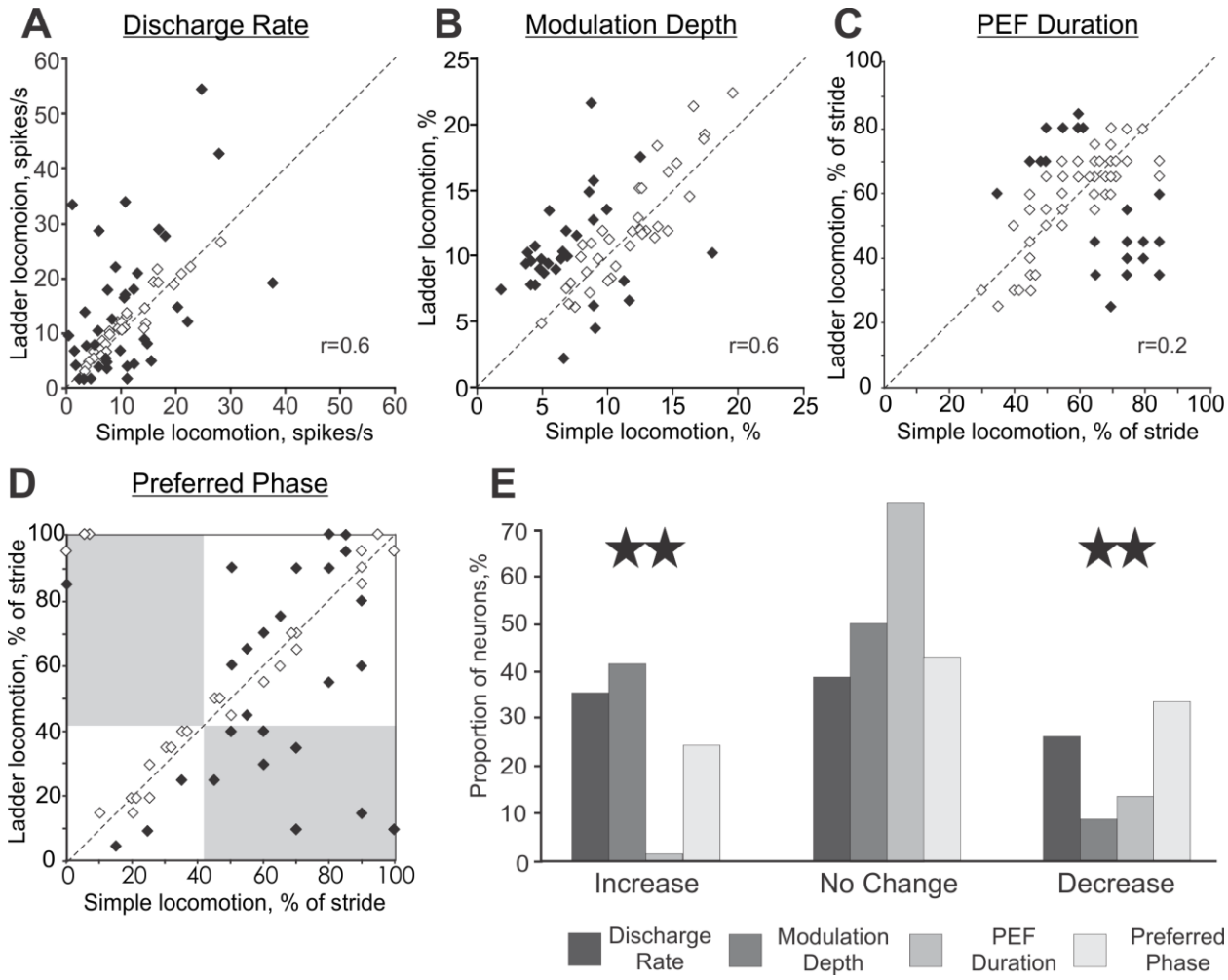

Figure 7. Comparison of activity characteristics of individual slow-conducting PTN between simple and ladder locomotion (organized in the same fashion as in Fig. 6). In E, darker stars indicate that significantly more neurons increased than decreased the depth of modulation, and lighter stars indicate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

narrower distribution of modulation depths during ladder as compared to simple locomotion (Fig. 6*B*). The frequency-based coefficient of modulation *M* for the fast-conducting population was, however, higher than during simple locomotion as was the *M* for the slow-conducting PTNs (*t* test, $p<0.05$; Table 2).

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

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

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

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

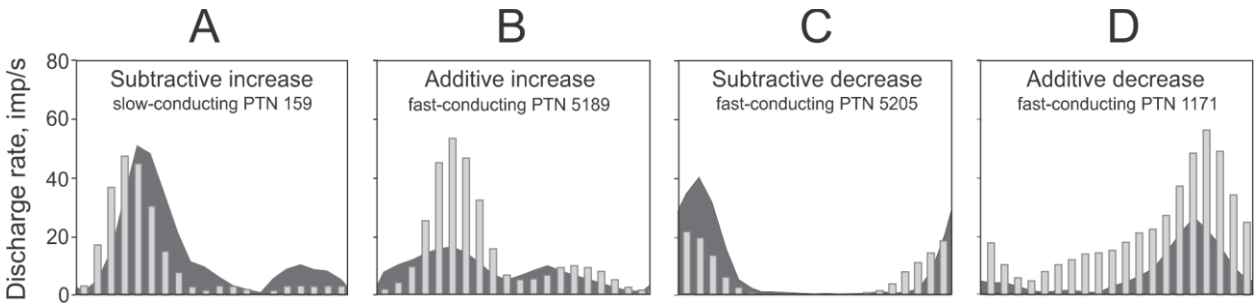

Figure 8. Typical changes in the depth of modulation upon transition from simple to ladder locomotion. Area histograms show the activity of a typical PTN during simple locomotion. The histograms show activity of the same PTN during ladder locomotion. A, increase in the depth of modulation by a subtractive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

Fast- and slow-conducting PTNs recorded from the same track, simultaneously or sequentially, exhibited the same activity characteristics as the larger populations (Table 2). Upon transition from simple to complex locomotion, fast and slow PTNs recorded in the same track were more likely to exhibit the same changes to discharge rate than would be expected based on the characteristics of the overall population (*t* test for proportions, $p<0.05$), but were more likely to show *different* changes to modulation depth (*t* test for proportions, $p<0.05$). For PTNs recorded simultaneously, same changes were observed for 3 out of 5 pairs with regards to discharge rate and for 2 out of 5 pairs with regard to modulation strength.

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

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

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

3.5 DISCUSSION

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

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

greater role than fast-conducting PTNs in managing accuracy demands during locomotion.

The activity of fast- and slow-conducting PTNs during simple locomotion has been earlier compared by Armstrong and Drew (1984a). These authors also found that fast-conducting PTNs have higher mean and peak discharge rates than slow-conducting PTNs. Armstrong and Drew (1984a), however, reported that during locomotion there was a tendency for fast-conducting PTNs to discharge discrete step-related bursts of activity separated by near silence, while slow-conducting PTNs more often fired continuously throughout the cycle, exhibiting a lesser magnitude of frequency modulation. However, our data obtained from a significantly larger population of slow-conducting PTNs (n=70 *vs.* n=16) shows that the activity of slow-conducting PTNs is not any less modulated in relation to stride than that of fast-conducting PTNs. This result is based on two assessments of modulation magnitude, *dM* and *M*, and also on the proportion of neurons that were completely silent for any $1/20^{th}$ portion of the cycle. Our failure to find any tendency for slow-conducting PTNs to discharge more "tonically" or fast-conducting PTNs to be active more "phasically" during locomotion also contrasts with previously reported data on activities of these neuronal populations during isolated limb movements in primates. Specifically, in primates it was found that slow-conducting PTNs are typically active tonically at rest and respond with a sustained discharge to passive ramp-form displacements of the forearm whereas fast-conducting PTNs are usually nearly silent at rest and exhibit transient responses (Evarts, 1965; Fromm & Evarts, 1977, 1981; Tanji et al. 1978; Fromm

et al. 1984). The difference between this and our locomotion data is likely to be explained by the fact that during walking cats only made comparatively large amplitude movements that effectively activated both fast- and slow-conducting PTNs. When the activities of these PTN subpopulations were compared during this mutually engaging condition, they differed only in discharge rates, and not in strength of the stride-related frequency modulation. Apart from the discharge rate, the only other difference between the activity of fast- and slow-conducting PTNs during simple locomotion is the slightly different distribution of their preferred phases, which in the fast-conducting group show a mild concentration during the swing phase, while slow-conducting PTNs as a group discharge roughly evenly throughout the stride cycle (Figs. 4*F*,*G* and 5 *F*,*G*).

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

The ladder imposes accuracy constraints on the locomotion task, as cats have to step accurately on crosspieces. It was previously demonstrated that

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

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

that the motor cortex may play a role in control of position of paw landing during walking was also reached by Amos and Armstrong (1990) and Friel and colleagues (2007) based on results of movement perturbations and motor cortex inactivation experiments.

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

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

The observed differences in the activities of fast- and slow-conducting PTNs cannot be explained by the difference in their receptive field properties. Slow-conducting neurons tend to lack somatosensory receptive fields and one may suggest that their population activity profiles during simple and ladder

locomotion are due to the large proportion of non-responsive PTNs (Figs. 4*H* and 7*G* in Stout and Beloozerova 2012). However, we found that slow-conducting PTNs are the ones to most strongly increase the depth of locomotion-related modulation upon transition to accuracy demanding ladder task. This is opposite to the typical behavior non-responsive PTNs, who more often than any other PTNs decrease the depth of modulation on the ladder (Fig. 6*D* in Stout and Beloozerova, 2012). Similarly, the activity of fast-conducting PTNs, which were most likely to have receptive fields on the shoulder, cannot be explained by this bias. Their population activity profiles are dissimilar to shoulder-related PTNs, and do not show the pronounced response to accuracy demand of the ladder task exhibited by shoulder-related PTNs (Stout and Beloozerova 2012).

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

Fast- and slow-conducting PTNs differ in their connections to the spinal cord, such that fast-conducting PTNs preferentially influence distal muscle-related networks, while slow-conducting PTNs influence both proximal and distal muscle-related networks (Brookhart, 1952; Wiesendanger, 1981; Canedo, 1997).

Therefore, more intensive involvement of slow-conducting PTNs in control of accuracy of movements during locomotion means that the accuracy of stepping is predominantly achieved not by adjustments of movements in distal limb segments, but by a more careful planning of the whole limb transfer, in which proximal limb-related networks significantly participate. It was shown that during limb movements, individual joints make unique contributions to the overall movement, as proximal joints greatly affect movements of distal joints, while distal joints have only small influence on movements of proximal joints (e.g. Grillner & Rossignol, 1978; Galloway & Koshland, 2002; Dounskaia, 2005).

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

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

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

PTNs have the most selective relations to accurately controlled movements by data from a targeted experiment.

We want to note that most studies of the discharges of individual neurons in the motor cortex over years have been strongly biased toward fast-conducting PTNs, on account of their comparatively large size, and thus relative ease of recording. With a recent wide adoption of commercially available chronically implantable microarrays for cortical neuronal recording, this biasing has become an even larger issue. However, the vast majority of PTNs are of the slow-conducting variety (Calvin & Sypert, 1976; Humphrey & Corrie, 1978), and these neurons have anatomical and physiological properties that are quite distinct from those of fast-conducting PTNs. Fast- and slow-conducting PTNs have different dendritic field ranges (Deschenes et. al. 1978; Sakai, 1982), different distributions throughout the motor cortex (Towe et al. 1968; Takahashi, 1965), and may receive input of different types (Deschenes et al. 1982). In addition, neurons of the two types influence one another in different ways: fast-conducting PTNs commonly make inhibitory disynaptic connections to slow-conducting PTNs, while slow-conducting PTNs often make excitatory monosynaptic connections to fast-conducting PTNs (Takahashi, 1965; Tsukahara et al. 1968; Ghosh & Porter, 1988; Canedo, 1997). While neurons of either type are equally likely to synapse upon the spinal cord, and both produce facilitation of their target muscles (Fetz & Cheney, 1982), the facilitation produced by fast-conducting PTNs is larger (Lemon et al. 1993). These differences in biophysical and connective properties strongly suggest that fast- and slow-conducting PTNs may have quite distinct

functional roles in the control of movements. The results of our study suggest that they may have different roles during accuracy-constrained stepping.

CHAPTER 4

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

Under review at Journal of Neuroscience (Stout et al. 2015)

4.1.1 ABSTRACT

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

We found that when the crosspiece was displaced before the cat stepped onto the ladder, kinematic modifications were complex and involved alterations of dynamics of all forelimb joints. When the crosspiece displaced unexpectedly while the cat was on the ladder, kinematic modifications were minimalistic and primarily involved distal joints. The activity of M. triceps and M. extensor digitorum communis differed based on the direction of displacement. Out of 151 neurons tested, 69% responded to at least one condition. Neurons were more

likely to respond when the crosspiece displacement was unexpected, and the specific changes to neuronal activity varied based on how much time the cat had to prepare before stepping onto the displaced crosspiece. These results suggest that different neural mechanisms and motor control strategies are used to overcome constraints for locomotor movements depending on whether they are known or unexpectedly emerge.

4.1.2 INTRODUCTION

The motor cortex is highly involved in the control of single limb movements, locomotion and posture. During locomotion, nearly all layer V neurons of motor cortex discharge in rhythm with the step cycle (Drew, 1993; Fitzsimmons et al., 2009; Stout and Beloozerova, 2012, 2013), and the characteristics of this activity are often specialized to the specific task being performed. In many behaviors, visual information about the environment must be used in order to navigate obstacles, accurately guide foot placement, or reach a stationary or moving target. The contributions of the motor cortex are essential in managing the complexities posed by irregular surfaces, including those found in the natural environment: when the motor cortex is inactivated or ablated, subjects lose the ability to successfully walk over complex terrain (Trendelenburg, 1911; Liddell and Phillips, 1944; Chambers and Liu, 1957; Friel at al, 2007).

During planned gait adaptations, kinematic adjustments will often be made in preparation of the adaptation (Mohagheghi et al., 2004). Frequently, the smallest kinematic adjustments that meet the adaptive constraint are preferred (Patla et al., 2004). During unexpected or emergent disturbances, preparatory movements are impossible, and strategy selection may be further constrained (Patla, 1999). However, during unexpected or emergent obstacle avoidance during walking or reaching tasks, the latency of obstacle avoidance behaviors is shorter than the latency of voluntary movement modifications (Pettersson et al., 1997; Weerdesteyn et al., 2004), suggesting that distinct neuronal processes are

taking place. Indeed, it was recently shown that during reaching to unexpectedly shifting targets, differential neuronal processing occurs in each of the displacement conditions (Ames et al. 2014). Therefore, it appears likely that the neuronal motor control strategies employed to overcome task-related constraints may be dependent on the amount of time between constraint perception and motor adaptation, as well as whether the constraint is known or unexpected. Little is known, however, about how the motor cortex functions to compensate for emergent or unexpected changes in the movement environment during locomotion. To investigate this function, the activity of motor cortical neurons was recorded as cats walked along a raised horizontal ladder, a complex locomotor task that involved accurate limb placement. One of the crosspieces was motorized, and could be displaced either prior to the cat stepping on the ladder or at different points along the cat's progression along the ladder, either towards or away from the cat. To successfully continue along the ladder, cats needed to make a longer or shorter step. Forelimb kinematics, EMGs, and motor cortex activity during shorter or longer steps with these various displacement timings were compared.

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

4.1.3 METHODS

Recordings were obtained from two adult male cats (weight 11 and 8.5 lb). Methods of data collection and spike trains analysis have been previously

reported (Prilutsky et al., 2005; Beloozerova et al., 2010; Stout and Beloozerova, 2012, 2013) and will be described briefly below. All experiments were conducted in accordance with NIH guidelines and with the approval of the Barrow Neurological Institute Animal Care and Use Committee.

Locomotion tasks

Positive reinforcement (food) was used to adapt cats to the experimental situation and to engage them in locomotion (Skinner, 1938; Pryor, 1975). A walkway, 2.5 m long and 0.3 m wide on each edge, served as an experimental chamber (Fig. 1A). Cats passed sequentially and repeatedly through the two corridors of the chamber in a counter-clockwise direction. In one of the corridors, the floor was flat, while the other corridor contained a horizontal ladder with 10 crosspieces. Crosspieces were spaced 25 cm apart, which is half of the mean stride length observed in the chamber during locomotion on flat floor at a self-selected pace (Beloozerova and Sirota, 1993; Beloozerova et al., 2010). The tops of crosspieces were flat and 5 cm wide. The width of the crosspieces was chosen to slightly exceed the cat's mean foot length (3 cm), so that cats had full foot support on a crosspiece. Crosspieces were elevated 6 cm above the floor of the

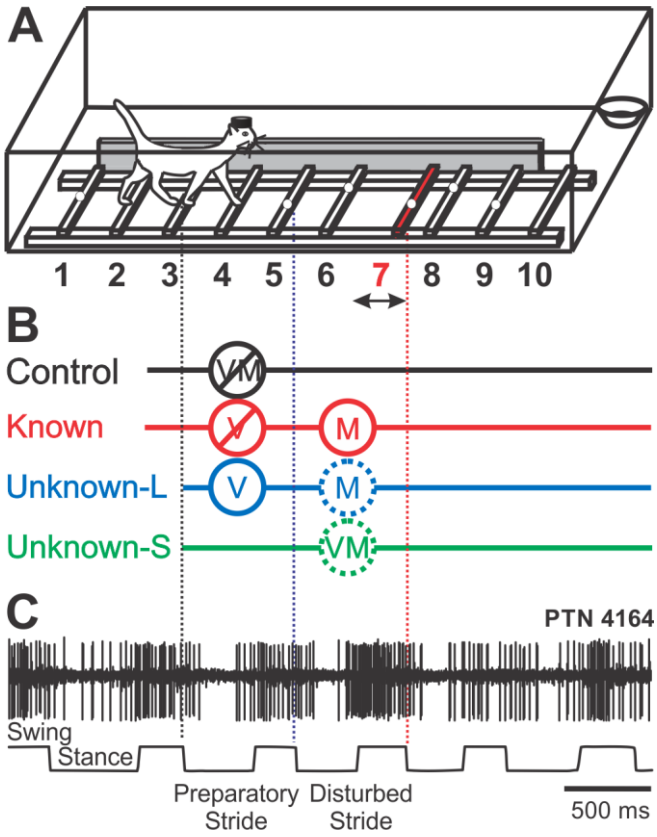

Figure 1: Experimental Design. A: Cats walked through a rectangular, two-side chamber. One side contained a raised horizontal ladder, with one motorized crosspiece (#7, red) that was displaced at different times as the cat walked in the chamber. B: A total of seven conditions were analyzed: a control condition with the crosspiece remaining in its central position, when all crosspieces were equally spaced 25 cm apart, and crosspiece movements away or towards the cat either before the cat stepped on the ladder (two "known" displacement conditions, Kn), or one stride away from it (two "unknown" long-notice displacement conditions, Ul), or during the current stride while the cat was about to initiate limb transfer to crosspiece #7 (two "unknown" short-notice displacement conditions, Us). Circles represent where the cat was along the ladder when the crosspiece displaced ("V" – visual stimulus) and when the step onto the disturbed rung was made ("M" – motor adaptation). C: An example of activity of a neuron (pyramidal tract neuron, PTN 4164) during locomotion along the ladder in the Ul long step condition.

chamber. One crosspiece (the seventh from the left side of the ladder, Fig 1A) was connected to an electric motor. When displaced, it was shifted 5 cm in either direction, such that there was no overlap between the crosspiece's position before or after the displacement. Displacement was completed within 145 ms of initiation. On the side of the crosspiece facing the cat, there was a yellow LED lamp. It was lit as soon as the triggering of the crosspiece displacement occurred, regardless of the direction of the initiated move. This illumination attracted the cat's attention to the crosspiece when it was displacing. Auditory cues from the activation of the motor also alerted the cat to a rung displacement. Regardless of the crosspiece's displacement or the cat's performance, after each round of walking, the cat received food in a feeding dish located in one of the chamber's corners.

This apparatus allowed us to compare several locomotion tasks by displacing the crosspiece at various time points along the cat's progression. Only passages where the cat stepped on the displaceable crosspiece with right feet were studied. Seven conditions were used (Fig. 1 B): control, when the crosspiece remained in its original location; and three groups of conditions where the crosspiece was displaced either toward or away from the cat at different times along the cat's progression through the chamber, and the cat had to make a larger or smaller step to successfully traverse the ladder. In "known displacement" (Kn) conditions, the crosspiece was displaced while the cat was at the feeder. In these conditions, the cat did not see movement of the crosspiece, as the ladder was in its final configuration when the cat stepped onto it. The cat had two full strides: a

stride from crosspiece #1 onto crosspiece #3, and a stride from crosspiece #3 onto crosspiece #5, before making a larger or smaller step to reach the displaced crosspiece #7. In unexpected "long-notice" conditions (Ul), the rung was displaced when the cat's right forelimb stepped on crosspiece #3. The cat had one full locomotion cycle to complete before needing to adjust. In unexpected "short notice" conditions (Us), the crosspiece was displaced when the cat's right forelimb stepped on crosspiece #5 and the very next transfer of the forelimb had to be adjusted. . A sequence of 21 conditions was repeated pseudorandomly by a computer program, occasionally resetting at random times, which were different for different experimental days and subjects. All conditions were presented an approximately equal number of times and the cat could develop no fore-knowledge of which condition would be presented.

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

Surgical procedures

After cats were trained, surgery was performed under isoflourane anes¬thesia using aseptic procedures. The skin and fascia were removed from the dorsal surface of the skull. At ten points around the circumference of the head, stainless steel screws were screwed into the skull and connected together with a wire; they served as a fixation and a common ground. The screw heads and the wire were inserted into a plastic cast to form a circular base. Later, while searching for neurons before locomotion tests, awake cats were rigidly held by this base. The base was also used to fixate connectors, a miniature micro-drive, a pre-amplifier, contacts for stimulating electrodes, and a protective cap. A portion of the skull and dura above the left motor cortex (approximately 0.6 cm^2) were removed. The area of the motor cortex was identified by the surface features and photographed (Fig. 2A). The aperture was then covered by a 1 mm thick acrylic plate. The plate was pre-perforated with holes of 0.36 mm in diameter spaced 0.5 mm, and holes were filled with bone wax. The plate was fastened to the surrounding bone by orthodontic resin (Densply Caulk).

For muscle activity recordings, a pair of leads constructed from Teflon-insulated multistrand stainless steel wire (AS632, Cooner Wire, Chatsworth, CA) was implanted into m. triceps lateralis and m. extensor digitorum communis. The electrode placements were verified by stimulation through the implanted wires before closure of the incision. The wires were led subcutaneously and connected

to sockets on the head base. Immediately after surgery, and then 12 hours thereafter, an analgesic buprenorphine was administered intramuscularly.

Cell recording and identification

Experiments were initiated after several days of recovery when cats resumed their normal preoperative behavior. The animal was positioned in the restraining device, and encouraged to take a "sphinx" position. After the cat rested in this posture for several minutes, the base attached to the skull during surgery was fastened to an external frame so that the resting position of the head was approximated. Over several days, a number of sessions of increasing duration were used to accustom the cat to the head restrainer. Cats fast learned to sit quietly with their head restrained. They did not seem to be disturbed by the restraint because they frequently fell asleep.

Extracellular recordings were obtained using conventional tungsten varnish-insulated microelectrodes (120 μm OD, Frederick Haer & Co). The impedance of electrodes was 1-3 MΩ at 1000 Hz. A custom made light-weight (2.5g) manual single-axis micro-manipulator permanently affixed to the head base was used to advance the microelectrode. Signals from the microelectrode were pre-amplified with a miniature custom made preamplifier positioned on the cat's head, and then amplified with the CyberAmp 380 (Axon Instruments). After amplification, signals were filtered (0.3-10 kHz band pass), digitized with a sampling frequency of 30 kHz, displayed on a screen, led to an audio monitor, and recorded to the hard disk of a computer by means of data acquisition hard-

and software package (Power-1401/Spike-2 System, Cambridge Electronic Design, Cambridge, UK). An example of recording from a pyramidal tract neuron during locomotion is shown in Figure 1C.

A detailed description of the area of recording has been given previously (Beloozerova et al. 2005). In brief, the area immediately adjacent to and inside the lateral half of the cruciate sulcus in the cat is considered to be the motor cortex (Fig. 2A). This is based on a considerable body of data obtained by means of inactivation, stimulation and recording techniques (Nieoullon and Rispal-Padel, 1976; Vicario et al., 1983; Armstrong and Drew, 1985; Beloozerova and Sirota, 1993a; Drew, 1993; Martin and Ghez, 1993), as well as on histological considerations (Myasnikov et al., 1997; Ghosh, 1997).

Motion capture and kinematics analysis

Kinematics for the right forelimb were recorded using the computerized, active-marker three-dimensional real-time motion capture and analysis system Visualeyez (VZ-4000, Phoenix Technologies Inc., Canada). Six wide-angle LEDs were placed on the shaved lateral aspects of the right forelimb using double-side adhesive tape: the greater tubercle of the humerus (shoulder joint), approximate elbow joint center, ulna styloid process (wrist joint), base of the fifth metacarpals (metacarpophalangeal joints, MCP), tip of the middle toe, and the trunk anatomical landmark the right scapula. The definitions of forelimb joint angles and the segment orientation are shown in Fig. 2 B. Three-dimensional positions of LEDs were recorded at 111.1 Hz throughout the duration of the

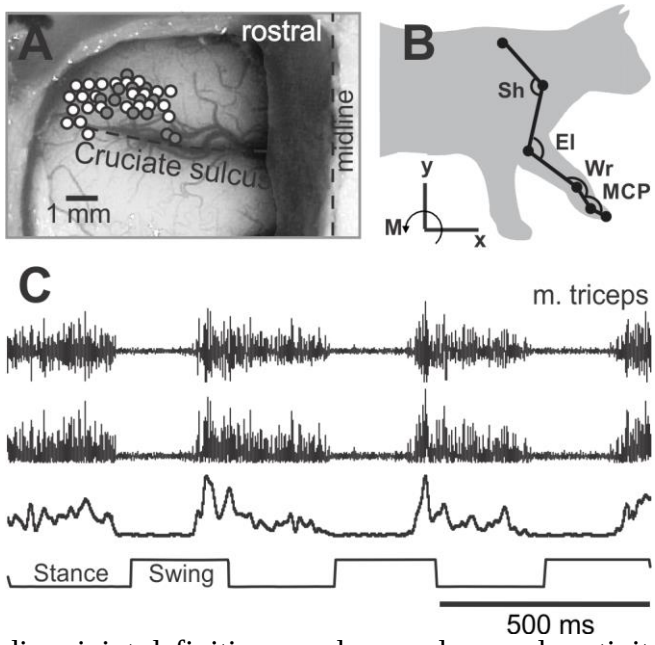

Figure 2: Area of recording, joint definitions, and example muscle activity. A: Area of recording in the forelimb representation of the left motor cortex. Microelectrode entry points into the cortex were combined from cat 1 (dark circles) and cat 2 (white circles) and superimposed on a photograph of cat 2 cortex. B: Markers placement for kinematics recording (see text for details) and definition of forelimb joint angles. C: An example of EMG recording and initial waveform processing. Raw EMG signal (top trace) was rectified (middle trace) and smoothed using central moving average with a time window of 20 ms (bottom trace) prior to analysis.

experiment. Accuracy of measuring distances on a rigid test object was better than 2.3 mm. Joint dynamics were calculated using provided functions from the VZ Analyzer software package. Kinematics were analyzed using a minimum of 10 strides of the same condition, all recorded during the same testing session, and compared between the tasks.

Processing of EMGs

Muscle activity was pre-amplified using miniature preamplifiers on the cat's backpack. The activity was additionally amplified and filtered (30 – 1500 Hz band pass) using CyberAmp 380 amplifier (Axon Instruments), sampled at 3 kHz, and stored on a computer hard drive. For analysis, raw EMGs were full-wave rectified and averaged using a central moving average with a time window of 20 ms (Fig. 2C). For each locomotor task (Fig. 1B), muscle activity was averaged over 10-40 strides recorded during the same testing session, and compared between the tasks.

Processing of neuronal activity

Neuronal data from steps that landed on the displaceable crosspiece #7 were analyzed. The onset of stance phase on crosspiece #5 was taken as the beginning of the stride to crosspiece #7. The duration of each stride was divided into 20 equal bins. Neuronal activity during strides in each of the seven conditions were compared for overall similarity using a support vector machine (SVM) trained on spiking activity during individual runs (Cortes and Vapnik, 1995; Stark

and Abeles, 2007; Jochumsen et al., 2013). Specifically, to test the similarity of a neuron discharge during a pair of conditions, data from each of the two conditions was segmented into two groups, one to train a SVM classifier (training group), and one to test the classifier (test group). To minimize uncontrolled variables such as walking speed, segmentation into training and test groups was stratified, with every other step being placed into the training (or test) group. Optimal splitting criteria between the two conditions were developed based on the neuronal activity in the training group (e.g., Figs. 3 A,B show individual traces on the top and average activity profiles at the bottom for two selected conditions). The splitting criteria were applied to the test group, and used to classify steps as belonging to one of the two conditions (Fig. 3C). Individual neuron responses were analyzed in a minimum of 20 strides, and compared between the tasks.

Histological procedures

At the termination of experiments, cats were deeply anaesthetized with pentobarbital sodium. Several reference lesions were made in the region of the motor cortex, from which neurons were sampled. Cats were then perfused with isotonic saline followed by a 3% formalin solution. Frozen brain sections of 50 μm thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet. Positions of recording tracks in the motor cortex were estimated in relation to the reference lesions.

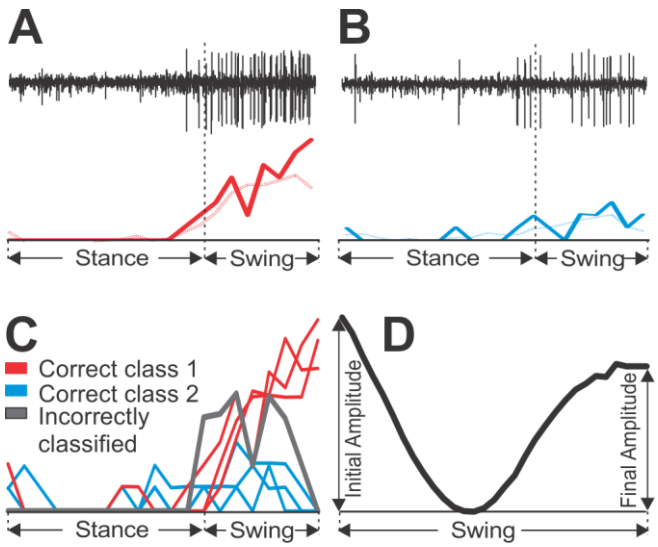

Figure 3: Support vector machine (SVM) methods and waveform analysis. A, B: Raw spiking activity of a neuron during a step cycle (top traces) recorded in two crosspiece displacement conditions, "a" and "b". The raw activity was converted into a frequency histogram of the neuron firing rate (bottom traces; thick line represents that individual step, thin line represents the average for all steps in the condition). Groups of strides made in each condition were split into training and test sets. Strides in the training set were used to develop SVM splitting criteria between the two conditions (see text for details). C: Neuronal activity during steps in the test set was classified according to these splitting criteria. If neuronal activity was correctly classified more often than would be expected by chance, the neuron was considered to discharge differently between the two conditions, thus exhibiting a "response". The classification accuracy in this example was 86%, so the neuron distinguishes between the two conditions ($p<0.05$; t-test for proportions). D: Profiles of joint angles and EMG activity were compared between conditions using the amplitude of the difference between the initial and minimum value (Initial Amplitude) and the difference between the minimum value and the final value (Final Amplitude) during the swing phase of the step cycle. A typical averaged trace of elbow joint movements during the swing phase is shown.

Statistical Analyses

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

Each recorded neuron was analyzed individually, and neuronal populations collected from each cat were compared in aggregate to ensure that neuronal properties were similar between subjects. To assess overall differences in neuronal activity between tasks, the prediction accuracy of SVM methods was tested. If SVM methods correctly identified which group a particular step belonged to more often than would be expected by chance (Fig. 3C), the neuron was considered to distinguish between the two conditions. Theoretical chance levels for classifying between conditions are 50%, and to test for classifier bias, a bootstrapping procedure with data from the same condition was performed. This

procedure produced mean classification accuracy of 50.3%, not significantly different from the theoretical chance level. The SVM procedure was repeated for all combinations of conditions (n= 21). To assess bin-wise differences in neuronal activity between tasks, an unpaired t-test was used with a significance level of $p<0.05$. To assess the significance of correlation, the t-test was applied to the Fisher transformation of Pearson's R coefficient.

4.1.4 RESULTS

Recordings of the activity of 151 neurons from layer V of the motor cortex, 2 forelimb muscles, and forelimb kinematics were obtained from two cats. The activity of 114 neurons was recorded during all seven conditions (Fig. 1B); the activity of the remaining 37 neurons was recorded only during control and four unexpected displacement conditions.

Movement adaptation strategies between known and unexpected perturbations are distinct

In each condition, the kinematics of the stride to the displaceable crosspiece were adjusted such that the limb could successfully land on the displaced platform. The kinematic strategies used, however, differed among the crosspiece displacement timing during adjustments of steps in both directions. During the unexpected stride length modification, to make the step either smaller or larger than normal, the cat produced accurate steps by altering the duration of the swing, making it shorter or longer, respectively (Fig. 4A). In the condition

when the displacement of the crosspiece was known as soon as the cat emerged on the ladder, however, the cat produced accurate steps by increasing or decreasing limb transfer velocity, respectively, without altering the duration of the swing phase (Fig. 4B). Additionally, in this condition, there was evidence of planning: during the stride preceding the disturbed one, the cat stepped on the crosspiece #5 either slightly further along in the direction of motion (when a larger step on crosspiece #7 was upcoming) or less far along, when a smaller step on crosspiece #7 was required (Figs. 4C).

The joint kinematics of disturbed steps also differed based on condition (Figs. 4D-K). In the shoulder joint, for example, there was a significant difference in joint position between the known disturbance and control conditions in the middle of the swing phase, denoted with a red star (Figs. 4D, H). In addition, the initial and final amplitudes of the joint movements was significantly higher for the known disturbance condition (Figs. 4L,M). Across all joints and conditions, two major differences were found during the swing phase of the disturbed step. First, during the known displacement condition, kinematic alterations were observed during the early parts of the swing phase (red stars in Figs. 4D-G, H and J), while during the unexpected displacement conditions, kinematic adaptations only began immediately prior to footfall (Figs. 4G, H). Second, while most joints exhibited changes during perturbed steps in the known displacement condition (Figs. 4 D-F, H and J – red stars), alterations during the unexpected displacement conditions that allowed less time for adaptation were largely restricted to more distal joints (Figs. 4 G,K,O).

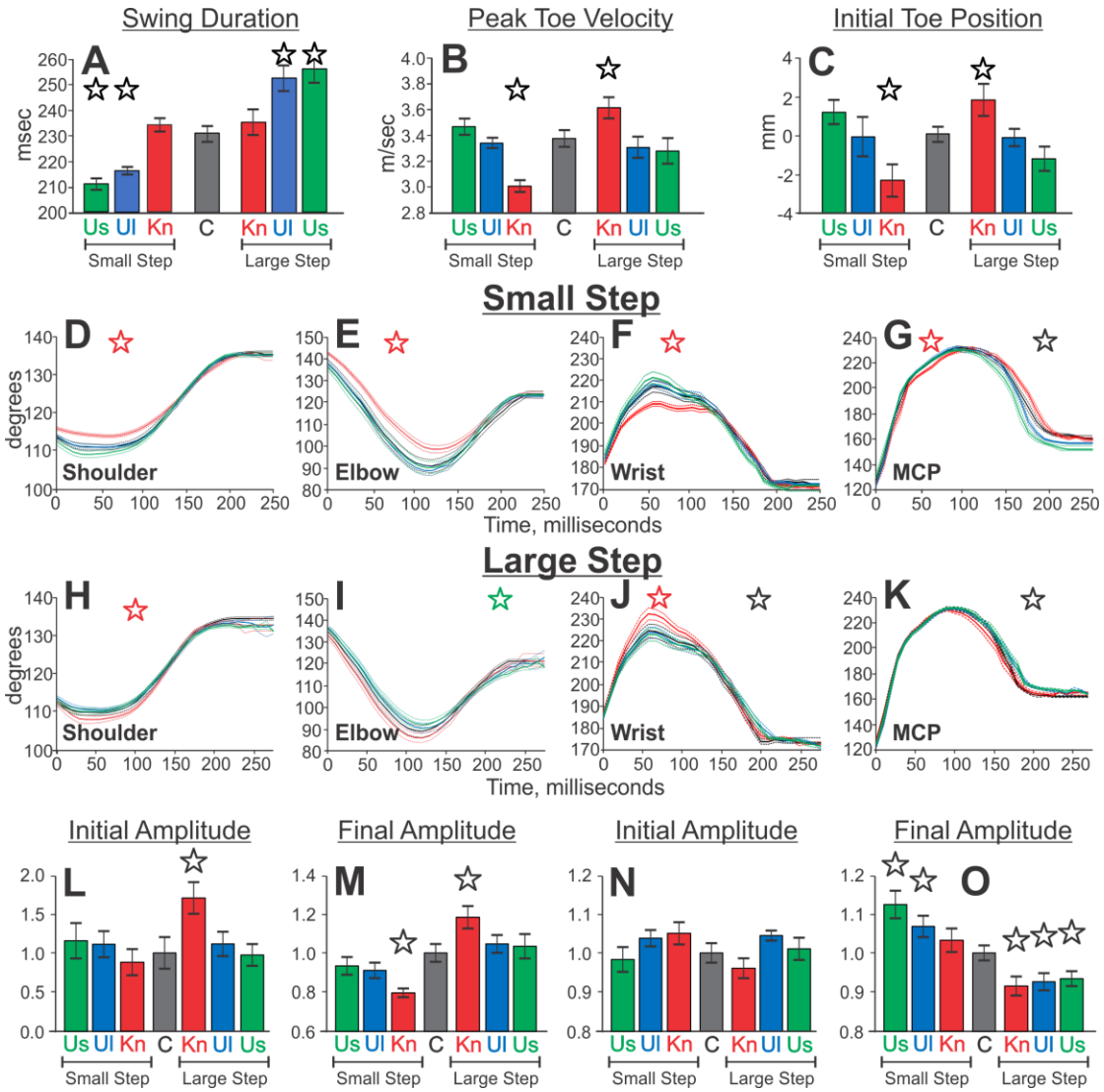

Figure 4: Kinematic strategies for making perturbed steps. A: Duration of the swing phase. B: Peak velocity of the toe during the swing phase in the direction of cat motion. C: Initial position of the toe on the crosspiece in the direction of motion, relative to control. D: Shoulder joint angle throughout the swing phase for control and smaller than normal steps. E: Elbow joint angle throughout the swing phase for control and small steps. F: Wrist joint angle throughout the swing phase for control and small steps. J: Metacarpophalangeal joint angle throughout the swing phase for control and small steps. H-K: Shoulder, elbow, wrist, and MCP joint angles throughout the swing phase for control and larger than normal steps. In D-K: representative examples obtained from one cat on one testing session are shown L, M: The initial and final amplitude for the shoulder joint angle in different conditions. N, O: The initial and final amplitude for the MCP joint angle in different conditions. Black represents the control condition (50 cm distance between crosspieces), red represents a known displacement requiring a small or large step (45 or 55 cm distance between crosspieces, respectively), blue represents an unexpected long-notice disturbance requiring such a step, and green represents an unexpected short-notice disturbance. Stars represent significant differences against the control condition; colored stars represent significant differences between a single condition and control.

Muscles respond to a change in the size of the stride

The activity of both recorded muscles (elbow extensor m. triceps, and wrist extensor EDC) during the entirety of the preparatory step from crosspiece #3 on crosspiece #5 (Fig. 1B) and the stance phase of the disturbed step on crosspiece #7 were similar among conditions (not illustrated). Both muscles, however, exhibited changes to activity during swing phase of the disturbed step, decreasing it during a small step and increasing during a large step (Fig. 5A-D). These changes were observed regardless of the crosspiece displacement condition. This could be expected, as both muscles are primarily active during the late swing and early stance phases, when kinematics were similar between conditions. However, the observed changes in terms of initial and final amplitude during the swing phase were generally consistent between unexpected long- and short-notice conditions - in 7/8 comparisons, either both were significantly different from control, or neither were (Fig. 5E-H). This degree of correspondence was not found with the muscle activity during the known displacement condition. Only in 5/8 comparisons were the changes observed in the known displacement condition in common with those in the unexpected displacement conditions.

Motor cortex neurons respond to adaptation of movement

Neuronal data was collected from 37 tracks through the motor cortex: from 13 tracks in cat 1 and 24 tracks in cat 2, sampling similar areas of the motor cortex (Fig. 2A). The activity of a total of 151 neurons (59 from cat 1 and 92 from cat 2) was analyzed. Neuronal response characteristics, as assessed by mean

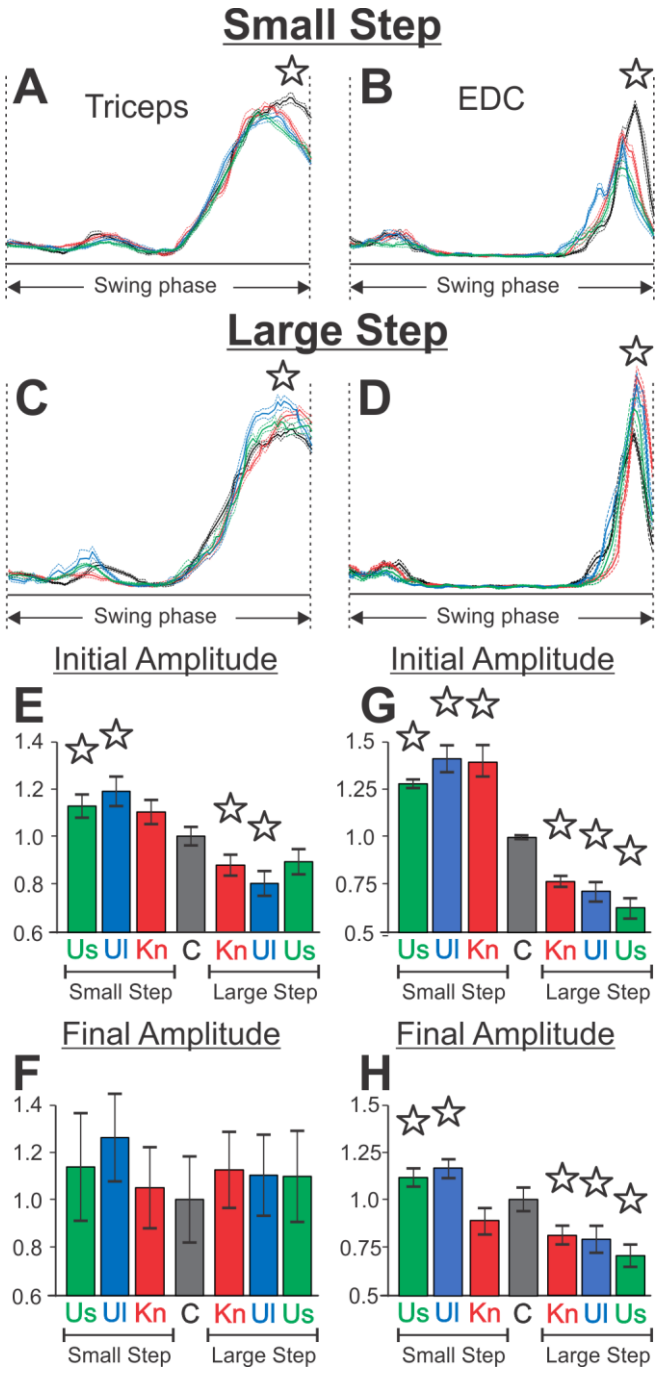

Figure 5: Muscle activity during perturbed steps. A, B: Traces of EMG activity in the right triceps medialis (A) and right extensor digitorum communis (B) muscles during swing phase of small steps. C, D: Traces of EMG activity for triceps medialis and EDC during swing phase of large steps. In A-D: representative examples obtained from one cat on one testing day are shown. E-H: The initial and final amplitudes for right triceps (E-F) and EDC (G-H) EMG activity during steps. Other designations as in Figure 4.

SVM prediction accuracy between the control and test conditions, were similar between the neuronal populations collected from each cat (57.2±3.1% vs. 58.7±2.5%, $p>0.05$). Sixty nine percent of all neurons (91/151) responded to the disturbance of the stride on the motorized crosspiece. Neurons exhibiting a response fell into two major categories. Unidirectional neurons, representing 40% of the total population, responded only to large or small steps, but not both, and bidirectional neurons, representing 30% of the population, responded to both large and small steps, most often increasing activity during large steps and decreasing activity during small steps. Examples of each response type are shown in Figures 6A and 6B, respectively.

Neuronal response likelihood depends on whether disturbance is known or unexpected

The percentage of neurons responding during the disturbed step varied with the timing of crosspiece displacement. The likelihood of a neuronal response under either of the unexpected displacement conditions were similar, and were considerably more common than responses under the known displacement condition, especially during large steps (Fig. 6C). However, neurons commonly responded to more than one displacement condition, and more than 20% of the total population responded to steps over the displaced crosspiece during all three timing conditions. Of those that responded to only two timing conditions, neurons responding to both unexpected displacement conditions were the most common subtype, composing 11% of the total population (Fig. 6D).

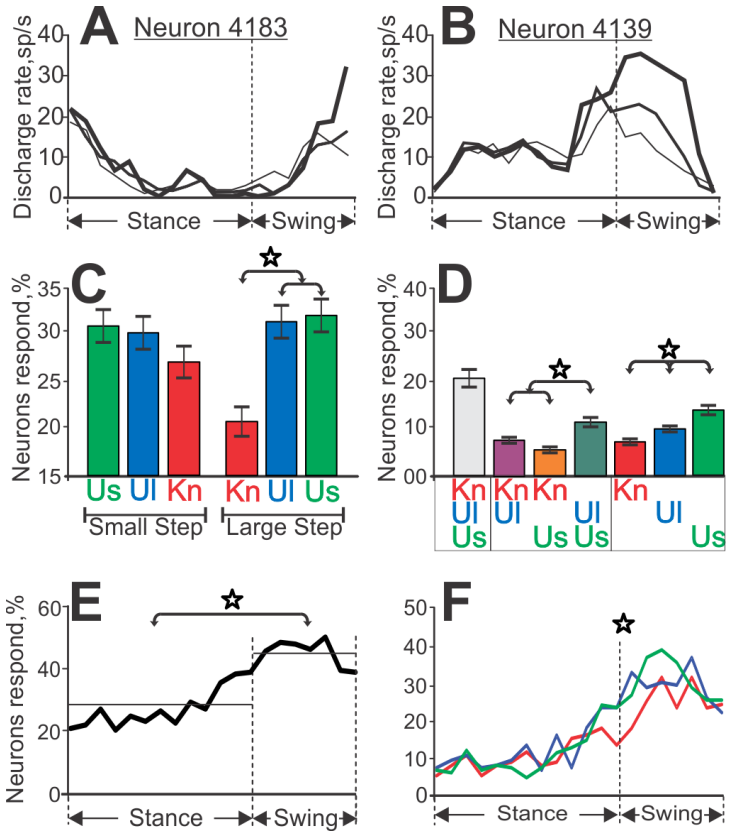

Figure 6: Neuronal response characteristics during perturbed steps. A, B: Example of SVM-identified neuronal responses to stepping over a disturbed rung for a unidirectional neuron (Neuron #4183, A) and a bidirectional neuron (Neuron #4139, B). Thick traces show mean activity during large steps (higher activity), thin traces show that during small steps (lower activity), and medium-thick traces show mean activity during control steps. C: Percentage of neurons showing a response during the disturbed step in the known, unexpected long-notice, or unexpected short-notice crosspiece displacement condition for small, normal, and large steps. D: Percentage of neurons showing a response to single or multiple displacement conditions during the disturbed step. For example, the orange bar shows the percentage of neurons responding to the known and short-notice unexpected conditions, but not the long-notice unexpected condition. E: Percentage of neurons exhibiting significantly different activity (t-test, $p<0.05$) during different phases of the disturbed step. Horizontal bar represents the mean percentage responding during the stance and swing phases. F: Percentage of recorded neurons exhibiting significantly different activity (t-test, $p<0.05$), during the disturbed step in each bin between the control condition and crosspiece displacement conditions.

With regard to the stride cycle, neuronal responses during the disturbed step were considerably more common during the swing phase. as the percentage of neurons exhibiting a response during this phase was nearly double the proportion responding during the stance phase (Fig. 6E). While this characteristic was observed across all conditions, responses during the late stance and early swing phases were significantly more common for the unexpected displacement conditions (Fig. 6F).

Neuronal responsivity is direction-sensitive but not latency-sensitive

The relationship between SVM classification accuracy during the various disturbed conditions was compared to determine if neurons that responded in one disturbance condition would respond to other disturbances that were similar, either in the direction of crosspiece displacement, or the timing at which the crosspiece displacement occurred. Representative scatterplots testing direction- and latency-sensitivity are shown in Figs. 7A,B. Neurons exhibiting a response during a short or long step were more likely to exhibit a response during steps of the same size. This relationship was uniformly stronger for larger-than-normal steps (Fig. 7C). However, neurons exhibiting a response during short or long steps displaced at a particular point along the cat's progression were no more or less likely to exhibit a response when the crosspiece was displaced in a different direction at the same point (Fig. 7D).

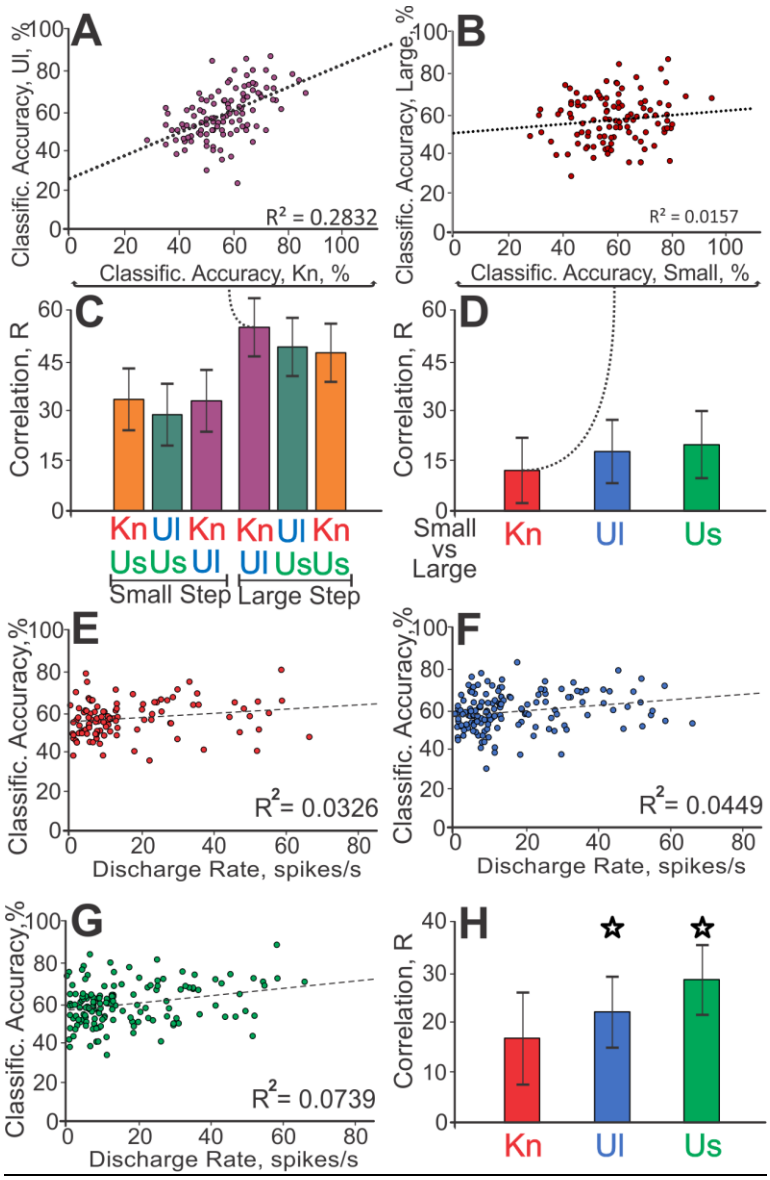

Figure 7: Characteristics affecting neuronal responsivity. A-B: Representative scatter plots comparing SVM classification accuracy for large steps in the known and unexpected long-notice conditions (A), and for small and large steps in the known condition (B). Dotted lines are the regression best-fit trend lines, with correlation R2 of the best fit shown in the bottom right. C-D: Comparison of Pearson correlation (R) between SVM classification accuracy for two crosspiece displacement conditions. E-G: Scatter plots comparing mean neuronal discharge rate during the swing phase of the disturbed step with SVM classification accuracy for the known-displacement condition (E), unexpected long-notice condition (F), and unexpected short-notice condition (G). Dotted lines are the regression best-fit trend lines, with correlation R2 of the best fit shown in the bottom right. H: Comparison of Pearson correlation (R) between neuronal discharge rate during the swing phase of the control condition and SVM classification accuracy for crosspiece displacement conditions. Color designations as in Figure 6.

Responses to unexpected disturbances preferentially involve neurons that are already active

The relationship between SVM classification accuracy and a variety of neuronal activity characteristics, including discharge rate, modulation with respect to the stride cycle, and preferred phase of discharge, were compared to determine which characteristics might predict a neuron's responses to disturbance in the stepping. Of these characteristics, only discharge rate was found to exhibit a consistent relationship with neuronal responses. Figures 8 A-C show scatter plots of neuronal discharge rate during swing phase and mean SVM classification accuracy for known, unexpected long- and short-notice displacement conditions. As the time available for stride modification decreased, swing discharge rate became increasingly related to the likelihood of neurons responding to a larger or smaller step (R^2=0.0326 vs. R^2=0.0449 vs. R^2=0.0739, respectively; Figs. 7E-G). However, this correlative relationship was only significant for unexpected disturbances (Fig. 7H). Therefore, in either unexpected displacement condition, the neurons which respond tend to be those which would be active even if the step were not disturbed, while in the known displacement condition, many neurons respond which would not be active if the step were not disturbed.

Neuronal responses to unexpected short-notice disturbances are often unique

The previous sections discussed the character of neuronal responses between the control condition and a disturbed condition involving crosspiece displacement. In this section, neuronal responses between two disturbed

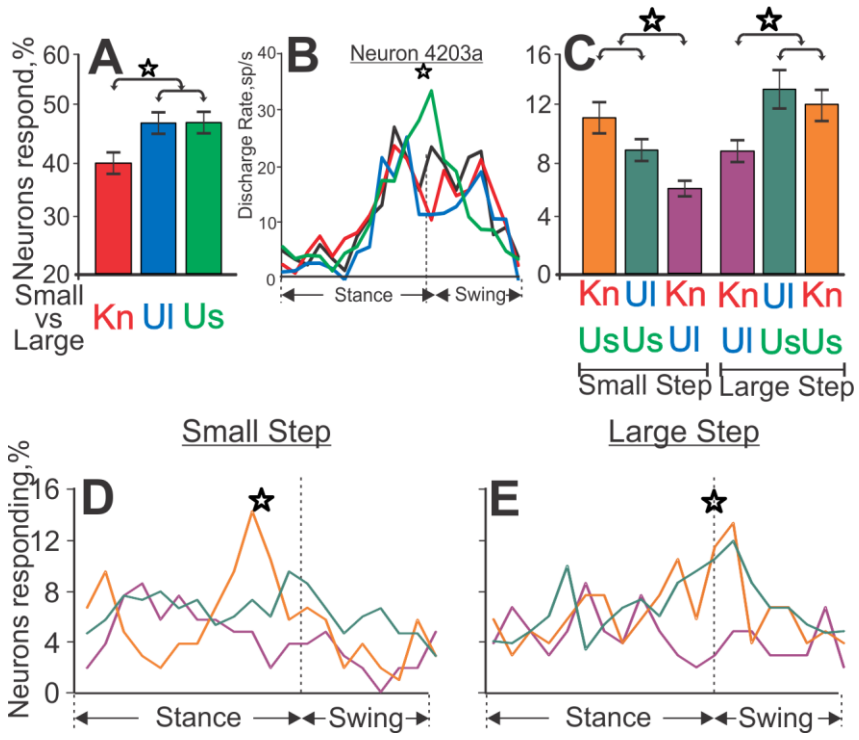

Figure 8: Directional sensitivity in neuronal responses. A: Percentage of neurons exhibiting a different response between crosspiece displacements occurring at the same point of the cat's progression along the ladder, but in different directions. B: Example neuron exhibiting a response only during the crosspiece's unexpected short-notice displacement. C: Percentage of neurons exhibiting a different response between crosspiece displacements in the same direction, but occurring at different times. D, E: Percentage of neurons exhibiting significantly different activity (t-test, $p<0.05$) during different phases of the disturbed step for small (D) and large steps (E).

conditions are compared. Most neurons distinguished between short and long steps when crosspiece displacement occurred at the same time (64% of neurons, 99/151). Such differences were more common in either of the unexpected displacement conditions (Fig. 8A). On the other hand, it was far less common for neurons to exhibit different responses to displacements occurring in the same direction, but with different timing: only 25% of neurons (38/151) responded in this manner. However, such "unique" responses between conditions involving steps of the same size did occur, and an example is shown in Fig. 8B. Unique responses were most common for the unexpected short-notice displacement condition (Fig. 8C). Most frequently, the difference in the neuronal responses occurred during the stance-to-swing phase transition, for both small and large steps (Figs. 8D-E).

4.1.5 DISCUSSION

It is apparent from our data that the strategies used to adapt to constraints in the walking environment differ depending on whether those constraints are known or unexpected. These differences were observed at all the kinematic, muscle, and neuronal levels. The strategies used were consistent for the same time condition whether crosspiece displacement caused steps to be made longer or shorter. The distinctions between strategies employed in different timing conditions for both long and short steps persisted despite the fact that those crosspiece displacement conditions imposed identical constraints on foot placement. These differences strongly suggest that distinct motor control

processes are at work to adapt to the constraints imposed in the known displacement conditions versus unexpected displacement conditions.

Although this study is the first to directly demonstrate that different neuronal, muscle, and kinematic mechanisms are employed between unexpected corrective gait modifications and planned gait modifications, its results agree with those found by others in investigations of movement adaptations. Similarly to Patla and colleagues (1999), we also found that the imposition of constraints to adaptive motor behaviors through known and unexpected displacement conditions did indeed lead to substantially different kinematic strategies. In temporally constrained conditions, such as the long- or short-notice conditions, the minimal kinematic adjustments necessary to successfully adapt to the disturbance were preferred as Patla and colleagues (2004) demonstrated for visually guided trajectory modifications during walking in humans, in contrast with the more extensive modifications observed in the known displacement condition. In addition, our results support the hypothesis of Weerdesteyn and colleagues (2004) that distinct neuronal mechanisms may be employed between unexpected and planned movement modifications, which was advanced based on biomechanics data.

The strategies we observed during unexpected and planned modifications are similar to those reported in other investigations. Similarly to Drew (1988), we found that gait adaptations involving increases to the length and trajectory of the step (large steps) involved increased EMG activity, and, commonly, increases in the discharge rates of motor cortical neurons. We also found that when the length

and trajectory of the step decreased (small steps), decreased EMG activity and neuronal discharge rates were often observed. During the known displacement condition, we found similar proportions (~40%, Fig. 6) of motor cortical neurons responding to volitional gait adjustments in landing on crosspieces located closer to or farther away from the cat, as Amos and colleagues (1990) found for landing on crosspieces displaced vertically higher or lower. The kinematic and EMG profiles shown in this report are consistent with those of previous reports from our laboratory and other investigators (Drew 1988; Prilutsky et al., 2005; Krouchev et al. 2006; Gregor et al. 2006; Beloozerova et al. 2010). However, while Marple-Horvat and colleagues (1993) commonly observed fast motor cortical responses to unexpected crosspiece displacement at approximately 40 ms following displacement onset, we observed no such response. This is likely due to the fact that their paradigm involved displacement of the crosspiece only after the forelimb was placed upon it, likely activating proprioceptive feedback circuits, while ours involved displacement in advance of paw placement.

The differences in neuronal adaptations found in this experiment suggest that a dynamical model of the motor cortex, which has been posed for reaching tasks (e.g. Churchland et al. 2010, 2012), could potentially be generalized to locomotion as well. Under this framework, there is an optimal neuronal preparatory state for the generation of future movement tasks (Churchland and Shenoy, 2007). When a preparatory state is incorrect, due to unexpected shifts in target location, neuronal activity rapidly adjusts to converge with the optimal preparatory state prior to initiation of reaching (Ames et al. 2014). We likewise

observed that neuronal responses to displacements in the same direction were often similar regardless of the amount of time for preparation (Fig. 6), suggesting that a similar transition is occurring for many motor cortical neurons. However, a substantial population responded differently to displacements in the same direction based on displacement timing, and when displacements were unexpected, neuronal responses preferentially involved neurons that were already active during the control condition (Fig. 8). This discrepancy may reflect a distinction between the tasks studied: in a reaching task, the body configuration is much more static prior to reaching initiation, while during the studied locomotion task, the body is undergoing continual motion.

The observed preference for already-active neurons in the unexpected displacement conditions may reflect complexities in integrating motor adaptations to movements that are currently in progress. It might be expected that the comparatively extensive alterations observed to kinematics in known-displacement conditions would require more substantive changes to motor cortex activity than in the unexpected displacement conditions. This was not the case. Rather, neuronal responses in the known condition were significantly less frequent than in unexpected displacement conditions (Fig. 6C). This apparent discrepancy could involve differences in how corrective motor commands are generated in these two situations. The posterior parietal cortex is involved in planning gait adaptations during complex locomotion tasks (Andujar et al. 2010, Marigold et al., 2011), and lesions to this structure compromise gait modifications (Lajoie and Drew, 2007). Because many neurons in the PPC discharge well in

advance of gait modifications, this structure may selectively activate efficient synergies of neurons (Drew et al. 2008), or activate alternate descending tracts involved in corrective motor commands, such as the rubrospinal (Pettersson et al. 1997) or reticulospinal tracts (Pettersson and Perfiliev, 2002). This would require less extensive motor cortical adaptations to successfully place the paw on the displaced rung. During the unexpected displacement conditions, due to time constraints, already-activated synergies could be modified to accommodate the rung displacement, regardless of whether these synergies are the most efficient for the task. This would entail modification of already active neurons in the unexpected displacement conditions, which was observed, and activation of otherwise inactive neuronal populations, as was observed in the known displacement conditions (Fig. 7E-H).

It is, however, difficult to reconcile the results of this experiment with the expected outcomes from optimal feedback control theory (OFCT) using an effort-minimizing cost function (e.g. Todorov, 2004; Diedrichsen et al., 2009). In the known displacement condition, one might expect that the trajectory modifications to step onto the displaced crosspiece would be "optimal" and involve the minimal energetic cost relative to the control step, and that the motor control strategy used in the short-notice condition might be "sub-optimal" and involve higher energetic cost, as the cat must adapt its walking trajectory immediately and has little time for preparation. However, the observed kinematic and muscle responses do not correspond to this prediction. The observed kinematic responses were far more extensive in the known condition, involving both proximal and distal joints, while

the responses in the unexpected condition involved only the most distal joints (Fig. 4), and EMG responses were generally similar regardless of displacement timing (Fig. 5).

It appears more likely that the global motor control strategy during locomotion, perhaps including selection of synergies, is determined well in advance of the step in question, and may not correspond to energetic cost minimization. This global strategy may then be tuned to arrive at a locally optimal control strategy based on any unexpected or emergent constraints imposed on the behavior. Local optimality may be defined by the minimal kinematic adjustment required to successfully accommodate the disturbance (Patla et al., 2004), the simplest adjustment to compute, given the hierarchical relationship between joints (Dounskaia, 2005), or the fastest modification to enact (Ghez and Gordon, 1995). However, it appears that there is a fundamental distinction between the neuronal, muscular, and kinematic motor control strategies employed when a constraint is known and planned for, and when one unexpectedly emerges and must be adapted to.

4.2 Additional Investigation

CONTROL OF INTERSEGMENTAL DYNAMICS DURING KNOWN AND UNEXPECTED PERTURBATIONS OF COMPLEX LOCOMOTION

4.2.1 ABSTRACT

Most natural movements require coordinated action at multiple joints to be successful. The roles of and interactions between joints in the forelimb of the cat were investigated during accuracy-dependent locomotion tasks involving known and unexpected trajectory modifications. Intersegmental dynamics of forelimb joints, including passive and active torques acting at each joint, were assessed as cats walked over a raised horizontal ladder. One of the crosspieces was motorized, and would displace before the cat stepped on the ladder or at different points along the cat's progression over the ladder, either toward or away from the cat. The cat was required to change the trajectory of the forelimb and make a shorter or longer step to land on the crosspiece.

We found that locomotor behaviors involved coordinated movements of joints during the swing phase of the step, consistent with a leading joint hypothesis (LJH) for joint control. Limb movements during the swing phase of the step were primarily produced by muscular contractions acting on the shoulder, while the passive torques acting on the elbow and wrist were regulated in order to stabilize limb trajectory to land on the ladder. When the crosspiece displaced, two types of motor adaptation strategies were used to successfully place the paw on the crosspiece, based on whether crosspiece displacement was known or unexpected. Responses to known and unexpected displacements exhibited hallmark features of feed-forward versus feedback-driven motor control

strategies, respectively. Both strategies involved coordinated adaptations to multiple joints and were consistent with LJH. Therefore, motor control processes that adapt locomotion to the constraints of the environment produce complementary modifications acting at multiple joints simultaneously, regardless of whether those adaptations are planned ahead of time or unexpectedly become necessary.

4.2.2 INTRODUCTION

A significant issue in motor control is that the multi-joint structure of limbs permits a large number of potential movement strategies to accomplish a given task, often referred to as the degrees of freedom problem (Bernstein 1967). As such, many investigations have focused on how tasks requiring movements at multiple joints are organized and coordinated (e.g. Hollerbach and Flash 1982; Galloway and Koshland 2002; Debicki and Gribble, 2004). Locomotion is a prime example of such a movement, and the kinetics governing locomotor movements in various scenarios have been investigated in humans (Patla and Prentice, 1995; Zernicke et al. 1991; Ulrich et al. 1994) and in the hindlimb of the cat (Wisleder et al. 1990; Hoy and Zernicke, 1985, Prilutsky et al. 2005; McFayden et al. 1999).

Many investigations have shown that intersegmental dynamics are exploited for production of movements involving multiple joints, in humans (Dounskaia et al. 1998; Galloway and Koshland, 2002; Hirashima et al. 2003) and cats (Hoy and Zernicke., 1996; Hoy et al. 1985). These and many other studies show that during multi-joint movements, one joint is commonly responsible for active generation of force, and provides motion at the other joints through mechanical interactions. This organization of control of multi-joint movements was summarized as the leading joint hypothesis (Dounskaia 2005; 2010). This type of control has been suggested to be feedforward (Dounskaia 2005; Goble et al. 2007), relying on the internal models of limb inter-segmental dynamics (Wolpert and Kawato 1998). However, most investigations have focused on

preplanned, unperturbed movements. It has never been studied whether joint control changes when movement quickly adjusts to unexpected perturbations. .

In this study, we addressed this question by investigating the intersegmental dynamics in the forelimb as cats walked along a raised horizontal ladder, a complex locomotor task that involved accurate limb placement. Accuracy constraints are quite common during behaviors in natural environments: locomotion often involves foot placement on support surfaces with limited length or width, and reaching and grasping objects relies on precise positioning and orientation of the hand. In this locomotion task, one of the crosspieces was motorized, and could be displaced prior to the cat stepping on the ladder or at different points during the cat's progression along the ladder, either towards or away from the cat. When crosspiece displacement occurred prior to the cat stepping on the ladder, the cat observed the final position of the crosspiece only in its final position, and could plan ahead. When crosspiece displacement occurred while the cat was approaching it on the ladder, the cat observed the crosspiece move, and needed to alter its trajectory to successfully land on the crosspiece in its new position. To successfully continue along the ladder, cats needed to make a longer or shorter step.

This experiment allowed us to pursue two goals. First, previous studies of inter-joint coordination during locomotion examined the locomotor task in a simplified and artificial environment, over a flat surface with no environmental complexities, both in humans (Zernicke et al. 1991; Ulrich et al. 1994) and cats (Hoy and Zernicke, 1985; Wisleder et al. 1990; Prilutsky et al. 2005). By analyzing

locomotion over a raised horizontal ladder, we were able to examine mechanisms underlying inter-joint coordination during locomotion with realistic complications and constraints. Second, the motor adjustments that the cat used when the crosspiece was displaced at various time points allowed us to study control of multi-joint movements performed in response to perturbations.

4.2.3 METHODS

Recordings were obtained from two adult male cats (weight 11 and 8.5 lb). Methods of data collection have been previously reported (Prilutsky et al., 2005; Beloozerova et al., 2010; Stout and Beloozerova, 2012, 2013) and will be described briefly below. All experiments were conducted in accordance with NIH guidelines and with the approval of the Barrow Neurological Institute Animal Care and Use Committee.

Locomotion tasks

Positive reinforcement (food) was used to adapt cats to the experimental situation and to engage them in locomotion (Skinner, 1938; Pryor, 1975). A walkway, 2.5 m long and 0.3 m wide on each edge, served as an experimental chamber (Fig. 1A). Cats passed sequentially and repeatedly through the two corridors of the chamber in a counter-clockwise direction. In one of the corridors, the floor was flat, while the other corridor contained a horizontal ladder with 10 crosspieces. Crosspieces were spaced 25 cm apart, which is half of the mean stride length observed in the chamber during locomotion on flat floor at a self-selected pace (Beloozerova and Sirota, 1993; Beloozerova et al., 2010). The tops of crosspieces were flat and 5 cm wide. The width of the crosspieces was chosen

to slightly exceed the cat's mean foot length (3 cm), so that cats had full foot support on a crosspiece. Crosspieces were elevated 6 cm above the floor of the chamber. One crosspiece (the seventh from the left side of the ladder, Fig 1A) was connected to an electric motor. When displaced, it was shifted 5 cm in either direction, such that there was no overlap between the crosspiece's position before or after the displacement. Displacement was completed within 145 ms of initiation. On the side of the crosspiece facing the cat, there was a yellow LED lamp. It was lit as soon as the triggering of the crosspiece displacement occurred, regardless of the direction of the initiated move. This illumination attracted the cat's attention to the crosspiece when it was displacing. Auditory cues from the activation of the motor also alerted the cat to a rung displacement. Regardless of the crosspiece's displacement or the cat's performance, after each round of walking, the cat received food in a feeding dish located in one of the chamber's corners.

This apparatus allowed us to compare several locomotion tasks by displacing the crosspiece at various time points along the cat's progression. Only passages where the cat stepped on the displaceable crosspiece with right feet were studied. Seven conditions were used (Fig. 1 B): control, when the crosspiece remained in its original location; and three groups of conditions where the crosspiece was displaced either toward or away from the cat at different times along the cat's progression through the chamber, and the cat had to make a larger or smaller step to successfully traverse the ladder. In "known displacement" (Kn)

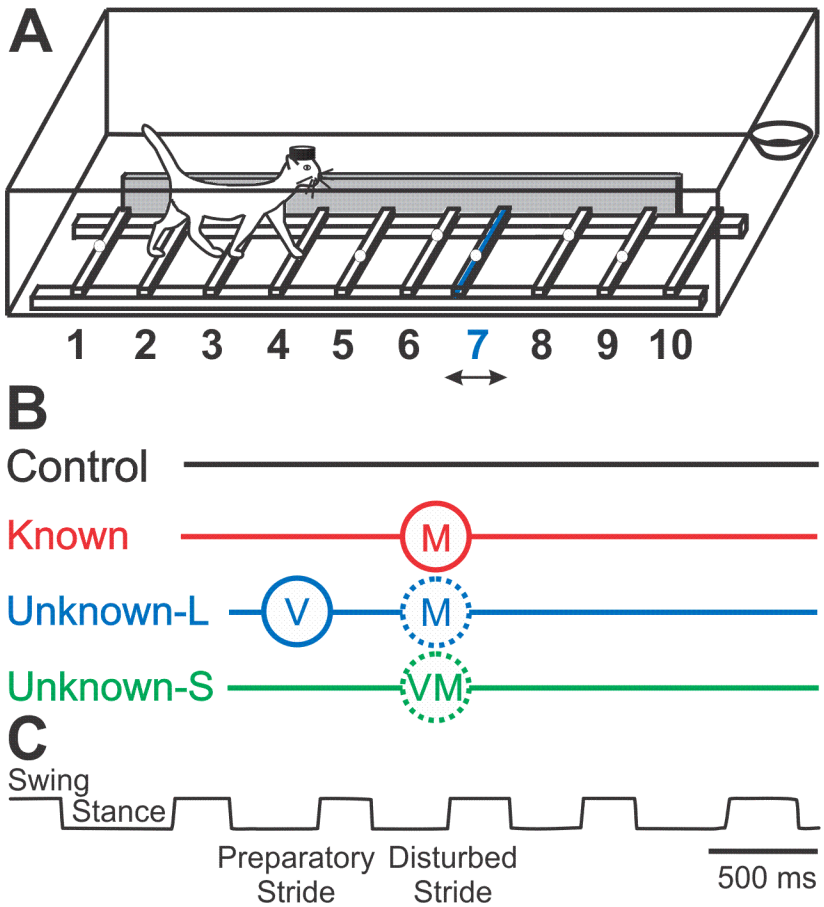

Figure 1: Experimental Design. A: Cats walked through a rectangular, two-side chamber. One side contained a raised horizontal ladder, with one motorized crosspiece (#7, red) that was displaced at different times as the cat walked in the chamber. B: A total of seven conditions were analyzed: a control condition with the crosspiece remaining in its central position, when all crosspieces were equally spaced 25 cm apart, and crosspiece movements away or towards the cat either before the cat stepped on the ladder (two "known" displacement conditions, Kn), or one stride away from it (two "unknown" long-notice displacement conditions, Ul), or during the current stride while the cat was about to initiate limb transfer to crosspiece #7 (two "unknown" short-notice displacement conditions, Us). Circles represent where the cat was along the ladder when the crosspiece displaced ("V" – visual stimulus) and when the step onto the disturbed rung was made ("M" – motor adaptation). C: Example of the step cycle during locomotion on the ladder.

conditions, the crosspiece was displaced while the cat was at the feeder. In these conditions, the cat did not see movement of the crosspiece, as the ladder was in its final configuration when the cat stepped onto it. The cat had two full strides: a stride from crosspiece #1 onto crosspiece #3, and a stride from crosspiece #3 onto crosspiece #5, before making a larger or smaller step to reach the displaced crosspiece #7. In unexpected "long-notice" conditions (Ul), the rung was displaced when the cat's right forelimb stepped on crosspiece #3. The cat had one full locomotion cycle to complete before needing to adjust. In unexpected "short notice" conditions (Us), the crosspiece was displaced when the cat's right forelimb stepped on crosspiece #5 and the very next transfer of the forelimb had to be adjusted. A sequence of 21 conditions was repeated pseudorandomly by a computer program, occasionally resetting at random times, which were different for different experimental days and subjects. All conditions were presented an approximately equal number of times and the cat could develop no fore-knowledge of which condition would be presented.

Cats were accustomed to wearing a cotton jacket, a light backpack with connectors and preamplifiers, and an electro-mechanical sensor on the right paw for recording duration of swing and stance phases of stride. They were also trained to wear LEDs on lateral aspects of the right forelimb. The floor in the chamber and the crosspieces of the ladder were covered with an electro-conductive rubberized material. During locomotion the duration of the swing and stance phases of the right forelimb was monitored by measuring the electrical resistance between the right foot and the floor with the electromechanical sensor

(Fig. 1 C). The passage of the cat through the beginning and end of each corridor was monitored using infrared photodiodes.

Motion capture and kinematic analysis

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

Body segment parameters, including forelimb segment masses and moments were estimated according to regressive relationships (Hoy & Zernicke 1985). Body characteristics for each cat, including body mass, segment lengths, estimated segment mass, and estimated moment of inertia for each forelimb

segment are shown in Table 1. In addition, contributions of individual joint movements to endpoint velocity of the paw in the direction of motion were assessed. These were computed using formulae adapted from Kim et al. (2009) to incorporate wrist contributions, as well as the contributions of body translation in the direction of motion. These formulae are included in Appendix A.

Kinetic Analysis

Kinetic analysis was employed to assess intersegmental dynamics and joint control strategies during the tested locomotor tasks. Inverse dynamics equations adopted from Hirashima et al (2003) were used to calculate torques at the shoulder, elbow and wrist. Four components of torques were calculated at each joint: net torque (NET_i, i = S, E, and W for shoulder, elbow, and wrist, respectively), interaction torque (INT_i), gravity torque (GR_i), and muscle torque (MUS_i). The four torque componens are defined by the following relationship:

$$NET_i = MUS_i + INT_i + GR_i$$

Net torque is proportional to the angular acceleration occurring at a given joint. Interaction torque represents passive torques generated by mechanical interactions among the body, upper arm, forearm, or intersegmental dynamics. Gravity torque represents passive torques generated by gravitational force acting on the limb's segments. Muscle torque was computed as the difference among NET, INT, and GR. It represents active torques generated by contractions of muscles acting on the joint as well as passive torques caused by elasticity of muscles and ligaments at the joint. The signs of the torques were determined by the definition of the joint angles (Fig. 2). Torques acting into joint flexion were positive at the shoulder and

elbow and negative at the wrist. When comparing the differences between torques generated during perturbed steps and the control condition, gravity torque and interaction torque were combined together into a passive torque (PT_i).

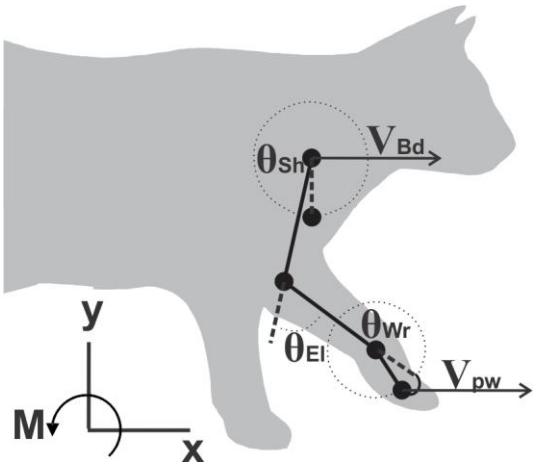

Figure 2: Joint angle definition and velocity components. Joint angle definitions were used to match those of Hirashima (2003) for calculation of torque components. Contributions to endpoint (paw velocity, Vpw) were calculated for each joint, as was the contribution of body motion (Vbd).

Table 1: body parameters for cats 1 and 2.

Cat 1	Length (cm)	Mass (g)	Moment	Center of Mass (cm)
Scalpula	9.0	97.9	677.4	4.29
Shoulder	10.3	112.0	905.6	5.03
Elbow	11.0	60.7	691.1	5.00
Wrist	3.5	13.5	10.6	1.83
Paw	3.5	7.3	22.3	1.75
Cat 2	**Length (cm)**	**Mass (g)**	**Moment**	**Center of Mass (cm)**
Scalpula	7.5	77.8	488.1	3.57
Shoulder	10.0	97.2	788.2	4.88
Elbow	10.0	48.2	452.0	4.54
Wrist	3.0	9.7	7.8	1.57
Paw	3.0	6.0	13.6	1.50

RESULTS

Recordings of forelimb kinematics were obtained from two cats. Figure 3 shows typical joint movements during the swing phase of the control step. The shoulder initially produced little motion and then flexed starting from the middle of the swing phase (Fig. 3A). Both the wrist and the elbow underwent flexion during the beginning of the swing phase, followed by extension (Fig. 3B,C). This pattern of joint motions during the swing phase of the forelimb is similar to that previously reported by Prilutsky et al. (2005).

Torques around each joint and contributions to endpoint velocity during the control condition are examined first, and changes to torques and endpoint velocity contributions in each of the test conditions are examined after.

Endpoint velocity contributions during the swing phase of the control step

Torques generated around the shoulder, elbow, and wrist joints, as well as endpoint velocity contributions during the swing phase of the step are shown in Fig. 4. The endpoint accelerated in the direction of motion during the early portion of the swing phase, attaining maximum speed at about 20% of the way through the swing phase, held this speed for about 50% of the cycle, and decelerated during the final 30% of the swing phase (Fig. 4A). The contribution to this velocity due to translation of the body was roughly consistent throughout the entire swing phase. Increases and decreases in endpoint velocity were primarily due to rotation of forelimb joints, principally at the shoulder and elbow joints. During the first 40% of the swing phase, the elbow joint rotation was primarily

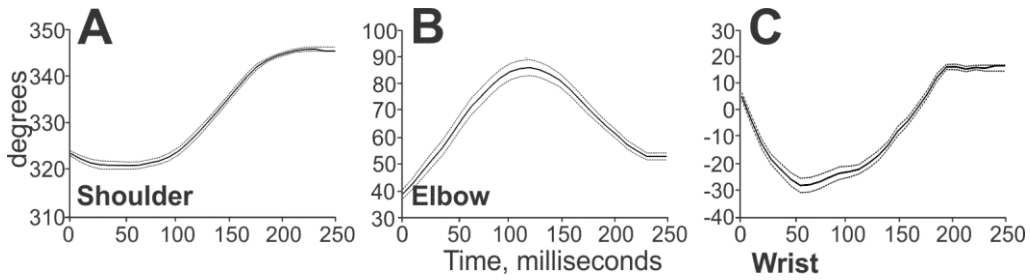

Figure 3: Joint angle movements during the swing phase of the control step. Joint angles were those defined in Figure 2 for shoulder (A), elbow (B), and wrist (C).

responsible for propelling the paw in the direction of motion, while shoulder joint rotation had little contribution. At 40% of the way through the swing phase, these roles switched: during the next 40% of the swing, shoulder joint rotation produced much of the paw velocity in the direction of motion. Wrist joint velocity contribution was initially negative and then remained near zero. During the final 20% of the swing phase, decreases in the contributions of shoulder joint rotation were responsible for the deceleration of the paw while the contributions of the elbow joint were low and in the opposite direction, further reducing paw velocity.

Joint control during the control step

Profiles of NET and MUS, INT and GR that contributed to it, were computed for the shoulder, elbow, and wrist during the swing phase of the control step (Fig. 4B-D, respectively). Shoulder NET was positive (flexing) during the first 60% of the swing phase and negative (extending) during the rest of the movement (Fig. 4B). GR consistently contributed to the positive values of shoulder NET, with assistance of first MUS and then INT. The negative portion of shoulder NET was caused exclusively by MUS with IT being slightly resistive and GT being near zero. NET for the elbow was negative (extending) during the period 30-70% of the way through the swing phase, and close to zero during the rest of the phase (Fig. 4C). The negative NET was produced by INT and GT with MUS being opposite in sign. Wrist NET was low and mainly followed the sign of GT while INT and MUS compensated for one another (Fig. 4D). Thus, the movement was initially produced predominantly passively, mainly by GT at the shoulder and INT at the elbow. During the second movement portion, the

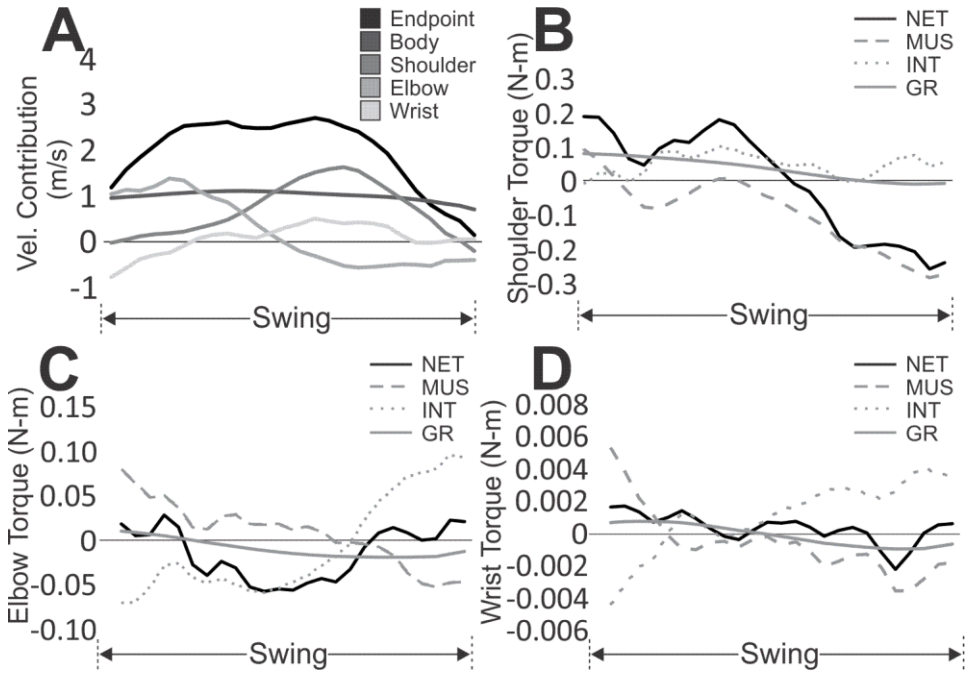

Figure 4**:** Joint torques and endpoint velocity contributions during the swing phase of the control step. **A:** Endpoint velocity contributions from each joint and body contribution throughout the swing phase of the control step. **B-D:** NET, MUS, INT, and GR torque components for shoulder (**B**), elbow (**C**) and wrist (**D**) joints. Positive values signify torque's action into extension at the shoulder and wrist and info flexion at the elbow.

shoulder was rotated actively, by MT, and the elbow continued to move primarily passively, due to INT.

Changes in endpoint velocity contributions during perturbed steps

The difference between endpoint velocity contributions in the control step and during perturbed steps that are shorter or longer than control are shown during the swing phase for the body, shoulder, elbow, and wrist (Fig. 5). Alterations to endpoint velocity were roughly symmetric between short and long steps. Substantial differences in how endpoint velocity was modified were observed for the known displacement condition and the unexpected displacement conditions. Body contributions to endpoint velocity during the known displacement condition were lower or higher at the beginning of the swing phase for short and long steps, respectively (Fig. 5A,E). In both unexpected displacement conditions, body contributions became lower or higher beginning 75% of the way through the swing phase. To compensate for the later onset of body contributions to velocity modifications, during the unexpected displacement conditions, changes to the contributions of the shoulder and elbow joints during the last 25% of the swing phase were of greater amplitude than those during the known displacement condition (Fig. 5B,F and C,G, respectively). As for body contributions, during the known displacement condition modifications of velocity contributions for shoulder and elbow joints were observed early in the swing phase. Modifications to the velocity contributions of the wrist were close to zero throughout the swing phase (Fig. 5D,H).

The total contributions of the body, shoulder, elbow, and wrist to lengthening or shortening of step size caused by changes in velocity contributions integrated throughout the entire swing phase are shown in Fig. 5I-L. Changes in step size were achieved almost exclusively through alterations to the contributions of the body and shoulder, regardless of whether the perturbation was known or unexpected (Fig. 5I,J). In the known displacement condition, the whole-body velocity alterations provided dominant contributions to changes in step size, and shoulder contributions were relatively small. However, in the unexpected displacement conditions, whole-body velocity was less altered, and lengthening or shortening of step size relied more heavily on changes to the extent of shoulder flexion. In nearly every condition, total elbow and wrist contributions to step length were random and minimal (Fig. 5K,L).

Changes in joint control during the swing phase of perturbed steps

During multi-joint movements, muscle activity is not the only cause of joint motions. Passive factors, such as gravity and motion-dependent mechanical interactions among the joints, contribute to the production of joint motions. To investigate the role of active control in the changes in the joint contributions to endpoint velocity, we compared the differences in torques generated at the shoulder, elbow, and wrist between the control and perturbed conditions. As for endpoint velocity contributions, the alterations to torques were roughly symmetric between short and long steps.

The difference between torques generated during the swing phase of steps in the control condition and during perturbed conditions are shown in Figure 6.

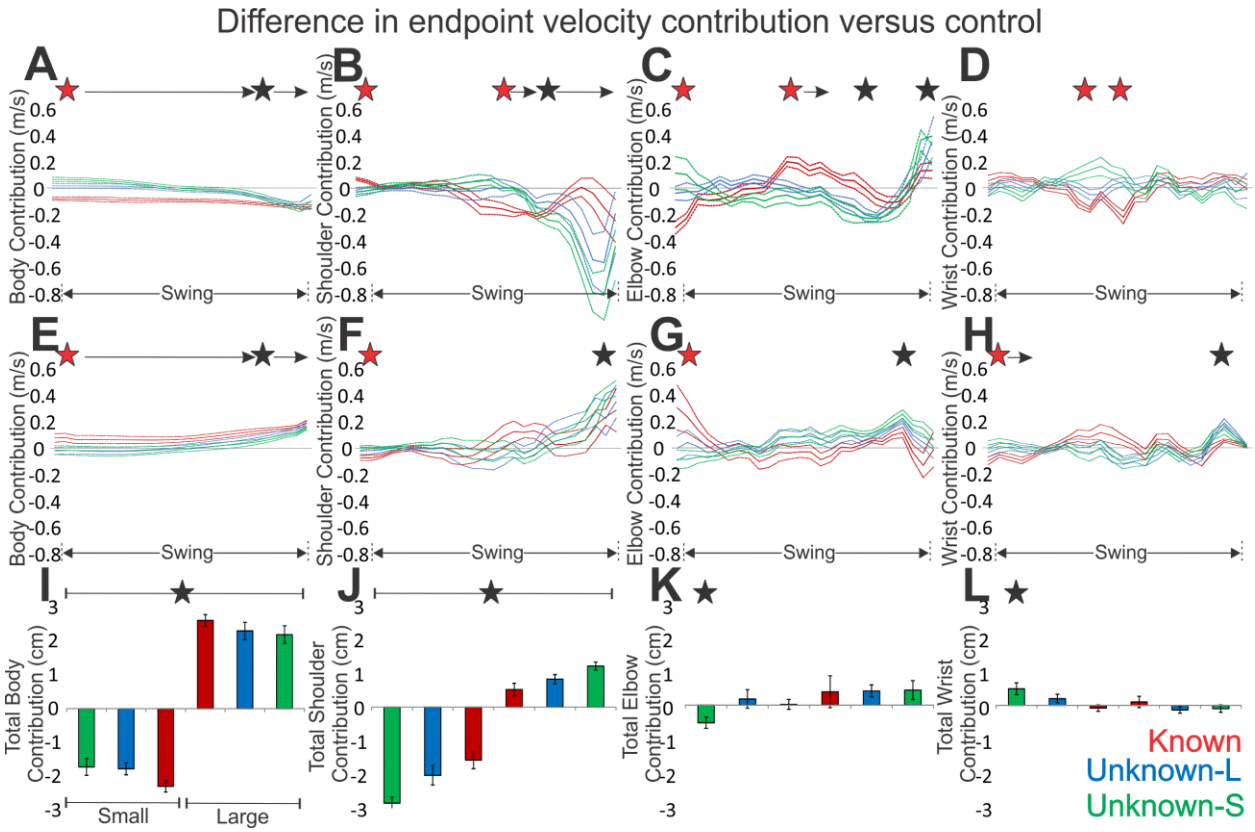

Figure 5: Changes in endpoint velocity contributions throughout the swing cycle of perturbed steps. Changes in endpoint velocity versus the control condition are shown for shorter-than-normal (A-D) and longer-than-normal (E-H) steps, including changes in body (A,E), shoulder (B,F), elbow (C,G), and wrist (D,H) contributions in the direction of motion. Total contributions of each component to lengthening (large) or shortening (small) of steps (I-L) were calculated by integrating the changes in endpoint velocity contributions across the full swing cycle of the perturbed step for the body (I), shoulder (J), elbow (K), and wrist (L). Coloration of traces represents perturbed steps made in the known displacement condition (red), unexpected long-notice displacement condition (blue), or unexpected short-notice displacement condition (green). Dashed lines above and below traces represent +/- 1 SEM. Stars represent significant differences in velocity contributions versus the control condition. Colored stars represent significant differences (t-test, $p<0.05$) in the corresponding condition only, while black stars represent significant differences in multiple conditions. Arrows represent the time-course exhibiting a significant difference.

Short (long) steps were produced through decreasing (increasing) net torque in the direction of shoulder flexion (Fig. 6A,D, respectively). During the known displacement conditions, the most pronounced changes in shoulder NET were at the very beginning and closer to the end of motion. Despite small contributions of the shoulder to endpoint velocity during the beginning of the movement period, changes in shoulder NET clearly show that the known displacement conditions elicited adjustments in shoulder motion already at the initiation of the swing phase. These initial changes were caused by both MUS and INT as the changes in these torques had the same sign as the changes in NET. The same can be concluded about the causes of changes in shoulder NET later during the motion.

Similar to endpoint velocity contributions, modifications in either of the unexpected displacement conditions were observed only during the last 25% of the swing phase. To compensate for the limited amount of time for modifications, the amplitude of torque changes was higher during the unexpected than expected displacement conditions. Changes to NET were primarily produced through changes to MUS (Fig. 6B,E), as follows from the same sign of changes to NET and MUS and primarily opposite sign and smaller amplitude of changes to PT (Fig. 6C,F).

For the elbow joint, during the known displacement condition, modifications to NET (Fig. 6G,J) were observed throughout the swing phase, while changes during the unexpected conditions were limited to the final 25% of the swing phase. In both cases, the changes in NET were matched by changes in

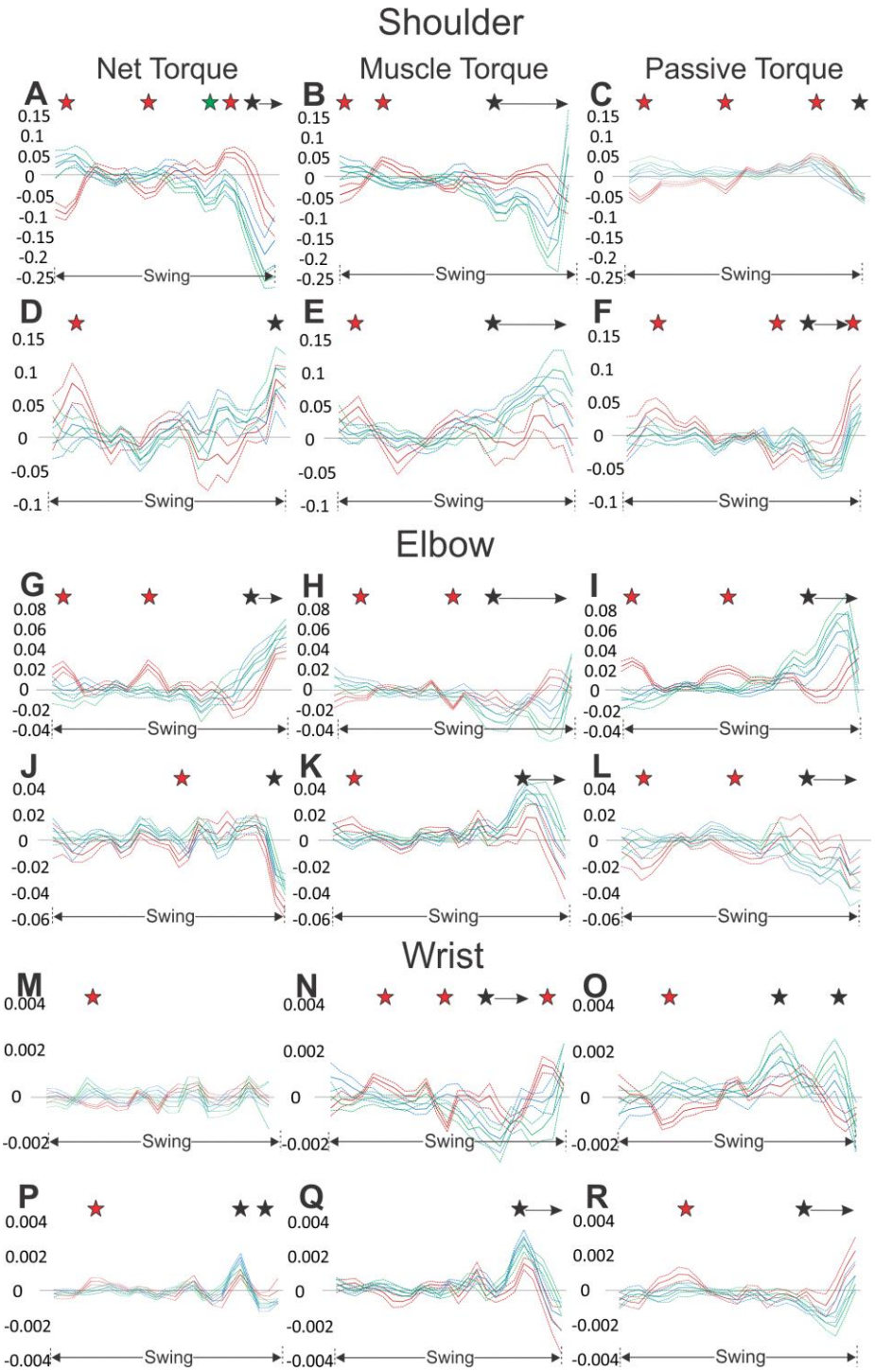

Figure 6: Changes in forelimb torques throughout the swing cycle of perturbed steps. Changes in shoulder torques are shown for shorter-than-normal (A-C) and longer-than-normal (D-F) steps, including changes in net torque (A,D), muscle torque (B,E), and passive torques (C,F), which was the combination of interaction and gravity torques, were shown throughout the swing cycle. Changes in elbow torques (G-L) and wrist torques (M-R) are shown in an identical format. Dashed lines above and below traces represent +/- 1 SEM. Coloration of traces and stars are as described in Figure 5.

PT (Fig. 6I,L) while changes in MUS (Fig. 6H,K) were relatively small and usually opposed the changes in NET. This indicates that changes to elbow NET were produced predominantly passively, due to PT, during both known and unexpected displacement conditions.

Changes to wrist torques were minimal throughout the swing phase in all perturbed conditions – changes in NET remained close to zero at nearly all points (Fig. 6M,P). However, changes to both MUS (Fig. 6N,Q) and PT (Fig. 6O,R) were significant, and these changes were observed throughout the swing phase during the known displacement condition, but only during the final 25% of the swing phase during the unexpected displacement conditions. The changes to MUS and PT were oppositional to one another and similar in amplitude, producing only minimal adjustments to the produced wrist torques. However, pronounced peaks in NET and MUS in the long step condition occurred close to the end of motion (Fig. 6P,Q) suggesting that MUS could sometimes be generated to produce brief last-moment corrections in wrist NET.

To summarize, the torque analysis confirms the results of the endpoint velocity analysis by showing that the changes in the limb motion during the swing phase were produced throughout the motion in the known displacement conditions and during the last 25% of motion in the unexpected perturbation conditions. The torque analysis also shows that during both known and unexpected perturbation conditions, most of the changes in the limb motion were produced through active control of the shoulder while the elbow passively responded to the changes in shoulder control. Contributions of the wrist to

movement changes were low, although brief, actively generated corrections in wrist motion could sometimes be produced.

DISCUSSION

The importance of controlling intersegmental dynamics during multi-joint movements has received considerable experimental attention in a variety of upper-limb tasks in humans (Hollerbach and Flash, 1982; Galloway and Koshland, 2002; Debicke and Gribble, 2004; reviewed in Dounskaia 2005). These investigations were critical for understanding how complex and coordinated behaviors are organized in a redundant motor control system where behaviors can be successfully executed in several different ways (Bernstein, 1967). Understanding locomotor movements is particularly important, not only because it is one of the most common behaviors observed in humans and animals, but also because these movements must be highly adaptable in order to overcome the complex and changing constraints posed by many natural environments. Unperturbed locomotion over a raised horizontal ladder is itself a complex locomotion task that requires precise foot placement in order to be successful. Locomotion over such complex terrain requires the involvement of supraspinal centers, such as the motor cortex, in order to be successful (Trendelenburg, 1911; Liddell and Phillips, 1944; Chambers and Liu, 1957; Friel at al., 2007). This study is the first to investigate joint control in the forelimb in cats during complex, accuracy-dependent locomotion tasks. This is also the first study to investigate how joint control is altered in response to both known and unexpected perturbations during locomotion.

Joint contribution to endpoint motion and control during the swing phase of the step

The major contributor to endpoint velocity at the paw was produced through translation of the body, which exhibited approximately constant velocity throughout the swing phase (Fig. 4A). The rest of endpoint velocity was produced initially by the elbow and then by the shoulder. The contribution of wrist motion to endpoint velocity was low throughout the swing phase (Fig. 4A).

Kinetic analysis revealed the organization of joint control underlying these joint contributions to endpoint velocity (Fig. 4B-D). Net torque was produced primarily by muscle and gravitational torques at the shoulder and by interaction torque at the elbow. At the wrist, muscle and interaction torque opposed one another and the profile of the net torque largely followed the profile of gravitational torque. Thus, the shoulder was the only joint that actively contributed to propelling the forelimb in space. Muscle torque at the elbow and wrist opposed passive torque that was the primary cause of acceleration/deceleration at these joints. The compensation of interaction torque by muscle torque at the wrist is consistent with control of the wrist typically observed in humans (Dounskaia and Wang, 2014; Galloway and Koshland, 2002) and may be achieved through simplified control mechanisms such as muscle co-activation and/or passive restrictions for motion at this joint (Gillard et al. 2000; Hirashima et al. 2003; Loeb et al. 1999).

The control strategy used during adaptations to both known and unexpected perturbations was similar to that used in the unperturbed condition.

Alterations in body motion provided pronounced contributions to changes in endpoint motion (Fig. 5I-L). This indicates that the entire body reacted to perturbations in all conditions in order to successfully place the forelimb onto the displaced crosspiece. The body contribution was especially high during known perturbations and it decreased during unexpected perturbations. The shoulder contributions were also substantial. They complimented the body contributions, increasing during unexpected perturbations (when body contributions were lower). The elbow and wrist contributions to endpoint motion were minor. Thus, in addition to modulations in body motion, the forelimb contributed to adjustments in step size during locomotion predominantly through active changes in shoulder rotation, and the contributions of the shoulder increased when the perturbation was unexpected.

Kinetic analysis supports the conclusion that the forelimb motion adaptations to perturbations were primarily mediated through alterations to the shoulder. Changes to shoulder net torque were large compared to the other forelimb joints, and primarily produced through alterations to muscle torque in all conditions (Fig. 6A,B and D,E). While passive torque (the sum of interaction and gravitational torque) assisted the shoulder net torque generation at the beginning of the swing phase in the known condition, it predominantly opposed the substantial changes made during the final 25% of the swing phase in all conditions. This indicates that during the late movement portion, muscle torque was the only source of changes in net torque and it also suppressed resistive passive torque. In contrast to the shoulder, changes in elbow net torque were

primarily passive: changes to net torque opposed elbow muscle torque but coincided in sign with changes in passive torque (Fig. 6G-L). Changes in net torque at the wrist were low, although muscle torque sometimes generated substantial peaks in wrist net torque during the final portions of the swing phase (Fig. 6P,Q), likely representing last-moment adjustments.

Thus, whether the perturbation was known or unexpected and the step was shortened or lengthened, the major changes in the forelimb motion were produced by the shoulder: Changes in the shoulder muscle torque caused changes in shoulder motion which resulted in changes in interaction torque that played the primary role in motion production at the other joints, especially at the elbow. It is likely that the same joint control structure is used during stepping on a flat service examined by Prilutsky et al. (2005), as suggested by the similarity of joint kinematics observed during the swing phase of forelimb motion in that and our study.

The consistency of the global organization of joint control in the variety of the tested conditions is in agreement with the existence of motor synergies (Ting and McKay, 2007) and an interpretation that movement adaptations could be achieved through adjustments to muscle synergies that produce coherent whole-limb movements (Drew et al. 2008). However, the idea of muscle synergies does not account for the exact organization of the revealed joint control, i.e., active rotations of the shoulder and predominantly passive motion of the elbow and wrist observed in all tested conditions. This structure is consistent with the leading joint hypothesis, which suggests that movements are produced primarily through active

control of a single joint, called the leading joint, while interaction torque caused by leading joint motion plays a cardinal role in motion production at the other ("subordinate" or "trailing") joints (Dounskaia, 2005; 2010). This organization of control has been demonstrated in a variety of multi-joint movements in humans (e.g. Dounskaia et al. 1998; Galloway and Koshland 2002; Hirashima et al., 2003, 2007; Ambike and Schmiedeler 2013). According to the leading joint hypothesis, the shoulder acts as the leading joint during the swing motion of the forelimb, and the elbow and wrist joints are trailing joints.

Our results emphasize that kinematics analysis is not sufficient for revealing the organization of joint control, and the analysis of joint torques is necessary. Indeed, analysis of joint kinematics for this task suggested that in the unknown displacement conditions, changes in angular amplitudes were more pronounced at the distal than proximal joints (Stout et al. in review). The kinetic analysis presented here shows that the distal joints moved predominantly passively in all conditions, and thus, the pronounced changes in amplitudes of these joints were to a large extent a consequence of changes in motion of the proximal joints.

Differences in adaptations to expected and unexpected perturbations

In addition to the commonality in joint control across all conditions, there were significant differences in timing of motor adaptations between the known and unexpected displacement conditions. During the known displacement condition, changes to endpoint velocity contributions and joint kinetics occurred at points throughout the entire swing phase of the step onto the displaced

crosspiece, while during the unexpected displacement conditions, alterations were restricted to only the final 25% of the swing phase. The distinct strategies observed in the known and unexpected perturbation conditions were consistent between both directions of crosspiece displacement, and the strategy used in the unexpected displacement conditions was identical, regardless of the amount of time the cat had between crosspiece displacement and stepping onto the displaced crosspiece.

These differences likely reflect differences in the global motor control processes used to make movement adaptations. The similarity between unexpected displacement conditions, but differences versus the known displacement condition, reflect hallmark features observed in feed-back and feed-forward driven motor control processes, respectively. Motor adaptations during the known displacement condition exhibit trajectory planning in advance of the step onto the displaced crosspiece, as well as differences throughout the movement, which is consistent with a forward-modeled motor plan (Wolpert and Kawato, 1998; Kawato, 1999). Motor adaptations during unexpected perturbations of locomotion follow a classical profile observed in many feedback-driven human reaching tasks, in which corrective motor adaptations are made during the final portions of a visually-guided, accuracy-dependent movement (Woodsworth, 1899; Milner 1992; Meyer et al. 1988), thought to be produced through an interaction of forward modeling and sensory feedback loops (reviewed in Desmurget and Grafton, 2000).

The use of feed-forward versus feedback-driven motor control between the known and unexpected conditions could be caused by constraints in strategy selection. One possibility is that the unexpected displacement conditions imposed a temporal constraint that limited the set of permissible motor adaptation strategies (Patla et al. 2004). If the whole-body locomotion trajectory was determined prior to crosspiece displacement in the unexpected displacement conditions, only feedback-driven modifications would be possible. Another possibility is that feed-forward motor planning is still possible in the long-notice unexpected displacement condition, but observing crosspiece displacement automatically activates a feedback-driven control strategy. During the known displacement condition, crosspiece displacement occurs when the cat is in a side chamber, and the cat does not directly observe crosspiece displacement. In contrast, during the unexpected displacement conditions, the crosspiece displaces while the cat is on the ladder, and the cat can directly observe the movement. It is possible that direct observation of changes in the environment causes a feedback-driven motor control mode to be activated, even if there is still enough time for the feed-forward planning of a motor response to the environmental disturbance.

The persistence of the dominant role of the shoulder for the production of the forelimb swing during feedforward and feedback-driven perturbed locomotion tasks is surprising because the trunk motion and the availability of the three joints provided substantial redundancy of degrees of freedom during the translation of the paw to the displaced crosspiece. This redundancy would allow, at least in some conditions, modification of step length by the distal rather than proximal

joints. Such control pattern would be advantageous in terms of reducing inertia of the controlled limb, and hence, decreasing muscle effort. It is often hypothesized that minimization of muscle effort is one of the major factors determining movement control in case of motor redundancy (Diedrichsen et al. 2010; Hatze and Buys 1977; Prilutsky and Zatsiorsky 2003; Todorov 2004). Our finding that the entire body was most involved in locomotion changes during predicted perturbations, even though corrections could be made with much less changes in the trunk motion as it was observed during unexpected perturbations, shows that alterations to the movements of the entire body that had high inertia was the preferred control strategy, which does not comply with the principle of muscle effort minimization or a related principle of minimal muscle torque change (Nakano et al. 1999).

Rather, Dounskaia and Shimansky (submitted) suggest that the leading/trailing organization of joint control reduces neural resources required for joint coordination during multi-joint movements. This is achieved by low precision of the leading joint control and the use of the trailing joints to increase precision of the entire movement. According to this interpretation, the shoulder was responsible for gross adjustments in the forelimb motion during the perturbed conditions. While the distal joints moved predominantly passively, their musculature could provide small corrections in the positioning of the paw on the crosspiece, as supported by the small peaks in wrist muscle torque that we observed during some movements. Our previous study of neuronal responses during the same task supports this interpretation, and additionally suggests that

feedback-driven corrections may require increased neural involvement relative to feedforward corrections. About 40% of motor cortical neurons exhibited a response during the known displacement condition (Stout et al. in review), but during both unexpected displacement conditions, neurons with somatosensory receptive fields at the elbow or wrist became more responsive, with nearly 70% of these subpopulations exhibiting a response, likely to provide feedback-driven corrections (Stout et al. in preparation).

Dounskaia and Shimansky (submitted) also predict that neural resources used for movement control increase if the task requires a modification of passive motion at the trailing joints through use of active muscle torque. The ability of humans to predict and flexibly regulate and shape the passive motion of trailing joints during upper-limb movements has been demonstrated (Dounskaia et al. 1998, 2002, Galloway and Koshland 2002; Gribble and Ostry 1999). Such fine-grained control may be mediated through direct connections between corticospinal neurons to spinal motoneurons, which humans and higher primates possess, but cats and other lower vertebrates lack (Landgren et al. 1962; Clough et al. 1968; Fetz et al. 1976; Bortoff & Strick, 1993; Lacroix et al. 2004; Rosenzweig et al. 2009). Most of these corticomotoneuronal neurons possess somatosensory receptive fields on distal portions of the limb (Sakata and Miyamoto 1968; Rosen and Asanuma 1972; Murphy et al. 1975), suggesting they may be involved in regulating passive torques acting on these joints. Indeed, in the variety of movement conditions tested in the present study, cats used muscle torque only to oppose passive torque in trailing joints or to make brief movement

corrections near the target. The ability to actively modify passive motion of trailing joints through feed-forward control may be a feature of coordinated multi-joint movements that accounts for the more versatile motor repertoire in humans compared with animals.

In conclusion, planned and unplanned adaptations were produced through feedforward and feedback-driven processes, respectively. Gait adjustments during locomotion, whether planned or unplanned, were generated by the same control mechanisms used to produce locomotor behaviors, and fundamentally account for intersegmental dynamics. These mechanisms appear to be used not to minimize energetic cost, but rather because they simplify control and are less costly for the nervous system to compute.

CHAPTER 5

MOTOR CORTICAL SUBPOPULATIONAL RESPONSES TO PERTURBATION OF THE ENVIRONMENT DURING LOCOMOTION

In preparation for submission to Journal of Neuroscience

5.1 ABSTRACT

Corrective movements during locomotion through complex natural environments require coordinated adaptations from multiple body parts. The neuronal contributions underlying such corrections are poorly understood, and very little is known about the relative contributions of neurons influencing different parts of the body. In this study, we identified motor cortical neurons on the basis of their somatosensory receptive field as receptive to the shoulder, elbow, or wrist joint of the contralateral forelimb, as well as whether or not their axons projected through the pyramidal tract. The activity of these neurons was recorded in cats performing a skilled locomotion task involving adaptations to changes in the environment. Cats walked along a raised horizontal ladder with a motorized crosspiece, which would displace either before the cat stepped on the ladder or unexpectedly at different points along the cat's progression over the ladder, either toward or away from the cat. Activity of each motor cortical neuron subpopulation during these tasks was compared.

We found substantial differences in the responses of each motor cortical population. Neurons with no receptive field or a shoulder-related receptive field, as well as non-PTNs, responded to observation of crosspiece displacement ahead on the track. Neurons with elbow- or wrist-related receptive fields did not respond to remote events but were recruited when the crosspiece displaced

unexpectedly and an immediate gait adjustment was required. These results suggest that the association with a specific part of the limb plays a significant role in determining their involvement in producing corrective adjustments during locomotion in response to unexpected stimuli.

5.2 INTRODUCTION

The activity of the vast majority of neurons in layer V of the motor cortex during locomotion exhibits a strong relationship the stride (Beloozerova and Sirota 1993; Drew 1993; Marple-Horvat and Armstrong 1999; Fitzsimmons et al. 2009; Stout and Beloozerova 2012, 2013). This relationship is significantly stronger during complex locomotion tasks requiring accurate foot placement, such as locomotion over barriers or along a raised horizontal ladder. The motor cortex plays a critical role in enabling subjects to traverse complex natural environments: following inactivation or ablation of the motor cortex, subjects become unable to navigate complex terrain (Trendelenburg, 1911; Liddell and Phillips, 1944; Chambers and Liu, 1957; Friel et al, 2007). However, during many behaviors, especially those in a natural setting, environments are not static; rather, motor commands must be continually updated and corrected as the environment changes. Little is known about how motor cortical neurons accommodate disturbances in the environment during movements. There have only been limited investigations of neuronal responses to perturbations of locomotion, in which obstacles or other constraints are unexpectedly introduced to the environment. Only Marple-Horvat and colleagues (1993) have investigated the response of motor cortical neurons during perturbations of locomotion, observing fast responses on the order of 20-40 ms in the cat, similar to other investigations involving corrections of reaching tasks in the monkey (Evarts, 1973; Omrani et al. 2014). Investigations into perturbations of locomotion in humans have shown

that the kinematic strategies used differ based on whether perturbations are known or unexpected; during unexpected perturbations, kinematic adjustments are often constrained compared to perturbations that are known ahead of time, and involve only limited adjustments in specific parts of the limb (Patla, 1999; Patla et al. 2004; Stout et al., 2015). Yet, despite these differences in kinematic strategies, nothing is known about whether motor cortical neurons influencing different parts of the body may play different roles in these adjustments.

In this study, we investigated whether or not motor cortical neurons associated with different parts of the body played different roles in accommodating perturbations to the environment during locomotion, and how these roles differed based on whether the perturbation was known and could be planned for, or unexpected and required immediate accommodation. We leveraged the fact that motor cortical neurons projecting to the spinal cord (pyramidal tract neurons, PTNs) influence the same part of the body they receive somatosensory information from (Asanuma et al. 1968; Murphy et al. 1975; Rosen and Asanuma 1972; Sakata and Miyamoto 1968; discussed in Stout and Beloozerova, 2012), enabling us to infer the portion of the body a motor cortical neuron influenced based on the location of its somatosensory receptive field. The activity of such identified motor cortical neurons was recorded as cats traversed a raised horizontal ladder, a complex locomotor task requiring accurate placement of limbs. One of the crosspieces was motorized, and could be displaced either prior to the cat stepping on the ladder or at different points along the cat's progression along the ladder, either towards or away from the cat. To

successfully continue along the ladder, cats needed to make a longer or shorter step. Neuronal activity among these various known and unexpected perturbations of locomotion was compared for subpopulations of neurons with different receptive fields and axon conduction properties.

5.3 METHODS

Recordings were obtained from two adult male cats (weight 5 and 4.7 kg). Methods of data collection and spike trains analysis have been previously reported (Prilutsky et al., 2005; Beloozerova et al., 2010; Stout and Beloozerova, 2012, 2013) and will be described briefly below. All experiments were conducted in accordance with NIH guidelines and with the approval of the Barrow Neurological Institute Animal Care and Use Committee.

Locomotion tasks

Positive reinforcement (food) was used to adapt cats to the experimental situation and to engage them in locomotion (Skinner, 1938; Pryor, 1975). A walkway, 2.5 m long and 0.3 m wide on each edge, served as an experimental chamber (Fig. 1A). Cats passed sequentially and repeatedly through the two corridors of the chamber in a counter-clockwise direction. In one of the corridors, the floor was flat, while the other corridor contained a horizontal ladder with 10 crosspieces. Crosspieces were spaced 25 cm apart, which is half of the mean stride length observed in the chamber during locomotion on flat floor at a self-

selected pace (Beloozerova and Sirota, 1993; Beloozerova et al., 2010). The tops of crosspieces were flat and 5 cm wide. The width of the crosspieces was chosen to slightly exceed the cat's mean foot length (3 cm), so that cats had full foot support on a crosspiece. Crosspieces were elevated 6 cm above the floor of the chamber. One crosspiece (the seventh from the left side of the ladder, Fig 1A) was connected to an electric motor. When displaced, it was shifted 5 cm in either direction, such that there was no overlap between the crosspiece's position before or after the displacement. Displacement was completed within 145 ms of initiation. On the side of the crosspiece facing the cat, there was a yellow LED lamp. It was lit as soon as the triggering of the crosspiece displacement occurred, regardless of the direction of the initiated move. This illumination attracted the cat's attention to the crosspiece when it was displacing. Auditory cues from the activation of the motor also alerted the cat to a rung displacement. Regardless of the crosspiece's displacement or the cat's performance, after each round of walking, the cat received food in a feeding dish located in one of the chamber's corners.

This apparatus allowed us to compare several locomotion tasks by displacing the crosspiece at various time points along the cat's progression. Only passages where the cat stepped on the displaceable crosspiece with right feet were studied. Seven conditions were used (Fig. 1 B): control, when the crosspiece

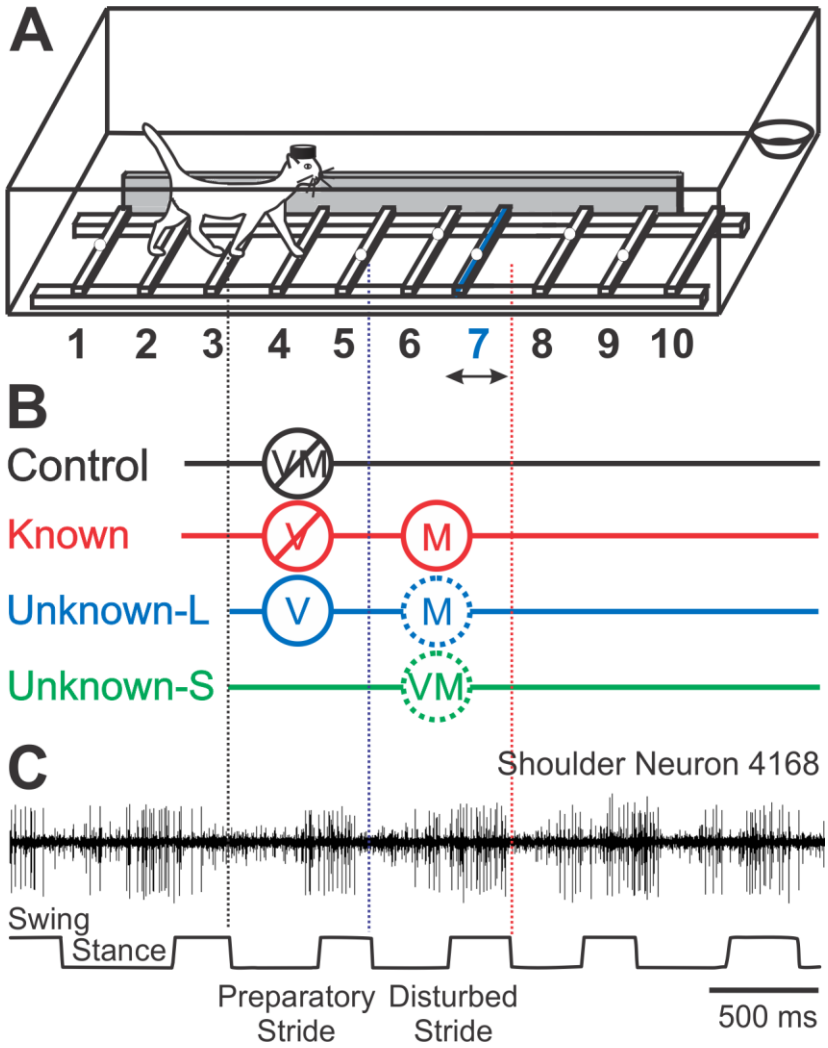

Figure 1: Experimental Design. A: Cats walked through a rectangular, two-side chamber. One side contained a raised horizontal ladder, with one motorized crosspiece (#7, red) that was displaced at different times as the cat walked in the chamber. B: A total of seven conditions were analyzed: a control condition with the crosspiece remaining in its central position, when all crosspieces were equally spaced 25 cm apart, and crosspiece movements away or towards the cat either before the cat stepped on the ladder (two "known" displacement conditions, Kn), or one stride away from it (two "unknown" long-notice displacement conditions, Ul), or during the current stride while the cat was about to initiate limb transfer to crosspiece #7 (two "unknown" short-notice displacement conditions, Us). Circles represent where the cat was along the ladder when the crosspiece displaced ("V" – visual stimulus) and when the step onto the disturbed rung was made ("M" – motor adaptation). C: An example of activity of a neuron (pyramidal tract neuron, PTN 4164) during locomotion along the ladder in the Ul long step condition.

remained in its original location; and three groups of conditions where the crosspiece was displaced either toward or away from the cat at different times, and the cat had to make a larger or smaller step to successfully traverse the ladder. In "known displacement" (Kn) conditions, the crosspiece was displaced while the cat was at the feeder. In these conditions, the cat did not see movement of the crosspiece, as the ladder was in its final configuration when the cat stepped onto it. The cat had two full strides: a stride from crosspiece #1 onto crosspiece #3, and a stride from crosspiece #3 onto crosspiece #5, before making a larger or smaller step to reach the displaced crosspiece #7. In unknown "long-notice" conditions (Ul), the rung was displaced when the cat's right forelimb stepped on crosspiece #3. The cat had one full locomotion cycle to complete before needing to adjust. In unknown "short notice" conditions (Us), the crosspiece was displaced when the cat's right forelimb stepped on crosspiece #5 and the very next transfer of the forelimb had to be adjusted. Presentation of conditions was generated pseudorandomly by a computer program, such that all conditions were presented an approximately equal number of times.

Cats were accustomed to wearing a cotton jacket, a light backpack with connectors and preamplifiers for electromyographic (EMG) signals, and an electro-mechanical sensor on the right paw for recording duration of swing and stance phases of stride. They were also trained to wear LEDs on lateral aspects of the right forelimb. The floor in the chamber and the crosspieces of the ladder were covered with an electro-conductive rubberized material. During locomotion the duration of the swing and stance phases of the right forelimb was monitored

by measuring the electrical resistance between the right foot and the floor with the electromechanical sensor (Fig. 1 C, the bottom trace). The passage of the cat through the beginning and end of each corridor was monitored using infrared photodiodes.

Surgical procedures

After cats were trained, surgery was performed under isoflourane anesthesia using aseptic procedures. The skin and fascia were removed from the dorsal surface of the skull. At ten points around the circumference of the head, stainless steel screws were screwed into the skull and connected together with a wire; they served as a fixation and a common ground. The screw heads and the wire were inserted into a plastic cast to form a circular base. Later, while searching for neurons before locomotion tests, awake cats were rigidly held by this base. The base was also used to fixate connectors, a miniature micro-drive, a pre-amplifier, contacts for stimulating electrodes, and a protective cap. A portion of the skull and dura above the left motor cortex (approximately 0.6 cm2) were removed. The area of the motor cortex was identified by the surface features and photographed (Fig. 2A). The aperture was then covered by a 1 mm thick acrylic plate. The plate was pre-perforated with holes of 0.36 mm in diameter spaced 0.5 mm, and holes were filled with bone wax. The plate was fastened to the surrounding bone by orthodontic resin (Densply Caulk). 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 PTNs in the awake animal. Immediately after surgery, and then 12 hours thereafter, an analgesic buprenorphine was administered intramuscularly.

Cell recording and identification

Experiments were initiated after several days of recovery when cats resumed their normal preoperative behavior. The animal was positioned in the restraining device, and encouraged to take a "sphinx" position. After the cat rested in this posture for several minutes, the base attached to the skull during surgery was fastened to an external frame so that the resting position of the head was approximated. Over several days, a number of sessions of increasing duration were used to accustom the cat to the head restrainer. Cats fast learned to sit quietly with their head restrained. They did not seem to be disturbed by the restraint because they frequently fell asleep.

Extracellular recordings were obtained using conventional tungsten varnish-insulated microelectrodes (120 μm OD, Frederick Haer & Co). The impedance of electrodes was 1-3 MΩ at 1000 Hz. A custom made light-weight (2.5g) manual single-axis micro-manipulator permanently affixed to the head base was used to advance the microelectrode. Signals from the microelectrode were pre-amplified with a miniature custom made preamplifier positioned on the cat's head, and then amplified with the CyberAmp 380 (Axon Instruments). After

amplification, signals were filtered (0.3-10 kHz band pass), digitized with a sampling frequency of 30 kHz, displayed on a screen, led to an audio monitor, and recorded to the hard disk of a computer by means of data acquisition hard- and software package (Power-1401/Spike-2 System, Cambridge Electronic Design, Cambridge, UK). An example of recording from a pyramidal tract neuron during locomotion is shown in Figure 1C.

A detailed description of the area of recording has been given previously (Beloozerova et al. 2005). In brief, the area immediately adjacent to and inside the lateral half of the cruciate sulcus in the cat is considered to be the motor cortex (Fig. 2A). This is based on a considerable body of data obtained by means of inactivation, stimulation and recording techniques (Nieoullon and Rispal-Padel, 1976; Vicario et al., 1983; Armstrong and Drew, 1985; Beloozerova and Sirota, 1993; Drew, 1993; Martin and Ghez, 1993), as well as on histological considerations (Myasnikov et al., 1997; Ghosh, 1997).

All encountered neurons were tested for antidromic activation with pulses of graded intensity (0.2-ms duration, up to 0.5 mA) delivered through the bipolar stimulating electrode in the medullary pyramidal tract. The criterion for identification of antidromic responses was the test for collision of spikes (Bishop et al. 1962; Fuller and Schlag 1976); it is illustrated in Fig. 2B. Neurons were checked for antidromic activation before, during, and after testing during locomotion.

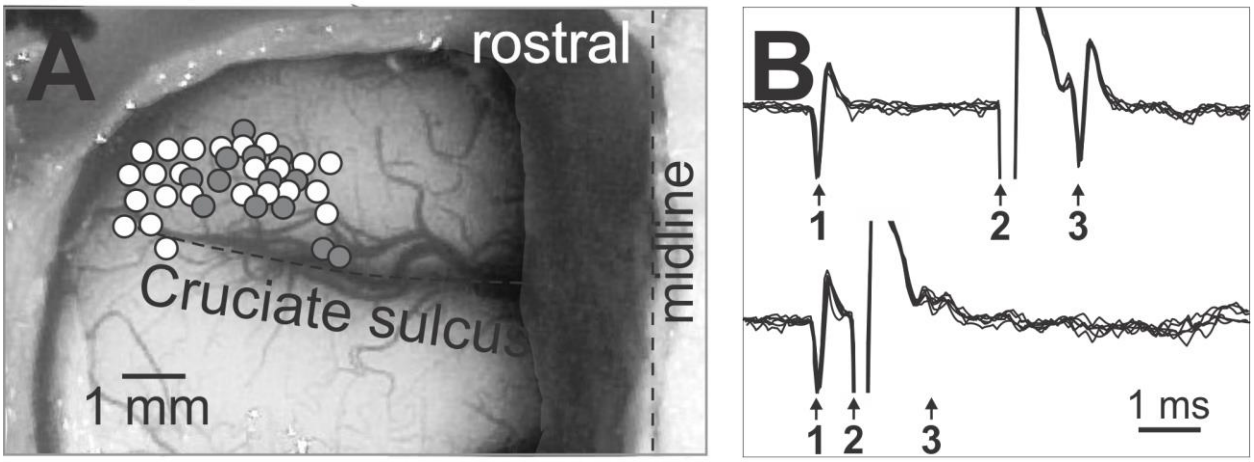

Figure 2: Area of recording in the motor cortex, and test for collision of spikes. A: Area of recording in the forelimb representation of the left motor cortex. Microelectrode entry points into the cortex were combined from cat 1 (dark circles) and cat 2 (white circles) and superimposed on a photograph of cat 2 cortex. B: Collision test for PTNs. Top trace, the PTN spontaneously discharges (arrow 1), and the pyramidal tract is stimulated 3 ms later (arrow 2). The PTN responds with latency of 1 ms (arrow 3). Bottom trace, the PTN spontaneously discharges (arrow 1) and the pyramidal tract is stimulated 0.7 ms later (arrow 2). PTN does not respond (arrow 3) because in 0.7 ms its spontaneous spike was still en route to the site of stimulation in the pyramidal tract, and thus collision/nullification of spontaneous and evoked spikes occurred.

Receptive Field Classification

The somatic receptive fields of the PTNs were examined in animals sitting on a comport pad with their head restrained. Stimulation was produced by palpation of muscle bellies and tendons and by passive movements of joints. For any region found to consistently elicit action potentials, the extent of the receptive field was determined by listening to the audio monitor and determining the entire expanse that the cell was responsive to. For this study, only neurons with the following somatosensory receptive fields were included in the analysis. 1) The shoulder-related group included PTNs responsive only to passive movements in the shoulder joint and/or palpation of upper back, chest, or lower neck muscles. 2) The elbow-related group included PTNs responsive only to passive movements in the elbow joint and/or palpation of upper arm muscles. 3) The wrist-related group included PTNs responsive only to passive movements in the wrist joint and/or palpation of distal arm muscles and/or to stimulation of the palm or back of the paw. 4) The nonresponsive group included neighboring PTNs that showed no somatosensory responses. PTNs that had receptive field spanning more than one forelimb segment, for example, those responsive to movements in both wrist and elbow joints, were not included in the analysis. Neurons responsive to movements of toes or claws were not included.

Processing of neuronal activity

Neuronal responses to two events during the cat's progression along the ladder were identified and characterized: a visual response to displacement of the moving rung in the long-notice unexpected displacement condition after the cat stepped upon crosspiece #3; and a motor adaptation response when the cat adjusted the length of its stride to land on the displaceable crosspiece #7. The onset of stance phase was taken as the beginning of the stride. The duration of each stride was divided into 20 equal bins. Neuronal activity during strides in each of the seven conditions were compared for overall similarity using a support vector machine (SVM) trained on spiking activity during individual runs (Cortes and Vapnik, 1995; Stark and Abeles, 2007; Jochumsen et al., 2013). Specifically, to test the similarity of a neuron discharge during a pair of conditions, data from each of the two conditions was randomly and equally segmented into two groups, one to train a SVM classifier (training group), and one to test the classifier (test group). Optimal splitting criteria between the two conditions were developed based on the neuronal activity in the training group (e.g., Fig. 3 A,B show individual traces on the top and average activity profiles at the bottom for two selected conditions). The splitting criteria were applied to the test group, and used to classify steps into one of the two conditions (Fig. 3C). If SVM methods correctly identified which group a particular step belonged to more often than would be expected by chance (Fig. 3C), the neuron was considered to distinguish between the two conditions. Theoretical chance levels for classifying between conditions are 50%, and to test for classifier bias, a bootstrapping procedure with data from the same condition was performed. This procedure produced mean

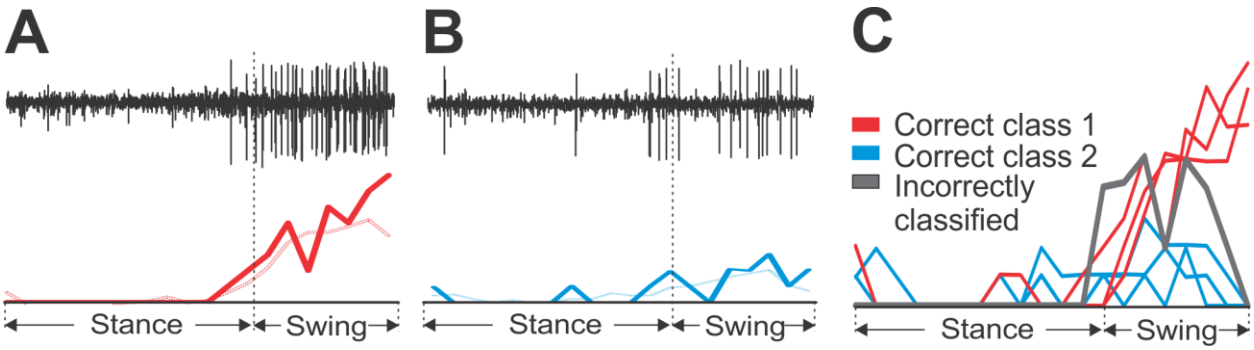

Figure 3: Support Vector Machine (SVM) classification of neuronal activity. A, B: Raw spiking activity of a neuron during a step cycle (top traces) recorded in two crosspiece displacement conditions, "a" and "b". The raw activity was converted into a frequency histogram of the neuron firing rate (bottom traces; thick line represents that individual step, thin line represents the average for all steps in the condition). Groups of strides made in each condition were split into training and test sets. Strides in the training set were used to develop SVM splitting criteria between the two conditions (see text for details). C: Neuronal activity during steps in the test set was classified according to these splitting criteria. If neuronal activity was correctly classified more often than would be expected by chance, the neuron was considered to discharge differently between the two conditions, thus exhibiting a "response". The classification accuracy in this example was 86%, so the neuron distinguishes between the two conditions ($p<0.05$; t-test for proportions).

classification accuracy of 50.3%, not significantly different from the theoretical chance level. The SVM procedure was repeated for all combinations of conditions (n= 21). To minimize uncontrolled variables such as walking speed, segmentation into training and test groups was stratified, with every other step being placed into the training (or test) group. To assess bin-wise differences in neuronal activity between tasks, an unpaired T-Test was used with a significance level of $p<0.05$.

Histological procedures

At the termination of experiments, cats were deeply anaesthetized with pentobarbital sodium. Several reference lesions were made in the region of the motor cortex, from which neurons were sampled. Cats were then perfused with isotonic saline followed by a 3% formalin solution. Frozen brain sections of 50 μm thickness were cut in the regions of recording and stimulating electrodes. The tissue was stained for Nissl substance with cresyl violet. Positions of recording tracks in the motor cortex were estimated in relation to the reference lesions. The position of stimulation electrodes in the medullar pyramids was verified by observation of electrode track gliosis.

5.4 RESULTS

Recordings of the activity of 151 motor cortical neurons were collected. The activity of 114 neurons was recorded during all seven conditions; the activity of the remaining 37 neurons was recorded only during control and four

unexpected displacement conditions. The number of steps recorded with each neuron in each condition varied from 20 to 50. Analysis of the motor cortical neuron population without regard to receptive field or projection characteristics has been reported previously in abstract form (Stout and Beloozerova, 2013); as such, the present results will be restricted to a comparative analysis of motor cortical neuron subpopulations.

Neuronal data was collected from 37 tracks through the motor cortex: from 13 tracks in cat 1 and 24 tracks in cat 2. The activity of a total of 151 neurons (59 from cat 1 and 92 from cat 2) was analyzed. The number of PTNs and non-PTNs, as well as the number of neurons with somatosensory receptive fields located at the shoulder, elbow, or wrist joints are shown in **Table 1**. While neurons were recorded without selection for PT projections or somatosensory receptive field location, nearly all (90%, 71/79) neurons with a forelimb-located somatosensory receptive field were also PTNs.

	Shoulder	**Elbow**	**Wrist**	**NoRF**
PTNs	29	19	23	27
Non-PTNs	3	2	3	67

Table 1: Number of recorded neurons of each subpopulation. Individual neurons may have somatosensory receptive fields at multiple body locations.

Non-PTNs, Shoulder-receptive, and Non-receptive Neurons exhibit visual responses

Neuronal responses to visual perception of a disturbance were uncommon, and occurred primarily in the 250-350 ms following crosspiece displacement. An example of a visual response is shown in Figure 4A. This neuron decreased its activity following rung displacement, and exhibited a similar response regardless of the direction of displacement. Non-PTNs were significantly more likely to exhibit a visual response than PTNs, and neurons either lacking a receptive field or shoulder-receptive were significantly more likely to exhibit a visual response than elbow- or wrist-receptive neurons (T-Test, $p<0.05$; Fig. 4B). Indeed, non-receptive, shoulder-receptive, and non-PTN neurons all exhibited a peak in responses during the 250-350ms window (Figs. 4C-E, respectively), while PTNs, elbow-, and wrist-receptive neurons exhibited baseline (random) levels of responsivity throughout the stride cycle (Fig. 4F). Responses from shoulder-receptive neurons preceded responses from non-PTNs and non-receptive neurons by 10-30 ms.

PTNs, Elbow-receptive, and Wrist-receptive neurons exhibit most commonly exhibit motor adaptation responses

Neuronal responses during the stride over the displaced crosspiece were quite common among all neuronal types. An example of a motor adaptation response is shown in figure 5A. When making a larger-than-normal stride, this

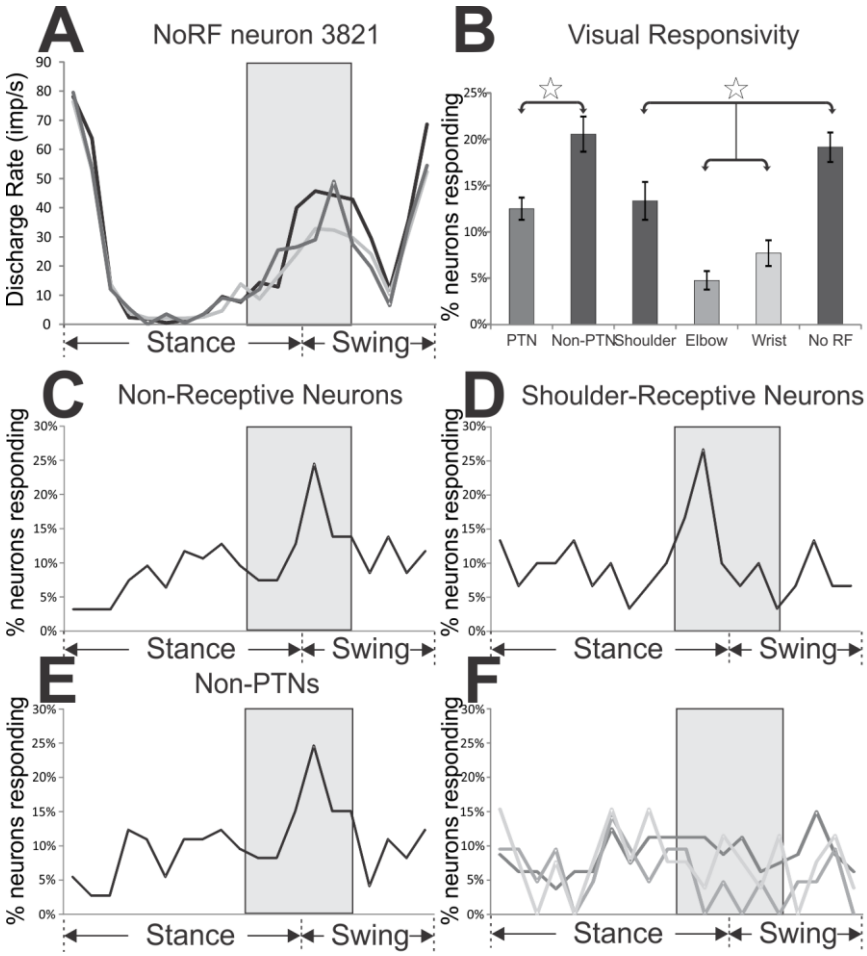

Figure 4: Visual responses to crosspiece displacement. A: Example of a non-receptive neuron responding to crosspiece displacement. Black trace represents neuronal activity during the control condition. Light and dark grey represent neuronal activity when the crosspiece displaced either away from or towards the cat, respectively. Neuronal activity decreased significantly 250-350 ms following crosspiece displacement. B: Percentage of neurons from each population producing a visual response. C-E: Percentage of neurons of the non-receptive, shoulder-receptive, and non-PTN populations exhibiting a response to crosspiece displacement across the step cycle, respectively. Light grey bar represents the 250-350 ms time window for responses. F: Percentage of neurons from the PTN, elbow-receptive, and wrist-receptive populations exhibiting a response to crosspiece displacement. Colors as shown in (B).

neuron increased activity during the swing phase, but exhibited no changes to activity during a smaller-than-normal stride. The majority of neurons of every type exhibited a motor adaptation response against one of the crosspiece displacement conditions, but PTNs were significantly more likely to exhibit a response than non-PTNs, and elbow- or wrist-receptive neurons were significantly more likely to exhibit a response than shoulder- or non-receptive neurons (Fig. 5B). These neurons could exhibit one of two response patterns: unidirectional neurons respond to either large steps or small steps, but not both; bidirectional neurons respond to both large and small steps. The neuron shown in Figure 5A is of the unidirectional type. PTNs, shoulder-receptive, and non-receptive neurons were significantly more likely to be of the unidirectional type (Fig. 5C). For the neurons with a receptive field, as the location of the field became more distally located on the limb, bidirectional neurons became increasingly frequent. Neuronal responses primarily occurred during the swing phase for all neuronal types. The shoulder-receptive population is shown as an example (Fig. 5D); however, the response profile was similar for all groups.

Neuronal subpopulations exhibit differing responsivity to differently timed crosspiece displacements

The percentage of neurons from each group exhibiting a motor adaptation response varied with the timing of crosspiece displacement. Regardless of pyramidal tract projection status or receptive field, similar proportions of neurons responded to the known displacement condition (Fig. 6A). However, elbow- and

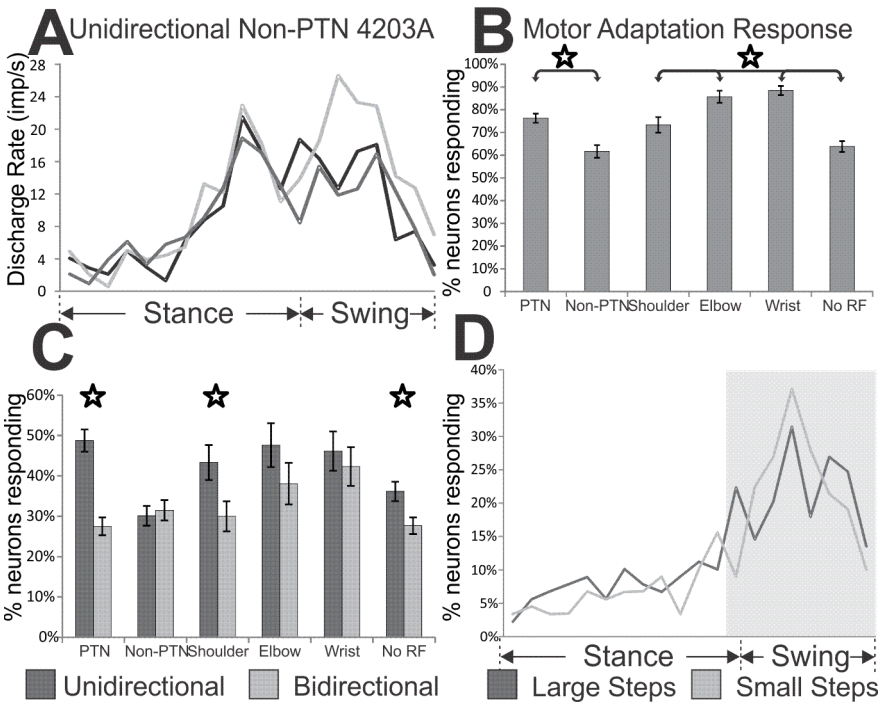

Figure 5: Motor adaptation responses over disturbed steps. A: An example of a non-PTN neuron exhibiting a motor adaptation response during the swing phase. Colors as in Figure 4A. B: Percentage of neurons of each type exhibiting a motor adaptation response to some disturbed condition. Stars represent a significant difference ($p < 0.05$, t-test). C: Percentage of neurons of each type exhibiting unidirectional (dark grey) or bidirectional (light grey) responses (see text). D: Example of motor adaptation responses of the shoulder population across the step cycle. Dark grey trace represents a smaller-than-normal step, and light grey trace represents a larger-than-normal step. Responses profiles across the step cycle were similar for all populations.

wrist-receptive neurons were significantly more likely to respond to either of the unexpected displacement conditions than the known displacement condition, and PTNs were significantly more likely to respond to the unexpected long-notice displacement condition. Similar proportions of Non-PTN, shoulder-receptive, and non-receptive neurons responded to disturbances at all timings.

Individual neurons could exhibit a response at one, two, or all of the crosspiece displacement timing conditions. Non-PTNs were significantly more likely than PTNs to exhibit responses at all timing conditions, but no differences were observed among neurons with or without receptive fields, regardless of location (Fig. 6B). Among neurons exhibiting responses to two timing conditions, it was most common for neurons to respond to both unexpected displacement conditions rather than any other combination. An example of this type of response for an elbow-receptive neuron is shown in Figure 6C. For PTNs, elbow-receptive, and wrist-receptive neurons, this type of response was significantly more common than the other combinations, although the effect was far more pronounced for elbow- and wrist-receptive neurons (Fig. 6D). For neurons exhibiting a response at only a single displacement timing, responses to one of the unexpected displacement conditions was most common. An example of this response type for a non-PTN is shown in Figure 6E. For non-PTNs and non-receptive neurons, responses during only the unexpected short-notice displacement condition were the most common by far. For neurons with a forelimb receptive field, responses during only the unexpected long-notice

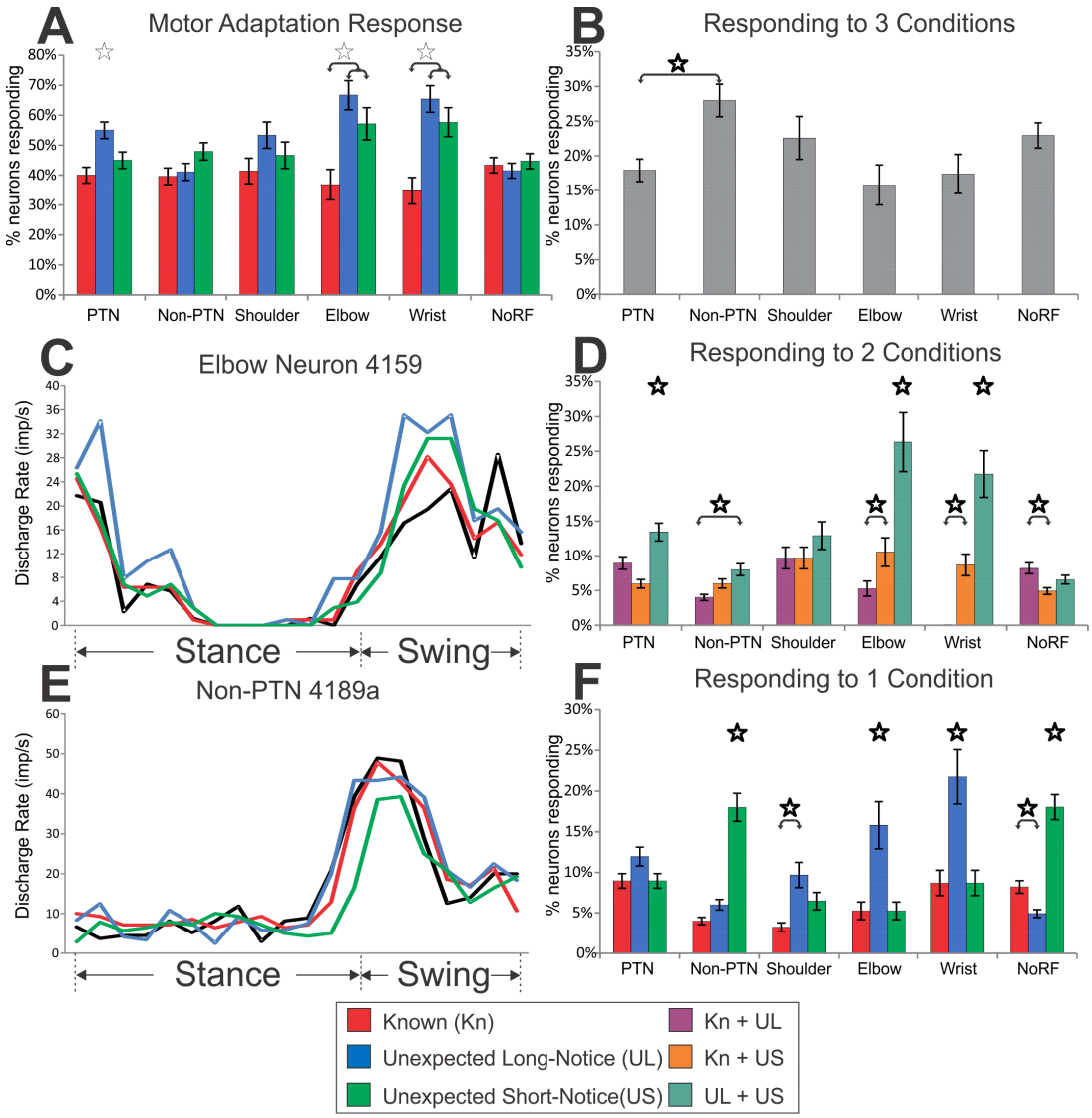

Figure 6: Population motor adaptation response characteristics during the disturbed step. A: Percentage of neurons of each type exhibiting a motor adaptation response at each timing condition. Red represents a known displacement requiring a small or large step (45 or 55 cm distance between crosspieces, respectively), blue represents an unexpected long-notice disturbance requiring such a step, and green represents an unexpected short-notice disturbance. Stars represent significant differences against the control condition; colored stars represent significant differences between a single condition and control. B: Percentage of neurons of each type exhibiting a motor adaptation response to all timing conditions. C: Example of a neuron exhibiting a response to only two timing conditions. Black represents the control condition (50 cm distance between crosspieces), D:, Percentage of neurons of each type exhibiting a response to only two timing conditions. Purple represents both known and unexpected long-notice conditions, orange represents known and unexpected short-notice, and teal represents both unexpected displacement conditions. E,F: Example of a neuron exhibiting a response to only a single timing condition (E), and percentage of neurons of each type exhibiting a response to only two timing conditions (F).

condition were the most common, particularly for the elbow- and wrist-receptive populations (Fig. 6F).

Neurons with forelimb receptive fields commonly exhibit directional preferences

Neuronal populations commonly exhibited directional preferences; that is, neurons more commonly responded to disturbed strides that were smaller-than-normal or larger-than-normal. PTNs, elbow-receptive, and wrist-receptive neurons were more likely to respond to large steps, while non-PTNs, shoulder-receptive, and non-receptive neurons exhibited no overall preference (Fig. 7A). However, directional preferences varied by the timing of crosspiece displacement. During the known-displacement condition, every neuronal population except for non-PTNs exhibited a direction preference (Fig. 7B). For these populations, all except for the wrist-receptive population was more likely to respond to large steps, while the wrist-receptive population was more likely to respond to small steps. However, during the unexpected displacement conditions, directional preferences were far less common. While all neuronal populations with forelimb somatosensory receptive fields exhibited a preference for large steps, this relationship was only significant for shoulder-receptive neurons (Fig. 7C). During the unexpected short-notice condition, only wrist-receptive neurons exhibited a directional preference: these neurons were more likely to respond to small steps (Fig. 7D). It may seem surprising that wrist-receptive neurons were overall more likely to exhibit responses to large steps, but were more likely to respond to small steps in the known and unexpected short-notice condition. The

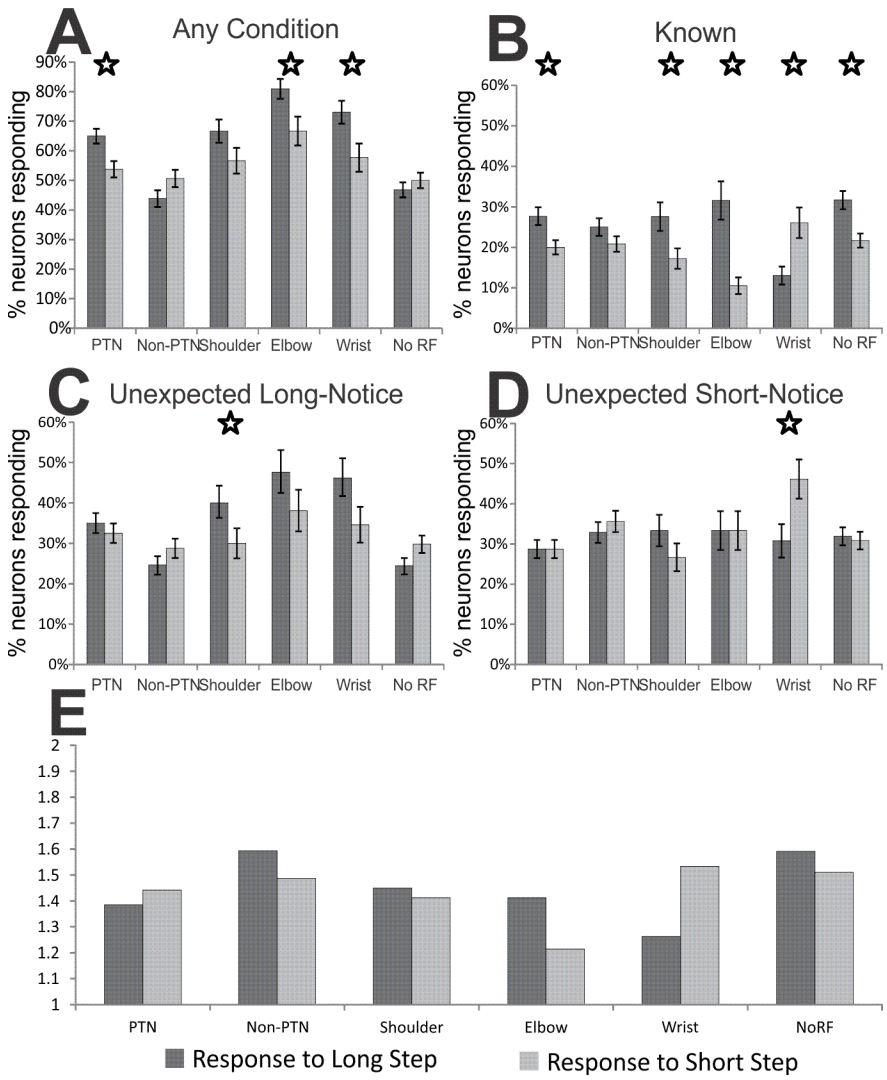

Figure 7: Populational responses to large and small steps. In all figures, dark grey bars represent large steps, and light grey bars represent small steps. A: Percentage of neurons of each type responding to large and small steps at all latencies. B-D: Percentage of neurons of each type responding to large and small steps during the known (B), unexpected long-notice (C), and unexpected short-notice (D) conditions. E: Average number of responses to large and small steps by neurons of each type, given that they exhibited at least one response to steps of that size.

reason for this is that the wrist-receptive neurons exhibiting responses to small steps tended to respond in multiple timing conditions, while wrist-receptive neurons responding to large steps were often did so only at a single timing condition (Fig. 7E).

Non-PTNs, and neurons with distal or no forelimb receptive field exhibit time-sensitive responses

Many neuronal populations exhibited time-sensitive responses; that is, if they exhibited a motor adaptation response during a small step when the crosspiece was displaced at a particular time, they were likely to respond to a large step displaced at the same time as well. To assess this, the correlation between SVM classification accuracy of small and large steps displaced at the same time was compared across neuronal populations and displacement timings. Scatter plots demonstrating these relationships are displayed in Figure 8A-R. Two major relationships were observed, and are shown in Figure 8S. Elbow- and wrist-receptive neurons exhibited time-sensitive responses for displacement conditions occurring at all (wrist-receptive) or most (elbow-receptive) timings. Non-PTNs and non-receptive neuronal populations exhibited responses that were increasingly time-sensitive; that is, as the amount of time shortened between when the crosspiece displaced and the step onto the displaced crosspiece occurred, these neurons were more likely to respond to both conditions or neither condition. PTNs and shoulder-receptive neurons were both time-sensitive for only a single condition.

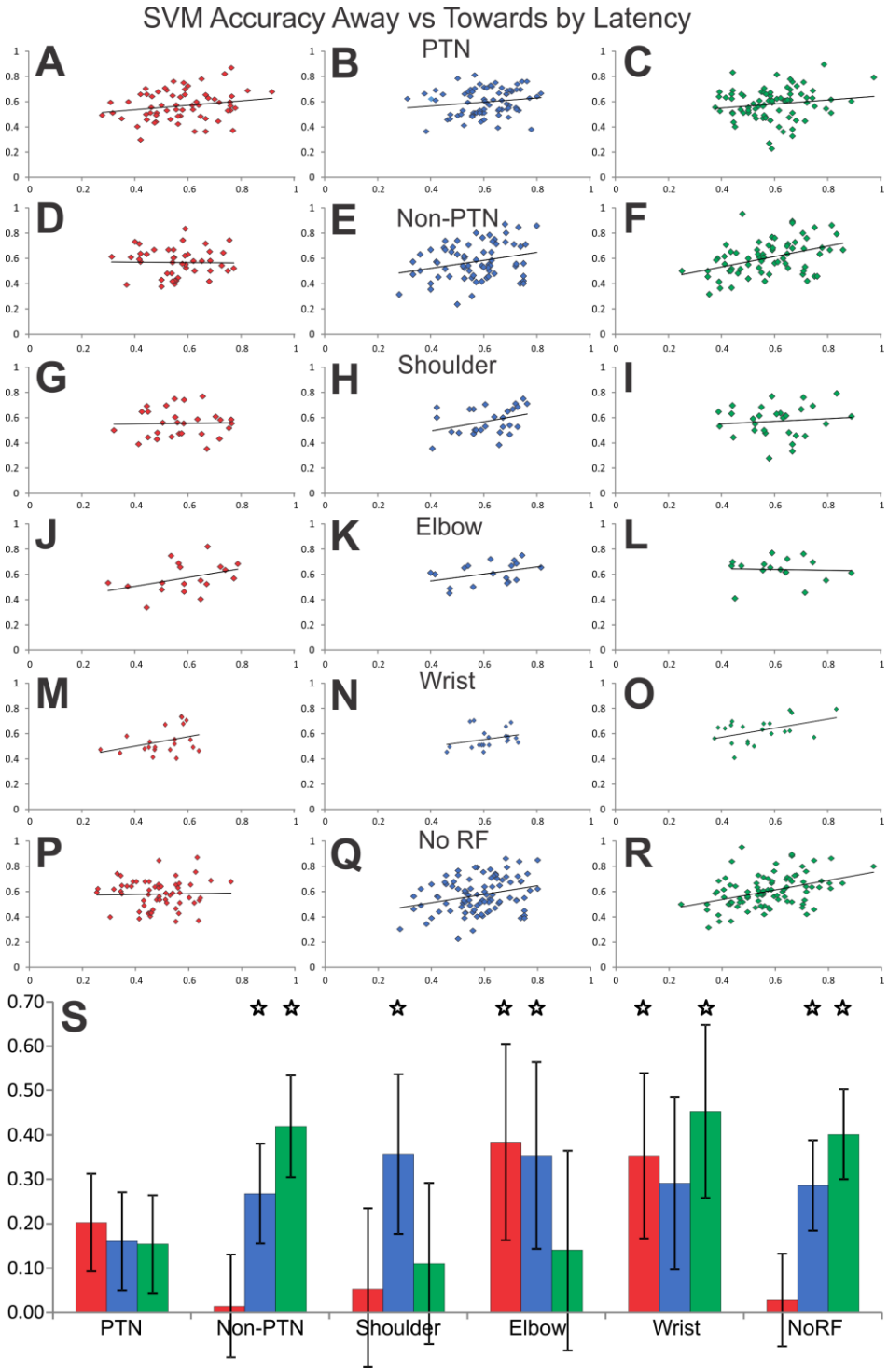

Figure 8: Time sensitivity in neuronal responses. A-R: Scatter plots demonstrating the relationship between SVM classification accuracy for large (abscissa) and small steps (ordinate) during each timing condition for PTNs (A-C), non-PTNs (D-F), Shoulder-receptive (G-I), elbow-receptive (J-L), wrist-receptive (M-O), and non-receptive (P-R) neuronal populations. S: correlation coefficient (Pearson R) for each neuronal population by timing condition. Colors for conditions as in Figure 6.

PTN and wrist-receptive population responsivity is activity-dependent

To determine what characteristics of neuronal activity other than pyramidal tract projection status and receptive field location might influence neuronal responsivity, the relationship between a variety of activity characteristics, including stride-phase modulation, period of elevated firing, preferred phase of discharge, and others. Of these, only discharge rate during the control condition was found to exhibit a consistent relationship with neuronal responsivity to crosspiece displacement, and only for the PTN and wrist-receptive neuronal populations. Scatterplots demonstrating this relationship are shown for each neuronal population and timing condition in Figure 9A-R. The strength of these relationships is shown in figure 9S. For the wrist-receptive neuronal population, at all timing conditions, neurons with higher discharge rates during the control condition were more likely to exhibit a response; that is, neuronal responses were primarily exhibited by neurons that were already active during the stride. For the PTN population, responsivity became increasingly activity dependent as the time between crosspiece displacement and the stride onto the displaced crosspiece shortened.

5.5 DISCUSSION

Motor cortical neurons have been found to exhibit strong involvement in a variety of skilled locomotion tasks (e.g. Drew et al. 1993; Beloozerova and Sirota, 1993; Beloozerova et al. 2010), and the role of pyramidal tract projecting neurons

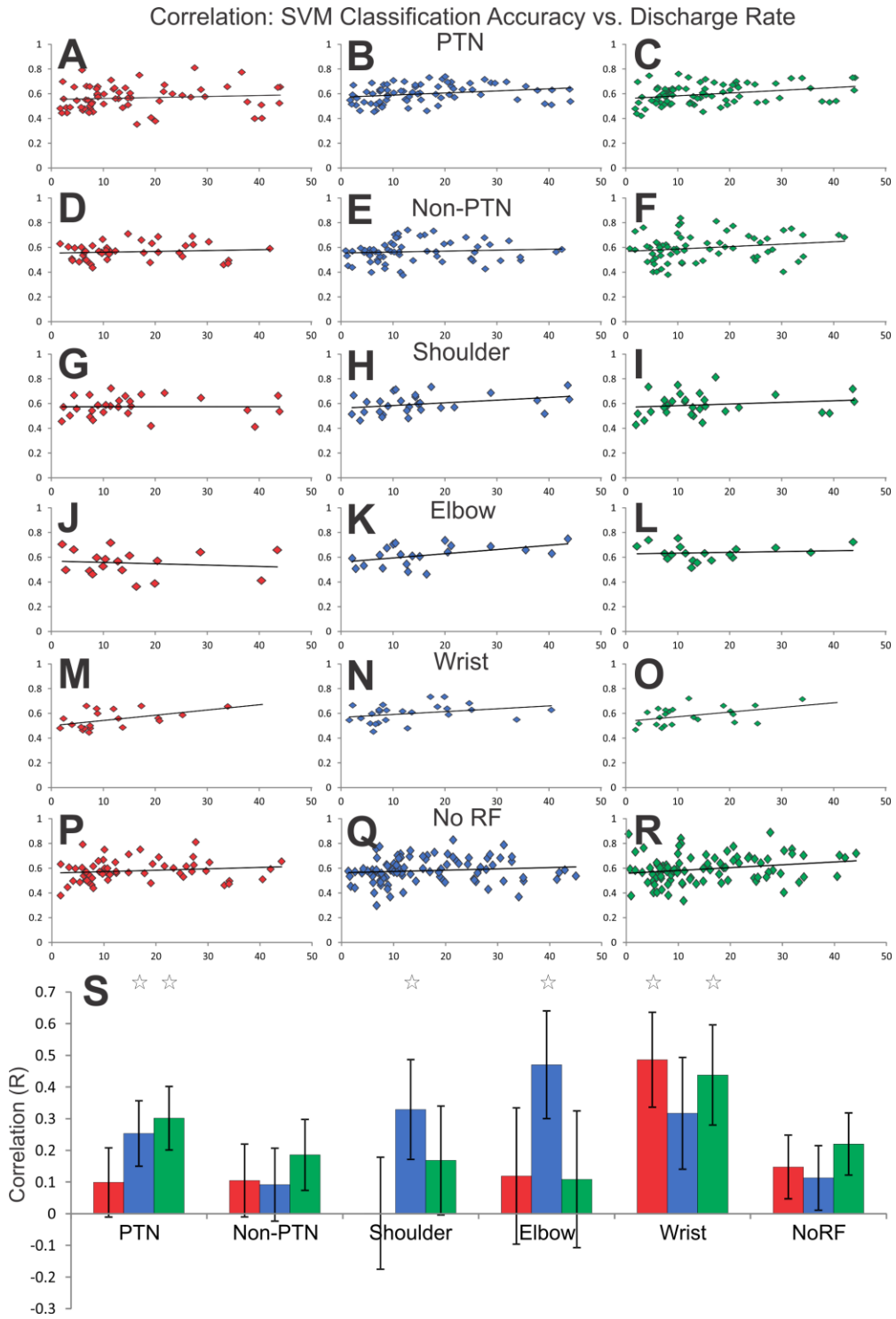

Figure 9: Relationship between discharge rate and SVM classification accuracy. A-R: Scatter plots demonstrating the relationship between discharge rate (abscissa) and SVM classification accuracy (ordinate) during disturbed steps at each timing condition for PTNs (A-C), non-PTNs (D-F), Shoulder-receptive (G-I), elbow-receptive (J-L), wrist-receptive (M-O), and non-receptive (P-R) neuronal populations. S: correlation coefficient (Pearson R) for each neuronal population by timing condition. Colors for conditions as in Figure 6.

in specific have long been the subject of strong interest due to their influence on spinal motoneuronal pools. Yet, there have been few investigations on the role of motor cortical neurons, let alone identified PTNs, in managing perturbations during skilled movement tasks, with the most investigations involving monkey reaching tasks. Only Marple-Horvat and colleagues (1993) have investigated perturbations during a skilled locomotion task in the cat. In their paradigm, a crosspiece of a horizontal ladder would unexpectedly depress following paw placement. They observed fast cortical responses to the task, on the order of 20-40 ms following paw placement, in agreement with the investigations performed in the monkey (e.g. Evarts, 1973; Omrani et al. 2014). We observed no response on such a fast time scale; rather, we observed perceptual responses on the order of 250-350 ms following rung displacement (Fig. 4), in agreement with other studies on visually-guided trajectory modifications (Carson et al., 1995). This is likely due to differences in the task presented: their paradigm involved depression of the crosspiece only after the paw was placed upon it, likely activating proprioceptive reflex pathways, while ours involved displacement of the crosspiece prior to footfall, likely activating visual and/or auditory pathways instead.

This is the first investigation to quantitatively compare the relative involvement of PTN and non-PTN neuronal populations, as well as motor cortical neuron populations with and without somatosensory receptive fields, in overcoming perturbations during skilled locomotion tasks. It is apparent from our data that the pyramidal tract projection and location of somatosensory receptive field on the limb play a significant role in determining the motor cortical neuron

responsivity to both the perception of a disturbance in the environment, and the subsequent motor adaptation to overcome that disturbance. Substantial differences were observed between groups with regard to their likelihood of exhibiting either a perceptual or motor adaptation response to the tasks (Figs. 4B,5B), as well as their involvement in managing perturbations occurring at different times (Fig. 6A).

Both long- and short-notice unexpected displacement conditions exert constraints on the amount of time cats have to produce trajectory modifications in order to place their forepaw upon the displaced crosspiece. Previous investigations in humans have demonstrated that in such constrained conditions, the smallest kinematic adjustments required to overcome the perturbation are preferred (Patla et al. 2004), in contrast to unconstrained or planned modifications, in which both preparatory and comparatively more extensive kinematic adjustments will often be used (Mohagheghi et al., 2004). Our own kinematic investigations of this task have revealed that the unexpected displacement conditions primarily involve kinematic adjustments to the distal joints of the forelimb, while the known displacement conditions involve complex modifications of the entire limb (Stout et al., *in review*). Given that motor cortical neurons tend to influence the same portion of the body that they receive somatosensory information from (Asanuma et al. 1968; Murphy et al. 1975; Rosen and Asanuma 1972; Sakata and Miyamoto 1968; discussed in Stout and Beloozerova, 2012), it might be expected that elbow- and wrist-receptive neurons would be highly involved during the unexpected displacement conditions, both

long- and short-notice. This was indeed the case; elbow- and wrist-receptive neurons become dramatically more responsive during the unexpected displacement conditions (Fig. 6A). The increase in responsiveness of the population is due to a substantial number of neurons in these populations which respond to both unexpected displacement conditions (Fig. 6C), and these elbow- and wrist-receptive neurons are typically involved regardless of whether the unexpected displacement requires a shorter or longer step (Fig. 8 J-O,S). For the wrist population, at least, the group of highly responding neurons appears to be primarily drawn from neurons which would be active anyway, producing high discharge rates during unperturbed locomotion (Fig. 9M-O,S).

Also of note is the significant and unique response that non-PTNs and neurons lacking somatosensory receptive fields exhibit during the unexpected short-notice condition. A substantial proportion of these neurons are responsive only during the unexpected short-notice condition (Fig. 6F), and often respond at this timing condition during both shorter and longer steps (Fig. 7S). There is substantial overlap between these two populations, with 90% of non-receptive neurons also being non-PTNs. Because this group is primarily composed of non-PTNs, these neurons do not directly project to the spinal cord through the cortico-spinal tract. Rather, these neurons likely either influence other motor cortical neurons, or they influence body movements through connections to other descending tracts. Previous investigations have shown that unexpected trajectory adjustments are initiated at shorter latencies than voluntary gait modifications (Patla 1991; Pettersson et al., 1997), and the involvement of subcortical structures

in during fast trajectory modifications have been proposed (Weerdesteyn et al. 2004), including major descending tracts known to be involved in corrective motor commands in the cat, such as the rubrospinal (Pettersson et al. 1997) or reticulospinal tracts (Pettersson and Perfiliev, 2002). Although not directly tested, it is quite likely that many of the non-PTNs are either corticorubral or corticoreticular neurons; corticorubral and corticospinal projections, in particular, have been previously found to be largely exclusive of one another (Palmer et al. 1981). Therefore, the specific responses of non-PTNs to the unexpected short notice condition could contribute to fast trajectory modifications mediated through subcortical structures.

The existence and timing of visual perceptual responses for specific neuronal populations provides some insight on what information such responses might contain. Coherent visual responses were observed from non-PTNs, non-receptive neurons, and shoulder-receptive neurons, but not PTNs, elbow-receptive, or wrist-receptive neurons. The restriction of visual sensitivity to only the most proximal joints may reflect the hierarchical relationship of joints (Dounskaia, 2005). Due to its proximal position in the limb, movements of the shoulder joint affect the dynamics of the elbow and wrist joints more strongly than their dynamics affect the shoulder. For this reason, during complex, multi-joint movements, such as locomotion, neural control of the shoulder joint may be more sensitive to environmental conditions and outside perturbations, while control of the elbow and wrist joints may be more dependent on the internal dynamics and body configuration. The consistent asynchrony in visual responses,

where responses exhibited by shoulder-receptive neurons precede responses exhibited by non-receptive neurons and non-PTNs by 15-45 ms, suggest that information about crosspiece displacement reaches each of these groups through different routes. While the source of this discrepancy is not known, it is possible that non-receptive and non-PTNs receive this information through collaterals from shoulder-receptive PTNs. Pyramidal neurons are known to form direction monosynaptic connections with other motor cortical neurons in the immediate area (Asanuma and Rosen, 1973; Lund et al. 1993; Keller and Asanuma, 1993), and produce EPSPs with a latency and duration consistent with the observed time lag (Matsumura, Chen, Fetz et al. 1996).

Previous studies have demonstrated that somatosensory afferentiation alone does not directly affect the discharge characteristics of motor cortical neurons during locomotion, as anesthetization of cutaneous somatosensory input evokes little effect on neuronal discharge characteristics (Armstrong and Drew 1984). Rather, neuronal activity characteristics during locomotion tasks are differentiated based on the portions of the body an individual motor cortical neuron exerts influence over (Stout and Beloozerova, 2012). The results of this investigation further demonstrate that such characteristics affect if, and how, motor cortical neurons contribute to overcoming perturbations during locomotion as well.

CONCLUSION

While the central role of the motor cortex in modification of motor behaviors has been established, the mode and mechanism of contribution of neurons with different anatomical and physiological characteristics had been far less clear. Investigations presented here have established that such characteristics, including somatosensory receptive field, and axonal conduction velocity, play important roles in determining neuronal contributions to skilled locomotion tasks, and in determining neuronal contributions in responding to emergent changes in the environment.

Information arising from somatosensory afferentiation may not play a direct role in producing activity of motor cortical neurons during locomotion (Armstrong and Drew, 1985), but the location of somatosensory receptive fields, or, equivalently, the part of the body a neuron controls, is important in determining the neuron's activity characteristics during simple and complex locomotion tasks. Association with a particular part of the limb is also important in determining if, and how, individual neurons respond to changes in the environment.

Fast- and slow-conducting PTNs are known to have different biophysical, anatomical, and physiological properties (Takahashi, 1965; Evarts, 1967; Calvin and Humphries, 1977). While different activity characteristics were observed during simple locomotion tasks (Armstrong and Drew, 1984), investigations in this dissertation extended this result by demonstrating that these two classes of

PTNs also exhibit differing activity characteristics during complex locomotor tasks.

During locomotion, emergent changes in the environment that require adaptation have been shown to evoke different responses based on whether the disturbance is known or unexpected (Patla et al. 1999, 2004). Results reported here have demonstrated that the neurons of the motor cortex exhibit different responses based on whether the disturbance is known or unexpected, and that these neuronal responses mirror the changes in kinematics of movements that occur to overcome such disturbances.

The results have shed light on the differing contributions of motor cortical subpopulations in control of locomotion behaviors. They have provided new insights into the understanding of motor control in intact, functioning systems. These results can also provide useful insights into the design and selection of motor rehabilitation strategies following traumatic brain injury, stroke, or degenerative disorders, and may also aid in the development of useful brain-machine interfaces.

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Institutional Animal Care and Use Committee

APPROVAL FORM

Protocol # 236 Animal Welfare Assurance # A3519-01

Grant # NIH (NINDS) –"Forebrain control of locomotion"

Investigator(s): Dr. Irina Beloozerova

Title of Project "Motor Cortex and Thalamocortical Network in Locomotion"

Species and Numbers of Animals: Cat - 15

This is to certify that the project identified above has been reviewed by the Institutional Animal Care and Use Committee which has considered specifically the compliance with applicable requirements of the Animal Welfare Act and pertinent state and local laws, regulations and adherence to the PHS Policy, and NIH Guide.

The proposed study has been approved by the IACUC and complies with the institutional assurance certification of the Barrow Neurological Institute of St. Joseph's Hospital and Medical Center.

Unless otherwise stated, this protocol has been approved by the Committee for a period of three years from the date noted below. The protocol is also subject to annual review on or before this date by this Committee.

Date of Committee Approval: May 21, 2012

COMMITTEE APPROVAL:

Thomas M. Hamm, Ph.D. Chairman - Institutional Animal Care and Welfare Committee

INSTITUTIONAL APPROVAL:

Ronald J. Lukas, Ph.D. Vice President of Research

c: Principal Investigator: You are reminded that modifications of any type in the above research project pertaining to animal experimentation requires re-review by this Committee.

APPENDIX A

ENDPOINT VELOCITY CONTRIBUTIONS BY JOINT

$V_{sh} = \delta_{sh} * \overrightarrow{r_{sh}} * \cos \theta_{sh}$
$V_{el} = \delta_{el} * \overrightarrow{r_{el}} * \cos(\theta_{sh} + \theta_{el})$
$V_{wr} = \delta_{wr} * \overrightarrow{r_{wr}} * \cos(\theta_{sh} + \theta_{el} + \theta_{wr})$
$V_{pw} = V_{sh} + V_{el} + V_{wr} + V_{bd}$

Symbols: V is the contribution to endpoint (paw) velocity in the direction of motion; δ is angular velocity; $\vec{r}$ is the distance from the joint center to the endpoint (paw); and θ is the angle of the joint.