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Discreteness in Spinal Motor Systems in the Rat and Frog

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B.A. Psychology
Wesleyan University, 1992

Submitted to the Department of Brain and Cognitive Sciences in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy in Neuroscience

at the
Massacusetts Institute of Technology
December 1997
[February 1998]

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ABSTRACT

We examined the organization of motor systems of the rat and frog spinal cord. In particular, we examined the hypothesis that spinal motor systems were organized from a small number of distinct types of movements. In the rat, we examined the responses evoked from microstimulation of spinal interneuronal regions. It appeared that only a small number of different types of responses were evoked from this type of stimulation. However, responses other than these few types could be produced by combining microstimulation at two different sites in the spinal cord and the resulting response could be described as a simple combination of the response evoked from each site separately. It also appeared that the anatomical organization of responses in the spinal cord paralleled the somatotopical organization of spinal cutaneous systems. We examined this relationship between the organization of responses from cutaneous stimulation and from spinal stimulation more extensively in a series of experiments carried out in the frog. We first characterized the organization of the withdrawal reflexes in the spinalized frog. We found that each hindlimb muscle was activated from a particular region of the hindlimb skin surface, such that distinct patterns of muscle activations were evoked from the foot, the front of the calf, and the back of the calf. These activation patterns appeared to be at least in part distinct from one another and each appeared to be controlled as a single functional unit. We then assessed the degree of similarity between the responses evoked from cutaneous and spinal stimulation using several different quantitative methods. Each method found that the two sets of responses were similar to one another, but not identical. Finally, we demonstrated that these distinct patterns of muscle activations could be combined flexibly in order to produce a wider range of movements. The results described in this thesis, therefore, suggest that only a few types of movements are explicitly encoded in the vertebrate spinal cord. These movements can then be used in combination in order to produce more complex behaviors.

Chapter 1: Background

The question of how descending systems act through the spinal cord in order to produce a wide range of movements is central to the study of the neural basis of motor control. Given that the vast majority of descending systems exert their actions through interneuronal systems located within the spinal cord(Dum and Strick, 1996; Shinoda et al., 1977), understanding how these spinal interneuronal systems contribute to the production of movement can help to guide the study of the control of movement by higher structures. This question has been studied from several different perspectives and several different hypotheses have been suggested as to how the rich repertoire of movements might be mediated by spinal interneuronal systems.

The majority of these hypotheses assume that the production of complex behaviors must be simplified in some way. One of the first explicit considerations of the difficulties involved in the production of movement was carried out by Bernstein(Bernstein, 1967). He pointed out that in general the number of variables that must be controlled by the nervous system is very large with respect to the constraints imposed by the task to be performed. For instance, when making simple reaching movements, the nervous system can accomplish the same movement of the hand using many different movements of the joint angles of the arm. The situation is even more complex at the level of the muscles: potentially, the nervous system must specify the activation level of each individual motor unit. One way that the nervous system might deal with these excess degrees of freedom is to couple groups of them together, an idea which was been termed a "synergy"(Lee, 1984; Macpherson, 1991). Such a synergy would simplify the control of movement by reducing the number of degrees of freedom of the motor system, making the specification of control variables more constrained by the demands of the desired movement.

Although the notion of a synergy was originally proposed in terms of coordination of kinematic degrees of freedom, the same idea has been extended to the coordination of muscle activations. As applied to the control of muscles, a "muscle synergy" implies that a group of muscles is activated together as a group. In the extreme, such a muscle synergy implies that a group of muscles will always be activated in a fixed balance, independent of the overall strength of the response. Further, in order to simplify the control of movement, there should only be a small number of such synergies, certainly less than the number of muscles. Otherwise, the number of degrees of freedom would remain unchanged and the problem of creating a desired movement would then be unconstrained. According to this hypothesis, the complex pattern of muscle activations observed during normal movements results from the specification of only a small number of muscle synergies.

On the other hand, complex patterns of muscle activations might result from the simultaneous specification of each individual degree of freedom. At the extreme, the number of these degrees of freedom is limited only by the number of motor units. The ability to coordinate and adapt each of these degrees of freedom to produce a desired behavior is clearly complex but not obviously impossible *a priori.*

The activation of each muscle or motor unit might be specified based on some simple control principle defined by the demands of the behavior. The pattern of muscle activations observed during the behavior would then result from the parallel and independent action of these control principles exerted on each muscle. In this context, the coordination of degrees of freedom of the motor system is a descriptive phenomenon: there is no overarching control law which coordinates the activation patterns of a group of muscles.

Clearly, these different hypotheses have different implications as to how the nervous system produces movements. If movements produced by the spinal cord are organized across a group of

muscles, descending systems must take these patterns of spinal coordination into account when creating arbitrary movements, either by exploiting this coordination or by breaking this coordination apart to allow for more precise control. If movements produced by the spinal cord are organized at the level of each individual muscle, on the other hand, descending systems must create the desired pattern of coordination *ab ovo,* specifying the details of muscle activations necessary to produce the desired movement.

Evidence in support of each of these different hypotheses of the organization of movement by the spinal cord has been found from several different lines of research. I will briefly review some of these research lines and then outline how the research in the present thesis examines these hypotheses.

Mammalian locomotion

Of all the behaviors that the neural networks in the mammalian spinal cord are capable of producing, perhaps the most complex is above ground locomotion(Grillner, 1981; Rossignol, 1996). In locomotion, there is a coordination across all four limbs, with an alternation of flexion and extension movements between the two hindlimbs and between the two forelimbs. Simultaneously, there is an alternation of flexion and extension between the limbs on the left side of the body and on the right side of the body. Thus, at a coarse level, locomotion can be divided into the two distinct phases of flexion and extension across all four limbs. The behavioral demands of the two phases are quite different. The extension phase supports the center of mass and propels the animal forward. In flexion, the limb is lifted off the ground and brought forward in preparation for the next step. These phases can be further subdivided on the basis of kinematic or dynamic variables or on the basis of muscle activations. Because of its fundamental nature across all mammalian species, locomotion has served as a behavior upon which many theories of the organization of movement by the spinal cord have been based.

Sherrington first described that the spinal cord isolated from the rest of the nervous system was capable of producing "spinal stepping"(Sherrington, 1910). In response to certain types of stimulation, he was able to evoke a series of rhythmical hindlimb movements which appeared to share many of the characteristics of locomotion produced by the intact animal. Later work described that the patterns of muscle activations underlying locomotion produced by the isolated spinal cord shared many of the characteristics of normal locomotion, although there are differences(Grillner and Zangger, 1979; Pearson and Rossignol, 1991). Sherrington also observed that the flexion and extension movements involved in locomotion resembled the flexion and extension movements produced during withdrawal reflexes. Based on this similarity, Sherrington proposed that the two phases of locomotion are produced by the same elements as those that produce withdrawal reflexes. The alternation between these two phases, he proposed, was regulated by the "reflex chaining" produced by patterns of afferent feedback during each phase. In this scheme, the transition from flexion to extension was accomplished by the afferent activity produced by the stretch of extensor muscles during the flexion movement. Similarly, the transition from extension to flexion might be accomplished by the afferent activity produced from the skin contact during the extension phase of locomotion. According to Sherrington's theory, locomotion consists of two basic muscle synergies, coupled together by afferent feedback.

Brown showed that the rhythmic alternation between flexion and extension was not critically dependent on the afferent feedback, demonstrating that at least a portion of the rhythmicity in locomotion was created centrally(Brown, 1911). He proposed a specific mechanism of flexion and extension "half-centers", coupled together by fatiguing inhibtion, which could account for the rhythmic alternation underlying locomotion.

Lundberg and Jankowska later identified a particular neural system which they proposed might subserve the alternation underlying locomotion(Jankowska et al., 1967; Lundberg, 1980). They examined the interneuronal systems recruited by activation of the "flexor reflex afferents" (FRA), consisting of high threshold muscle and cutaneous afferents(Eccles and Lundberg, 1959). They found that when animals were given L-DOPA, a noradrenaline precursor, stimulation of the FRA produced a rhythmic alternation between the activation of flexor and extensor motoneurons. Further, it appeared that there was a mutual inhibition between these flexion and extension movements, similar to that proposed in Brown's half-center hypothesis. Based on these observations, Lundberg and Jankowska proposed that the FRA systems which were activated following L-DOPA application might subserve the production of mammalian locomotion.

Such a clear and simple explanation for the production of locomotion was called into question when it became clear that the patterns of muscle activations underlying locomotion could be very complex(Grillner and Zangger, 1979; Pearson and Rossignol, 1991). Muscle activations did not clearly segregate into the flexion and extension groups proposed by Sherrington or in Brown's half center hypothesis. Instead, muscles could be active in both flexion and extension or only at the transition between the phases. Further, the timing of individual muscles within the locomotor cycle could be very different for each individual muscle. These details of muscle activations produced during locomotion were difficult to reconcile with the notion of the simple combination of separate flexion and extension synergies.

Lundberg proposed that these details of locomotion might be the result of afferent feedback modifying an underlying simple flexion and extension alternation(Lundberg, 1980). However, experiments performed in the paralyzed, fictive preparation and in deafferented animals demonstrated that these detailed muscle activations were in fact specified centrally in the spinal cord(Grillner and Zangger, 1979; Pearson and Rossignol, 1991). The details might still reflect a modification of a basic flexion extension alternation, but that modification must occur centrally.

Grillner suggested a hypothesis that might explain these details while preserving the simplifying notion of muscle synergies(Grillner, 1981). He proposed that there is a set of "unit bursters" organized within the spinal cord. Each unit burster activates a set of agonist muscles acting at the same joint and each is intrinsically rhythmical. These unit bursters can then be coupled in arbitrary ways in order to create the desired behavior. In this theory, a pattern of muscle activations is rarely the action of a single unit burster. Instead, the observed activations result from complex interactions between several different bursters. In support of this theory, research examining the activations of muscles underlying forward and backward locomotion found that the two different forms could be explained simply as a reordering of two basic muscle synergies. This finding is in agreement with the idea that a few basic units of muscle activations can be combined flexibly to produce different movements. A similar organization of spinal motor systems has been proposed by Jordan(Jordan, 1991). He suggested that there is a set of "modules" each of which consists of a set of neurons acting to activate a set of agonist muscles and inhibit their antagonists.

Each of these hypotheses of locomotion described above relies on some type of grouping of muscle activations, or muscle synergies, in order to simplify the production of locomotion. There are relatively fewer proponents of the idea that locomotion is specified at the level of each individual muscle.

One line of research which argues against this notion of a set of centrally specified pattern generators acting across a group of muscles to produce locomotion has been carried out by Pearson and his collaborators(Pearson, 1987). This work has emphasized the control of the locomotor rhythm by patterns of afferent feedback into the spinal cord. For instance, stretch flexor muscles during locomotion produces a strong inhibition of the extension phase(Hiebert et al., 1996). Similarly, the degree of loading in ankle extensors can have a profound impact on the locomotor

cycle(Pearson et al., 1992). This work implies that much of the locomotor rhythm might be the result of afferent feedback acting on the spinal cord. This afferent feedback would be distributed to a small subset of muscles which would be determined by the reflex pathway activated. The combined action of these reflexes would then create the observed pattern of locomotion.

Finally, there is some research suggesting that these two control strategies of individual muscle control or of muscle synergies might both be used to produce spinal locomotion. Loeb and colleagues recorded the activity of many muscles in the hindlimb of the cat during locomotion (Chanaud and Macpherson, 1991; Pratt, 1991; Pratt et al., 1991). They examined the detailed organization of these activations, examining different anatomical compartments of the same muscle and looking for the level of organization which is controlled during locomotion. They then compared these muscle activations to those produced by cutaneous stimulation during locomotion. From examination of these patterns of muscle activations, they came to several conclusions. For some groups of muscles during locomotion, there was evidence that they were controlled together as a single unit. For other muscles, however, the activation patterns appeared to be more complex and individuated. This unique control seemed to be especially true for broad, mutlifunctional muscles. Also, the functional subdivisions of muscles observed during locomotion corresponded to the functional subdivisions of muscles observed during cutaneous stimulation; i.e. those muscles activated as flexors in locomotion were excited by cutaneous stimulation while muscles activated as extensors were inhibited. This observation was consistent with Sherrington's hypothesis that two behaviors of locomotion and flexion withdrawal shared some of the same spinal substrates. For some muscles, however, the two types of responses did not appear to correspond to one another, suggesting that the overlap between the two behaviors was not complete. Taken as a whole, this body of work suggests that the patterns of muscle activations underlying locomotion are composed of the combined control of groups of muscles and of individual muscles.

Scratch reflexes

The concepts of unit bursters and modules of excitation and inhibition have found support from research into another common rhythmical behavior produced by the spinal cord: the scratch reflex. Like locomotion, the scratch reflex was first characterized by Sherrington at the turn of the century(Sherrington, 1910; Sherrington, 1906). In several different animals, light, repeated tactile stimulation results in a limb movement which attempts to remove the irritant from the skin surface. The movement is often repeated several times in order to effectively remove the irritant, resulting in a rhythmic activation of the hindlimb. This rhythmical activity consists of alternating flexion and extension but does not resemble the activations underlying locomotion, at least in the cat(Grillner, 1981).

The scratch reflex has been characterized extensively in two amphibians, the turtle and the frog. The organization of scratch reflexes in the turtle has been studied by Stein and his collaborators(Stein et al., 1986). The turtle shows three main forms of the scratch reflex depending on the location of the site of the skin surface stimulated(Mortin et al., 1985). Stimulation on the bridge of the turtle's shell evokes a 'rostral' scratch in which the irritant is removed with the foot. Stimulation of the skin in the region near the leg inside the animal's shell evokes a 'pocket' scratch, in which the side of the thigh or calf is used to remove the irritant. Stimulation near the base of the thigh or tail evokes a caudal scratch, in which the irritant is removed with the heel or the side of the foot. These three forms of the scratch reflex are each produced by a basic underlying alternation of hip protraction and retraction with a superimposed knee extension. The differences between each form of the scratch results from differences in which part of the hip protraction and retraction cycle the knee extension is activated(Robertson et al., 1985). This type of organization has been

interpreted in terms of a functional coupling between unit bursters or modules acting at the hip and knee joints: different forms of the scratch reflex result from the activation of the same set of elements, just coordinated in a different way.

Work from this group has also suggested that these modules might be organized by groups of neurons located in different parts of the spinal cord. By making a series of spinal cord lesions and examining the effects of these lesions on the scratch reflex, it was suggested that segments of the spinal cord are differentially specialized for the control of hip protraction and hip retraction(Mortin and Stein, 1989). There also appears to be a strong influence from the contralateral spinal cord, and that this influence can be exerted selectively on one particular component of the scratch reflex(Stein et al., 1995). This work as whole argues that the turtle spinal cord is organized into a number of distinct modules, both in terms of function and of anatomy, which can be combined flexibly in order to produce the different scratch reflexes.

The frog also is capable of producing a number of forms of scratch reflexes, again depending on the site of stimulation on the skin(Berkinblitt et al., 1989). The scratch reflexes of the frog have historically been called wipes. Stimulation of the back or forelimb produces a rostral wipe, in which the limb is flexed and the stimulus removed with the foot. Stimulation of the cloaca produces a wipe in which the stimulus is removed with the heel or ankle. Stimulation of the contralateral ankle produces a hindlimb-hindlimb wipe, in which both legs are extended and the unstimulated foot is used to remove the stimulus from the contralateral leg's ankle. Berkinblitt originally described that the rostral wipe could be divided into a number of distinct phases. These phases were defined on the basis of the kinematics of the movement: phases were separated from one another by a short pause in the movement of the limb. Each of the phases also appeared to be controlled independently: some phases could be deleted entirely from the behavior or their duration extended without a dramatic effect on the other phases. Based on these observations, Berkinblitt et al. proposed that the wipe reflexes in the frog were composed of a number of distinct movement patterns which were combined sequentially(Berkinblitt et al., 1986). Each pattern could be governed by a different control rule modifying its implementation in a particular context, such as the adaptation to the site of stimulation observed in the rostral wipe(Giszter et al., 1989; Sergio and Ostry, 1993). They also proposed that some of these phases might be shared by other behaviors. For instance, they proposed that the flexion phase of the rostral wipe might be produced by the same system producing the flexion withdrawal. Although it was later shown that the two movements are not in fact the same(Schotland et al., 1989; Schotland and Rymer, 1993), this idea of the production of complex behavior from the composition of simpler ones might still be valid in another formulation.

These two hypotheses of the organization of scratch reflexes in the turtle and the frog share the common idea that the scratch reflex is created by the composition of more basic muscle patterns. They differ, however, in the nature of these basic muscle patterns. In the turtle, these patterns represent subunits of behavior which can be simultaneously combined in order to produce the full behavior. In the frog, these patterns represent particular subdivisions of the behavior, each one having a distinct behavioral goal: there is no simultaneous combination of these patterns to produce more complex behaviors, only sequential combination. Thus, although both explanations suppose the existence of something like a muscle synergy, they differ considerably in how these synergies are utilized by the nervous system.

Withdrawal reflexes in the rat

Of all the behaviors organized in the spinal cord, perhaps the most basic is the withdrawal reflex. Sherrington originally described the flexion withdrawal as a "type reflex", in which the set of muscles activated was always the same(Sherrington, 1910). Similarly, the extension of the

contralateral limb which accompanied the ipsilateral flexion involved the activation of fixed set of muscles. It was these flexion and extension responses that he postulated comprised the flexion and extension phases of locomotion. In fact, later he classified muscles as being flexors if they were activated during withdrawal and extensors if they were inhibited during withdrawal. In later work, however, Sherrington described that the balance of muscle activations within the fixed set of muscles could be altered depending on the particular afferent nerve which was stimulated(Creed and Sherrington, 1926). For stimulation of a particular nerve, this balance of muscle activations was constant for different strengths of activation.

This latter work has been examined more extensively in the rat by Schouenborg and colleagues (Schouenborg et al., 1992; Schouenborg and Kalliomaki, 1990; Schouenborg and Weng, 1994). In this work, different regions of the hindlimb skin surface were stimulated and the activation of individual muscles was measured. They found that each muscle was activated from a particular region of the hindlimb surface. The region of skin from which a muscle was activated appeared to depend on the mechanical action of that muscle, such that a muscle was most activated from that region of the skin which it most effectively removed from stimulation. They also found that when the action of a muscle was altered by moving the attachment of one of its tendons, the region of the skin from which that muscle is activated is changed according to the muscle's new action(Holmberg and Schouenborg, 1996). The specificity of the excitation of muscles in the withdrawal reflexes was also observed for the inhibition of muscles: muscles were inhibited from those parts of the skin which would be toward the site of stimulation(Weng and Schouenborg, 1996). Based on these findings, Schouenborg proposed a model of the withdrawal reflexes in the rat in which each muscle is controlled according to its individual mechanical action. In this hypothesis, withdrawal reflexes do not consist of a constant balance of muscles but can be varied according to the site of stimulation on the skin surface. This variation is a consequence of the parallel and independent control of each individual muscle and not to a strategy coordinating the entire set of muscles.

Recruitment of muscle compartments

It has been known for a long time that muscles are not functionally uniform(Engberg and Lundberg, 1969; Hoffer et al., 1987; Sherrington, 1910). A number of studies have demonstrated that within a single muscle there can be several different domains, each with its own mechanical action, its own functional properties, and its own pattern of utilization during different behaviors. Even in one region of a muscle, different motor units might be differentially controlled. Based on these results, Loeb has proposed that spinal cord neural systems might be organized into a number of different "task groups".(Loeb, 1985) A task group consists of a set of motoneurons and interneurons devoted to a particular set of tasks which share common functional aspects. For instance, the control of muscles during tasks in which they are activated while lengthening might be considerably different during tasks in which they are activated while shortening. These different control regimes, according to this hypothesis, would be subserved by distinct neural systems, each effected by different populations of motor units. This hypothesis suggests that there is a coordination of control, but this coordination is across different motor units in the same muscle. In effect, the level of control is at most across units within an individual muscle.

Neuronal mediation of descending commands

The literature reviewed up until this point has mainly examined the organization of movement by the spinal cord by examining general behavioral outputs such as kinematics or dynamics or patterns of muscle activations. A number of hypotheses of the mediation of

descending movements by the spinal cord have resulted from the study of neuronal mechanisms within the spinal cord.

One extensive set of experiments has been conducted by Alstermark and his colleagues (Alstermark and Lundberg, 1992). This group has identified a population of interneurons in the C3-C5 segments (termed the 'C3-C4' propriospinal system, mainly for historical reasons) which appear to be a large degree specialized for mediating commands from descending systems. These neurons project directly to motoneurons and receive monosynaptic projections from several descending systems. They also receive input from sensory afferents, but this input appears weak in comparison to the descending input. When the output of these neurons is lesioned, cats become dysmetric in their reaching movements, even though they can still grasp and manipulate objects well. This same system also appears to be active only when animals are required to perform tasks requiring precise visuomotor control(Alstermark and Kummel, 1990). Individual C3-C4 propriospinal neurons appear to project to a set of motoneurons which can be related to particular components of reaching behaviors(Tantisira et al., 1996). These results suggest that descending movements might be mediated by a devoted spinal interneuronal system. Further, within this interneuronal system, this work suggests that individual neurons are specialized in the control of the groups of muscles involved in the production of particular phases of the reaching behavior.

Lundberg has also proposed how a particular spinal interneuronal system might help to mediate movements specified by descending commands (Lundberg, 1979; Lundberg et al., 1987). He has focussed on the flexion reflex afferent system (FRA). The FRA system is defined as the systems activated by stimulation of high threshold muscle and cutaneous afferents. Several characteristics of FRA system make it a candidate system for the mediation og descending commands. First, the FRA system is capable of producing a wide range of movements. Second, because of its multimodal inputs, the FRA can be regulated and controlled through feedback from the periphery. Third, the general types of responses observed from stimulation of descending systems are similar to those produced by stimulation of the FRA. Lundberg proposed that these characteristics of the FRA system might be exploited by descending systems in a type of servo mechanism. In this hypothesis, descending systems first activate the population of FRA neurons required to produce the desired behavior, causing the initiation of the movement. The reafference resulting from this movement is then channeled back onto the same neurons, resulting in a prolongment in their activity. As the movement reaches its goal, this reafferent activity decreases, resulting in the cessation of the movement. While there has been little evidence in support of this hypothesis, it does present another alternative theory of how movements can be created through the spinal cord.

Modularity of the frog spinal cord as assessed by microstimulation

A recent series of experiments by Bizzi and colleagues has examined the organization of the frog spinal cord by describing the movements evoked by intraspinal microstimulation (Bizzi et al., 1991; Giszter et al., 1993; Mussa-Ivaldi et al., 1994). In these experiments, the interneuronal regions of the frog lumbar spinal cord were stimulated with low levels of current. This stimulation resulted in the activation of a set of muscles in the ipsilateral hindlimb. The isometric force produced by these activated muscles was then measured at the ankle with the hindlimb fixed in a particular configuration. The hindlimb was then moved to a new configuration, the same site in the spinal cord was stimulated, and the evoked force measured. This procedure was then repeated for several different configurations of the hindlimb. The pattern of position dependent forces, or force field, measured in this manner was then used to characterize the responses evoked from this intraspinal stimulation.

The basic result of these experiments was that when many different regions throughout the spinal cord of the frog were stimulated, only a few types of force fields were measured. A similar result was observed following focal iontophoresis of NMDA into the spinal cord(Saltiel et al., 1996). This observation that only a few different types of movements were apparently encoded in the spinal cord is difficult to reconcile with the ability of the nervous system to produce a wide range of movements. A possible solution to this problem was suggested from the results obtained from stimulating two different regions of the spinal cord simultaneously(Mussa-Ivaldi et al., 1994). This costimulation resulted in a response which was the simple linear combination of each response evoked separately. It has subsequently been shown that the patterns of muscle activity underlying this costimulation also combine linearly. Additionally, the exact force field produced by costimulation can be controlled by applying different strengths of stimulation to each site, suggesting that these responses can be combined flexibly to produce a desired response. These results have led to the hypothesis that the nervous system produces desired movements by activating a combination of only a few types of force fields encoded in the spinal cord.

This work is consistent with the idea that the organization of movements in the spinal cord is based around a small number of patterns of muscle activation which can be combined flexibly in order to produce a wide range of movements. Essentially, this hypothesis is the same as the unit burster hypothesis of Grillner. The main difference between the two theories is that Grillner's hypothesis was intended to explain rhythmical behaviors such as locomotion, while the summation hypothesis of Bizzi was intended to explain trajectory formation and positional control. The proposed mechanism underlying each of them, however, is very similar.

Synthesis

The results of the research described above suggest, at least for some aspects of motor control, movements produced by the spinal cord are organized in terms of muscle synergies. There appears to be evidence for the control of individual muscles in some circumstances as well. This idea of muscle synergies has lead to several theories of the construction of complex movements. Each of these theories shares the common idea that these muscle synergies can be combined flexibly, either by sequentially piecing them together, as in the frog wipe reflexes, or by their simultaneous expression, as in Grillner's unit burster hypothesis or the summation hypothesis of Bizzi. In each case, the use of muscle synergies has been proposed as a mechanism by which the nervous system simplifies the control of many redundant degrees of freedom in order to create arbitrary movements.

Distinguishing whether a group muscles is controlled as a functional unit or whether muscles are controlled individually is very difficult, however. The simple observation that a set of muscles is activated at the same time is not evidence that those activations are being controlled as a unit: the observed coordination might simply emerge from the independent control of each muscle. Given that there will usually be a set of muscles which make similar contributions to a particular behavior (i.e. are mechanical 'synergists'), one might therefore expect those muscles to be activated in a similar manner, the activation of each muscle being related to its mechanical action. The consequent pattern of simultaneous activity observed across those muscles could then be interpreted as a muscle synergy, even though the underlying control strategy was at the level of each individual muscle.

Similarly, it can be difficult to determine conclusively that muscles are each controlled individually. If the unit burster or summation theory were true, it might appear that the organization of a particular behavior was at the level of each individual muscle even though the underlying control strategy was in terms of muscle synergies. For example, the activation of semitendinosus during locomotion in the cat often shows two bursts of activity, one during flexion and another during extension. This complex pattern of activation has been interpreted as evidence that this

muscle was controlled individually and, given other observations, it seems likely that this is the case. This observation on its own, however, is not sufficient to conclude that this muscle is controlled individually. These activations might arise if this muscle were associated with two different underlying muscle synergies, one produced during flexion, the other produced during extension. Similarly, the detailed temporal patterns of muscle activations observed during behaviors do not exclude the possibility that muscles are controlled as a unit. Such temporal patterns could be a stereotyped feature of the motor action produced by a particular coupling between muscles and not the independent specification of each separate muscle activation. It can therefore be difficult to assess the degree to which muscles are controlled independently or as a functional unit.

Outline of experiments described in this thesis

The experiments described in this thesis examine these same issues of the level of control of movement exerted by the vertebrate spinal cord. In particular, these experiments will extend the work described above examining the movements evoked from microstimulation of the frog spinal cord. We will elaborate the hypothesis produced from these experiments of a modular organization of movements by the spinal cord. In Chapter 2 we verify that the results found previously were not specific to the frog spinal cord, but can be found the mammalian spinal cord. That chapter will also examine whether the responses evoked from spinal stimulation can be related to known aspects of the organization of the vertebrate spinal cord. In particular, the anatomical organization of these responses will be compared to the somatotopical organization of the spinal cutaneous systems. Chapter 3 will then examine the organization of withdrawal reflexes produced by the spinalized frog, providing evidence that this behavior is organized into a small number of distinct patterns of muscle activations. Chapter 4 will then test the hypothesis that the responses observed from spinal microstimulation are similar to the responses observed from noxious cutaneous stimulation, examining the patterns of muscle activation produced by spinal stimulation and cutaneous stimulation within the same animal. These experiments will help to place the results obtained from spinal microstimulation in a physiological context. By doing so, the experiments will help to make the microstimulation responses more interpretable so that their contribution to complex behaviors can be better understood. Finally, we examine in Chapter 5 whether the responses from cutaneous stimulation can be described as the combination of a small number of muscle synergies. The results of this chapter provide evidence which supporting the hypotheses reviewed here that complex behaviors can created by the flexible combination of a small number of distinct muscle synergies. Each of the results described in this thesis, therefore, found in the rat and in the frog and from both intraspinal and cutaneous stimulation, supports the hypothesis that movements produced by the isolated spinal cord are organized discretely.

References:

Alstermark, B., and Kummel, H. (1990). Transneuronal transport of wheat germ agglutinin conjugated horseradish peroxidase into last order spinal interneurones projecting to acromio- and spinodeltoideus motoneurones in the cat. 2. Differential labelling of interneurones depending on movement type. Exp. Brain Res. *80*, 96-103.

Alstermark, B., and Lundberg, A. (1992). The C3-C4 propriospinal system: target-reaching and food-taking. In Muscle Afferents and Spinal Control of Movement (Oxford, New York: Pergamon Press).

Berkinblitt, M. B., Feldman, A. G., and Fukson, O. I. (1986). Adaptability of innate motor patterns and motor control mechanisms. Behavioral and Brain Sciences *9*, 585-638.

Berkinblitt, M. B., Feldman, A. G., and Fukson, O. I. (1989). Wiping reflex in the frog: movement patterns, receptive fields, and blends. In Visuomotor Coordination, J. P. Ewert and M. A. Arbib, eds. (New York: Plenum Press), pp. 615-629.

Bernstein, N. (1967). The coordination and regulation of movements (New York: Pergamon Press).

Bizzi, E., Mussa-Ivaldi, F. A., and Giszter, S. F. (1991). Computations underlying the execution of movement: a biological perspective. Science *253*, 287-291.

Brown, T. G. (1911). The intrinsic factors in the acto of progression in the mammal. Proc. Roy. Soc. Lond. *84*, 308-319.

Chanaud, C. M., and Macpherson, J. M. (1991). Functionally complex muscles of the cat hindlimb. III. Differential activation within biceps femoris during postural perturbations. Exp. Brain Res. *85*, 271-280.

Creed, R. S., and Sherrington, C. S. (1926). Observations on concurrent contraction of flexor muscles in the flexion reflex. Proc. Roy. Soc. Lond. *100B*, 258-267.

Dum, R. P., and Strick, P. L. (1996). Spinal cord teminations of the medial wall motor areas in macaque monkeys. J. Neurosci. *16*, 6513-6525.

Eccles, R. M., and Lundberg, A. (1959). Synaptic actions in motoneurones by afferents which may evoke the flexion reflex. Arch. Ital. Biol. *97*, 199-221.

Engberg, I., and Lundberg, A. (1969). An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. Acta Phys. Scand. *75*, 614-630.

Giszter, S. F., McIntyre, J., and Bizzi, E. (1989). Kinematic strategies and sensorimotor transformations in the wiping movements of frogs. J. Neurophys. *62*, 750-767.

Giszter, S. F., Mussa-Ivaldi, F. A., and Bizzi, E. (1993). Convergent force fields organized in the frog's spinal cord. J. Neurosci. *13*, 467-491.

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods, and fish. In Handbook of Physiology: Sect I. The Nervous System, V. B. Brooks, ed. (Bethesda, MD: American Physiological Society), pp. 1179-1236.

Grillner, S., and Zangger, P. (1979). On the central generation of locomotion in the low spinal cat. Exp. Brain Res. *34*, 241-261.

Hiebert, G. W., Whelan, P. J., Prochazka, A., and Pearson, K. G. (1996). Contribution of hind limb flexor muscle afferents to the timing of phase transitions in the cat step cycle. J. Neurophys. *75*, 1126-1137.

Hoffer, J. A., Sugano, N., Loeb, G. E., Marks, W. M., O'Donovan, M. J., and Pratt, C. A. (1987). Cat hindlimb motoneurons during locomotion. III. Functional segregation in sartorious. J. Neurophys. *57*, 554-562.

Holmberg, H., and Schouenborg, J. (1996). Postnatal development of the nociceptive withdrawal reflexes in the rat: a behavioral and electromyographic study. J. Physiol. *493*, 239-252.

Jankowska, E., Jukes, M. G. M., Lund, S., and Lundberg, A. (1967). The effect of DOPA on the spinal cord. 5. Reciprocal organization of pathways transmitting excitatory action to alpha motoneurons of flexors and extensors. Acta Phys. Scand. *70*, 369-388.

Jordan, L. (1991). Brainstem and spinal cord mechanisms for the initiation of locomotion. In Neurobiological basis of human locomotion, M. Shimamura, S. Grillner and V. R. Edgerton, eds. (Tokyo: Japan Scientific Societies Press), pp. 3-20.

Lee, W. A. (1984). Neuromotor synergies as a basis for coordinated intentional action. Journal of Motor Behavior *16*, 135-170.

Loeb, G. E. (1985). Motoneurone task groups: coping with kinematic heterogeneity. Journal of Experimental Biology *115*, 137-146.

Lundberg, A. (1980). Half-centres revisited. In Regulatory Functions of the CNS: Principles of Motion and Organization, J. Szentagothai, M. Palkovits and J. Hamori, eds. (Budapest: Pergamon Press), pp. 155-167.

Lundberg, A. (1979). Multisensory control of spinal reflex pathways. In Progress in Brain Research: Reflex control of posture and movement, R.Granit and O.Pompeiano, eds. (Amsterdam: Elsevier), pp. 11-28.

Lundberg, A., Malmgren, K., and Schomburg, E. D. (1987). Reflex pathways from group II muscle afferents. 3. Secondary spindle afferents and the FRA; a new hypothesis. Exp. Brain Res. *65*, 294-306.

Macpherson, J. M. (1991). How flexible are muscle synergies? In Motor Control: Concepts and Issues, D. R. Humphrey and H.-J. Freund, eds.: John Wiley & Sons Ltd.), pp. 33-47.

Mortin, L. I., Keifer, J., and Stein, P. S. G. (1985). Three forms of the scratch reflex in the spinal turtle: movement analyses. J. Neurophys. *53*, 1501-1516.

Mortin, L. I., and Stein, P. S. G. (1989). Spinal cord segments containing key elements of the central pattern generators for three forms of scratch reflex in the turtle. J. Neurosci. *9*, 2285-2296.

Mussa-Ivaldi, F. A., Giszter, S. F., and Bizzi, E. (1994). Linear combinations of primitives in vertebrate motor control. Proc. Nat. Acad. Sci. *91*, 7534-7538.

Pearson, K. G. (1987). Central pattern generation: a concept under scrutiny. In Advances in Physiological Research, H. McLennan, J. R. Ledsome, C. H. S. McIntosh and D. R. Jones, eds. (NY: Plenum Press), pp. 167-185.

Pearson, K. G., Ramirez, J. M., and Jiang, W. (1992). Entrainment of the locomotor rhythm by group Ib afferents from ankle extensor muscles in spinal cats. Exp. Brain Res. *90*, 557-566.

Pearson, K. G., and Rossignol, S. (1991). Fictive motor patterns in chronic spinal cats. J. Neurophys. *66*, 1874-1887.

Pratt, C. A. (1991). Functionally complex muscles of the cat hindlimb. I. Patterns of activation across sartorious. Exp. Brain Res. *85*, 243-256.

Pratt, C. A., Chanaud, C. M., and Loeb, G. E. (1991). Functionally complex muscles of the cat hindlimb. Exp. Brain Res. *85*, 281-299.

Robertson, G. A., Mortin, L. I., Keifer, J., and Stein, P. S. G. (1985). Three forms of the scratch reflex in the spinal turtle: central generation of motor patterns. J. Neurophys. *53*, 1517-1534.

Rossignol, S. (1996). Neural control of stereotypic movements. In Handbook of Physiology, Section 12. Exercise: Regulation and Integration of Multiple Systems, L. B. Rowell and J. T. Sheperd, eds.: American Physiological Society), pp. 173-216.

Saltiel, P., Tresch, M. C., and Bizzi, E. (1996). Modularity of the frog spinal cord motor system. In Society for Neuroscience Abstracts (Washington D.C.

Schotland, J. L., Lee, W. A., and Rymer, W. Z. (1989). Wipe and flexion withdrawal reflexes display different EMG patterns prior to movement onset in the spinalized frog. Exp. Brain Res. *78*, 649-653.

Schotland, J. L., and Rymer, W. Z. (1993). Wipe and flexion reflexes of the frog. I. Kinematic and EMG patterns. J. Neurophys. *69*, 1725-1735.

Schouenborg, J., Holmberg, H., and Weng, H.-R. (1992). Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. Exp. Brain Res. *90*, 469-478.

Schouenborg, J., and Kalliomaki, J. (1990). Functional organization of the nociceptive withdrawal reflexes. I. Activations of hindlimb muscles in the rat. Exp. Brain Res. *83*, 67-78.

Schouenborg, J., and Weng, H.-R. (1994). Sensorimotor transformations in a spinal motor system. Exp. Brain Res. *100*, 170-174.

Sergio, L. E., and Ostry, D. J. (1993). Three-dimensional kinematic analysis of frog hindlimb movement in reflex wiping. Exp. Brain Res. *94*, 53-64.

Sherrington, C. S. (1910). Flexion-reflex of the limb, crossed extension reflex and reflex stepping and standing. J. Physiol. *40*, 28-121.

Sherrington, C. S. (1910). Notes on the scratch-reflex of the cat. Quarterly Journal of Experimental Physiology *3*, 213-20.

Sherrington, C. S. (1906). Observations on the scratch-reflex in the spinal dog. J. Physiol. *34*, 1-50.

Shinoda, Y., Ghez, C., and Arnold, A. (1977). Spinal branching of rubrospinal axons in the cat. Exp. Brain Res. *30*, p203-218.

Stein, P. S. G., Mortin, L. I., and Robertson, G. A. (1986). The forms of a task and their blends. In Neurobiology of vertebrate locomotion, S. Grillner, P. S. G. Stein, D. G. Stuart, H.Forssberg and R. M. Herman, eds. (London: Macmillian Press), pp. 201-216.

Stein, P. S. G., Victor, J. C., Field, E. C., and Currie, S. N. (1995). Bilateral control of hindlimb scratching in the spinal turtle: contralateral circuitry contributes to the normal ipsilateral motor pattern of fictive rostral scratching. J. Neurosci. *15*, 4343-4355.

Tantisira, B., Alstermark, B., Isa, T., Kummel, H., and Pinter, M. (1996). Motoneuronal projection pattern of single C3-C4 propriospinal neurones. Canadian Journal of Physiology and Pharmacology *74*, 518-530.

Weng, H.-R., and Schouenborg, J. (1996). Cutaneous inhibitory receptive fields of withdrawal reflexes in the decerebrate spinal rat. J. Physiol. *493*, 253-265.

Chapter 2: Organization of movements evoked from spinal microstimulation in the rat

INTRODUCTION

As described in the previous chapter, a recent set of experiments has proposed a hypothesis as to how the nervous system produces movements by acting through the spinal cord. These experiments examined the movements evoked from microstimulation of the frog spinal cord (Bizzi et al., 1991; Giszter et al., 1993; Mussa-Ivaldi et al., 1994). These experiments demonstrated that there were only a small number of movements evoked from microstimulation of the interneuronal regions of the frog spinal cord. Movements other than these few types, however, could be produced by combining stimulation at multiple sites in the spinal cord and the movement produced by this combined stimulation could be predicted simply. These results suggested that descending systems might produce a wide range of movements by the coactivation of only a small number of movements encoded in the spinal cord. Such a scheme might greatly simplify the computational problem of creating an arbitrary movement by descending systems.

These results, however, were somewhat limited by the fact that they were performed in the frog. As a vertebrate, the frog spinal cord shares many characteristics with spinal cords of other animals, but there are also substantial differences (Simpson, 1976). Generalizing the results found in the frog to other vertebrate species, therefore, is not straightforward. There is also generally not as much known about the organization of the frog spinal cord as compared to the spinal cords of other vertebrate species, especially mammals.

For these reasons, we examined the responses obtained by applying microstimulation to the interneuronal regions of a mammalian spinal cord, that of the rat. The first motivation for these experiments was to test whether an organization similar to that of the frog could be observed in the rat spinal cord. The second motivation of these experiments was to attempt to place the results obtained from spinal microstimulation in the context of known aspects of the organization of the mammalian spinal cord. In particular, we examined the relationship between the responses evoked from spinal microstimulation and the spinal interneuronal systems activated by cutaneous afferents.

METHODS

Preparation

Results from 26 adult male and female Sprague-Dawley rats (250-400 g) are reported in this study. All procedures were approved by the Committee for Animal Care of M.I.T. All rats were chronically spinalized in the lower thoracic spinal cord, at the level of T10-T12. Rats were anesthetized with ketamine/xylazine (87/13 mg/kg i.p.) given along with atropine (0.02 mg/kg s.c.). Rats were also given methylprednisolone (35 mg/kg i.m.) presurgically in order to accelerate recovery after spinal transection (Behrman et al., 1994). Under aseptic conditions, the T8 spinal vertebra was opened dorsally, lidocaine was applied to the surface of the spinal cord and injected into the spinal cord, and the spinal cord was sectioned with iridectomy scissors. Completeness of the transection was verified by visually observing through a microscope the two cut ends of the cord withdraw to the caudal and rostral margins of the exposure. The wound was then closed in layers. Animals' temperature was regulated for several hours after surgery. After recovering from anesthesia, animals usually appeared bright and lively and began eating and drinking spontaneously. If animals appeared behaviorally depressed, however, they were given analgesics (buprenex 0.01 mg/kg s.c.) for 2-3 days following surgery. Antibiotics (ampicillin 25mg/kg i.m.) were given twice

daily for the first week after surgery. Fluids (Ringer's s.c.) were supplemented as necessary to ensure a daily fluid intake of 66cc/kg/day. Postoperatively, bladders were expressed at least twice daily, using gentle manual pressure. Male rats were initially used in this study but we later switched to female rats since their maintainance following spinalization was generally much easier. There were no apparent diferences between the results from male and female rats and so data from both was combined.

In some animals (n=6), we also performed a complete unilateral hindlimb deafferentation in the same surgical session as the spinal transection. After the animal was spinalized, vertebrae T13-L3 were exposed. The space between vertebrae was enlarged over the left half of the spinal cord by removing a portion of each vertebra and the dura was opened to expose the dorsal roots. Dorsal roots were distinguished from ventral roots by the amount of electrical stimulation (single .1ms pulses through a silver wire just touching the root) required to evoke a visible movement in the ipsilateral hindlimb. This procedure was necessary since we performed a limited laminectomy and since it was often difficult to distinguish spinal roots visually at these vertebral levels. Once the dorsal roots were identified, the roots were bathed in lidocaine in order to reduce injury discharge and the dorsal roots severed. Completeness of the deafferentation was verified postsurgically by the loss of ipsilateral cutaneous reflexes, during the acute experiment by the loss of neural activity evoked by sensory stimulation in the ipsilateral spinal cord, and by dissection of the spinal cord following the experiments. Only those animals with complete L1 to S1 deafferentations are reported in this study.

After a period of 1-3 weeks following the spinalization, animals were prepared for the acute experiment and data collection. Rats were anesthetized with a single dose of ketamine/xylazine (87/13 mg/kg i.p.) as well as with atropine (0.02 mg/kg s.c.). We also gave a dose of dexamethasone (18 mg/kg i.p.) in order to reduce edema of the neural tissue remaining after the decerebration. Both carotid arteries were exposed and sutures were loosely looped around both arteries. Rats were then transferred to a stereotaxic frame, supported by a platform which allowed the limbs to hang free, and the skull was immobilized with earbars. The sutures around the carotids were then attached to weights, constricting the vessels and cutting off circulation to the brain. A craniotomy was performed to expose the brain between bregma and lambda and the dura opened. Rats were then decerebrated precollicularly, using a scalpel along with an aspirator to remove tissue rostral to the superior colliculus. The wound was then loosely packed with powdered gelfoam. Anesthesia was discontinued after this point. After a period of 1 to 2 hours following the decerebration, the carotid arteries were released from constriction.

The lumbar spinal cord was exposed by opening T13, L1, and/or L2 vertebrae by dorsal laminectomy. In some animals only one or two vertebrae were opened and so only a portion of the lumbar spinal cord was examined. The exposed spinal cord was bathed in warm mineral oil using the skin surrounding the wound to form a pool. In most animals the dura was kept intact, although in a few animals dura was opened to allow for precise viewing of the surface of the spinal cord. Vertebrae immediately rostral and caudal to the exposure were clamped to secure the spinal cord and a coccyxgeal vertebra was also clamped to secure the hip.

Rats' temperature was monitored with a rectal thermometer and maintainted between 35.6 and 37.8^0 C using a heating lamp. Fluids were supplemented throughout the experiment (4.5cc/kg Dextrose s.c. approximately every two hours). The time between the dose of anesthesia and the beginning of data collection was usually 1.5 to 2 hours. The viability of the preparation was assessed throughout the experiment by monitoring heart and respiration rate, by the strength of responses to cutaneous stimulation, and in some animals by the neural responses to sensory stimulation. This preparation was typically viable for 8-10 hours although some rats survived for periods of longer than 24 hours.

Data collection

Microelectrodes (stainless steel, tip diameter .5-1μm, 10Mohm) were inserted into the spinal cord under visual inspection through a microscope. We applied trains of stimulation (cathodal currents of 2-30μA, 250-600ms, 70Hz, .3ms) through this electrode. Only responses evoked by currents of 15μA or lower are presented in this paper. The largest of these currents would be expected to activate directly a volume of tissue of about 200μm radius (Gustafsson and Jankowska, 1976; Ranck Jr., 1975). Sites between 300 and 800μm lateral to the cord midline were examined here. These electrodes usually penetrated the spinal cord without considerable dimpling of the spinal cord, as assessed by viewing the advance of the electrode through the microscope and by observing a similar pattern of responses at different depths with the dura removed.

The left hindlimb was attached to a 6 axis force transducer (ATI sensor, sampled at 86.5 Hz) using a cuff wrapped securely around the ankle. The transducer was mounted on a positioning device which allowed the ankle to be fixed in any position within the workspace of the hindlimb. The isometric force evoked by microstimulation was measured at the ankle by the transducer.

We examined how the response evoked from stimulating a particular site in the spinal cord varied with different limb configurations. With the electrode fixed in a single position in the spinal cord, the ankle was moved to several different locations in the workspace. The same site in the spinal cord was stimulated for each ankle position and the evoked force was measured. This pattern of position dependent forces constitutes a force field and concisely describes the response evoked from stimulating a site in the spinal cord (Bizzi et al., 1991; Giszter et al. 1993).

We also examined the interaction between the responses evoked from different sites in the spinal cord. Electrodes were placed in two different spinal sites and the force field from stimulating each site separately was measured. Microstimulation was then applied to both electrodes simultaneously and the force field resulting from this costimulation was measured.

Because measuring a force field as described above usually requires measuring forces for 10-20 ankle positions, collecting a force field can take a significant amount of time. In order to assess the responses evoked across many different sites in the spinal cord of a particular rat, we therefore measured the forces evoked by microstimulation at only a single ankle position. This ankle position was usually below the hip and with the leg nearly pendant, although different ankle positions were also tested in different animals. We tested different ankle positions in different rats in order to differentiate the different types of microstimulation responses from one another. Because there were differences between the ankle positions used in different rats, data from different animals are not combined in the present study. For these experiments in which many sites in the spinal cord were examined, the electrode was advanced in steps of 250μm from the dorsal surface of the spinal cord and currents of either 2, 8, or 15μA were applied at each site.

In some animals (n=7), we also examined the sensory receptive fields of different sites in the spinal cord. Neural activity in response to peripheral sensory stimulation was recorded through the same electrode as was used to apply microstimulation. Because the animals were unanesthetized, vigorous cutaneous stimulation often evoked a motor response, making the signals related to the sensory stimulus difficult to distinguish from the signals related to the production of the reflex. We therefore limited our sensory stimulation to light brushing of the hair and occasional light pressure on the limb. The receptive field from multiple units was usually recorded, although single units were isolated whenever possible. The region of skin from which responses could be obtained was mapped on standard drawings of the rat hindlimb. When microstimulation was applied to the same site in the spinal cord, the receptive fields were mapped prior to the stimulation, although we usually observed the same neural responses before and after microstimulation.

At the end of the experiment, we placed electrolytic lesions at sites of interest, either to mark locations at which particular responses were measured or to provide reference landmarks (10μA, 20s). Lesions were usually placed as the state of animals was rapidly deteriorating, so perfusions were only rarely performed. More often, we removed the spinal column, leaving the spinal cord within the vertebrae, and placed it in fixative (10% formalin) for a period of at least 4 weeks. These fixed cords were then cut into 80μm transverse sections and stained with Prussian blue or cresyl violet.

Data analysis

Although we measured the three dimensional forces evoked by stimulation, we generally only report the results based on an analysis of the force evoked in the rat's sagittal plane containing the hip. Forces out of this plane were not critical for the analyses performed in this study and including the extra dimension significantly complicates many of the analyses perfomed here. The background, resting force measured prior to stimulation was subtracted from all force measurements and the remaining active, evoked force was used for all subsequent analyses. Only force responses of a magnitude of greater than .022 N were used. Responses less than this magnitude could have large variations in their direction due to limitations of the force sensor's sensitivity. For most analyses we examined responses at a fixed latency from the onset of microstimulation. We chose a latency of 230ms since the response at this time was always within the train of microstimulation, and we found that responses at this latency were generally near their peak (see Results). Analyses of directional data were done using standard circular statistics (Fisher, 1993; Mardia, 1972).

CHANGE IN FORCE DIRECTION AT STIMULATION OFFSET. It appeared that in many cases the evoked force changed following the offset of the stimulation train. We examined this issue by comparing the forces measured just before and just after the end of the stimulation train. The two times were separated by 200ms. Since it appeared that the changes could be in either the direction or the magnitude of the force response, we measured the difference between force responses as the distance between the two force vectors. These differences were calculated for each force response in each animal. We compared these differences to the differences between two forces measured within the train of stimulation (separated by 200ms), again calculated for all responses in each animal. We then assessed whether, for a given animal, the differences between the evoked forces at times before and after the end of the stimulation train were greater than the differences between the evoked forces at times within the stimulation train, using a one tailed paired t-test.

VARIATION OF FORCE DIRECTION WITH DEPTH. We examined the variation of the evoked force direction at different distances from the dorsal surface of the cord. In particular, we wanted to determine whether the direction of force significantly changed at different depths along a given electrode penetration. In any individual electrode penetration, each depth was usually stimulated three times, once for each current level. Consequently, there was not enough data in any individual electrode penetration to allow for an adequate statistical analysis of the differences between depths. We therefore examined these differences in each rat with data combined from all electrode penetrations. For each electrode penetration, we took the mean direction of the most superficial site examined, usually 250μm from the top of the spinal cord. This mean direction was then subtracted from each direction along that electrode penetration, and the absolute value of these deviations taken. These deviations at different depths from the top of the spinal cord for each penetration were then combined for each animal. We then performed a statistical test to examine the effect of depth on the deviation of force direction from the top of the spinal cord for each

animal. We used a bootstrap test (500 bootstrap steps) using the nonparametric Y statistic for the comparison of multiple samples of circular data (Fisher, 1993), essentially a oneway ANOVA. If there was a significant effect of depth, pairwise comparisons of each depth to the most superficial depth were then performed, again using the Y statistic.

FORCE FIELDS FROM SUPERFICIAL AND DEEP SITES. We compared the force fields measured from superficial and deep sites within the spinal cord. It appeared that the change in the evoked force between different ankle positions was smoother for superficial sites than for deeper sites. To quantify this impression, we examined for a given force field how well the variation of evoked forces across the workspace could be described as a simple function of ankle position. We fit the change in force measured across the workspace as a second order polynomial function of ankle position, using standard regression techniques. The quality of the fit for a particular force field was then measured using the coefficient of determination, R^2, calculated from the residual sum of squares. We interpreted a large R^2 value as indicating that there was a smooth variation of the evoked force across the workspace. We then compared the R^2 values measured for force fields evoked from superficial sites with the R^2 values measured for force fields evoked from deep sites, using a bootstrap t-test.

COSTIMULATION OF SPINAL SITES. To examine the interaction between responses evoked from distinct sites in the spinal cord, we used the analysis of Mussa-Ivaldi et al. (Mussa-Ivaldi et al., 1994). We tested the hypothesis that simultaneous stimulation of two sites in the spinal cord produces a force field which is a simple linear combination of the force field produced by stimulation of each site separately. For this analysis we examined all three components of the measured force.

Force fields were represented as high dimensional vectors, where each component corresponds to the rostrocaudal, dorsoventral, or mediolateral component of the force produced at each ankle position: if a force field was measured at N (typically 10-20) ankle positions, this field vector would have 3N components. In this representation, each component of the force at each position constitutes a single dimension in a 3N dimensional space. Using this representation, therefore, two fields which are similar should point in the same direction in this high dimensional space. The similarity between two force fields can therefore be taken as the cosine of the angle between them, a value of 1 indicating identical responses, and a value of -1 indicating directly opposite responses.

We compared the force field produced by costimulation of two spinal sites to the force fields produced from separate stimulation of each site, and to the force field predicted if these two separate force fields combined linearly. The response evoked by costimulation was considered to be a good case of summation if two criteria were met: 1) the similarity measure between the observed costimulation force field and the force field predicted from a simple linear vector addition of each separate force field was greater than .9, and 2) this similarity measure was at least .1 greater than the similarity measure between the force field produced from costimulation and from stimulation of each separate spinal site. The first criterion ensures that the costimulation force field is similar to the predicted summation force field, while the second criterion ensures that the costimulation force field is not simply due to one of the two force fields having a larger magnitude than the other or to the two fields being very similar to one another to begin with. Meeting this second criterion proved to be quite difficult as finding two sites in the spinal cord which produced significantly different responses could be difficult.

DISTRIBUTION OF FORCE DIRECTIONS AT A SINGLE LIMB CONFIGURATION. The number of modes in the distribution of force directions evoked by microstimulation of many sites in the spinal cord and measured at a single ankle position was determined using a statistical test described by Hsu et al. (Hsu et al., 1986). The basic idea of this test is to fit the observed distribution of data to a mixture of n modes and calculate a goodness of fit statistic for this mixture: if the goodness of fit statistic is significantly bad, then there are at least n+1 modes in the distribution of data.

We assumed that the modes in the distribution of data were each described by a von Mises probability distribution (Fisher, 1993; Mardia, 1972). The von Mises distribution is the standard probability model for circular data and we have found that it generally describes the data obtained in our experiments well. A mixture of these von Mises distributions was fit using the Expectation Maximization (EM) algorithm (Dempster et al., 1977). The Appendix describes this method applied to circular data in more detail. EM generally performs better than other fitting procedures and the results obtained from using EM qualitatively give a good fit of the data. Once the optimal fit of n von Mises distributions was found using EM, the goodness of fit of this mixture was calculated using the U^2 statistic for circular data (Mardia, 1972). The significance of this statistic for a particular fit to the data was evaluated using a bootstrap procedure (see Appendix).

ANATOMICAL VARIATION OF FORCE DIRECTION To determine whether the direction of evoked force was related to the rostrocaudal or mediolateral coordinate of the stimulation site, we performed a regression analysis. We fit the direction of evoked force to a second order polynomial function of the anatomical coordinates of the stimulation site using standard regression techniques. We concluded that there was a significant relationship between the force direction and an anatomical coordinate if the 95% confidence interval of one of the regression parameters weighting that coordinate did not include zero.

RESULTS

Responses from microstimulation of the rat spinal cord

Microstimulation of the rat spinal cord produced forces measured at the ankle both during the stimulation train and also for a period of time following the offset of stimulation. An example of the time evolution of responses evoked at different depths along an electrode penetration through the spinal cord is illustrated in Figure 1A. As can be seen in the figure, at the offset of the microstimulation train the force measured at the ankle was often altered, showing an increase in magnitude or a change in direction. In the majority of animals examined (7/10), there was a significant change in the force measured following the offset of microstimulation ($p<.05$). When tested, this second response was clearly due to the offset of the stimulation train, as demonstrated by increasing the duration of the stimulation train. Because of the difficulty in interpreting these later responses, we describe only those responses evoked during the train of stimulation since those responses should more directly reflect the action of a particular site in the spinal cord.

Examination of Figure 1A also suggests two main regions from which responses could be evoked by microstimulation of the spinal cord: a region from the top of the cord to approximately 1000μm, and a deeper region starting at 1750μm. The unresponsive region in between these two regions was also commonly observed: when tested, stimulation in this region with currents of as high as 30μA was usually incapable of producing a measurable response. It appeared that, in any given electrode penetration, the responses throughout the more superficial region were more or less similar to one another but were often different from the responses evoked from the deeper regions.

To examine this observation we analyzed how the direction of force changed at different depths from the dorsal surface of the spinal cord. The majority of animals examined (7/11) showed the first significant change ($p<.05$) in force direction at depths of 1500μm from the top of the spinal cord, 3/11 showed the first change at 1250μm, and one rat showed a change at 750μm. This analysis is consistent with the observation that, in a given electrode penetration, the responses from superficial sites are similar to one another but are different from the responses from deeper sites.

Differences between superficial and deep sites could also be observed in the force fields evoked from each region. Figure 1B shows a force field evoked from the superficial region of the spinal cord, at a depth of 500μm. It can be seen that the response in Figure 1B is well structured throughout the workspace of the hindlimb, such that wherever the ankle is positioned initially, the force response brings the limb toward the same final configuration: the force field is convergent. Figure 1C shows a force field measured from a site 1500μm deeper along the same electrode penetration as the site in Figure 1B. In contrast to the more superficial force field, this deeper force field was poorly structured, with the force direction changing markedly between adjacent ankle positions. We quantified this difference between the force fields from superficial and deep sites by examining how well the change in evoked force across the workspace could be described as a simple function of ankle position. We found that the force fields evoked from superficial sites were significantly better fit as a second order polynomial function of ankle position than the force fields from deeper sites (superficial $R^2 = .83 \pm .01$, deep $R^2 = .70 \pm .16$, $t_{44} = 2.35$, $p<.05$, 1000 bootstrap steps). Figure 2 shows that the force fields evoked from these superficial regions were also well structured when their action in 3 dimensions was examined.

Examination of histological material suggested that this superficial region corresponded roughly to laminae I through V while the deeper region corresponded mainly to ventral lamina VII and lamina IX. Histology also suggested that the vast majority of electrode penetrations were made in the spinal grey, although in some cases sites were located in fiber tracts.

These basic features of the responses from microstimulation in chronically transected animals were preserved following a unilaterally complete chronic deafferentation of one to three weeks (n=6). Figure 3A shows that microstimulation could still evoke responses from superficial regions at similar stimulation strengths as used for animals with chronic spinal transection but with afferents intact. Figure 3B shows an example of a force field evoked from stimulation of this region of the spinal cord in a chronically deafferented animal.

Based on the results described in this section, we limit the data presented in subsequent analyses to those responses evoked during the train of microstimulation from the region between 250μm and 1250μm from the dorsal surface of the spinal cord.

Costimulation

It has previously been shown that the simultaneous stimulation of two sites in the spinal cord of the frog results in a force field which is a simple linear summation of the responses evoked from stimulation of each site alone (Mussa-Ivaldi et al., 1994). We examined this issue for the responses evoked by microstimulation of the rat spinal cord. An example is illustrated in Figure 4. Stimulation of site A produced a force field directed caudally and dorsally (Fig. 4A), while stimulation of site B evoked a force field directed ventrally (Fig. 4B). The force field predicted from a linear summation of each separate response is illustrated in Figure 4C. The force field observed from costimulation of the two sites is illustrated in Figure 4D. Qualitatively, the observed costimulation force field is very similar to that predicted by linear summation, but is different from the force fields evoked from separate stimulation of the spinal sites. Figure 5 shows the force field correlation measure (see Methods) for each of these comparisons through the period of microstimulation. It can be seen that the correlation of the costimulation response to the predicted

summation was very high throughout the train of stimulation and was higher than the correlations to either of the separate force fields. This example met both criterion described in the Methods for a good case of summation only in the initial phases of the response. In all, we examined 7 cases of costimulation of sites in the spinal cord. These sites were chosen at the time of the experiment on the basis of whether they appeared to produce sufficiently different responses to allow a good test of summation. In 6 of these cases the costimulation response met both criterion at some point during the stimulation train. In the other case, it turned out that the responses were too close to one another to allow for a good test of summation.

Distribution of microstimulation evoked forces

Figure 6 shows the range of force directions measured at a single ankle position evoked by microstimulation of many sites in the spinal cord. Data from three animals (Fig. 6A,B,C) are shown in this figure, for two different levels of current. The most striking characteristic of these distributions and of all the distributions we observed from spinal microstimulation is that they are strongly biased to responses which draw the limb upwards to the body. Closer examination of the distributions shown in Figure 6 suggested that the distributions in each animal and at each current level tested were multimodal, with two main peaks being evident in each of them. We have shown the distributions obtained from different levels of currents for each rat in order to illustrate the consistency of this multimodality and that it was not due to different stimulation strengths. Similar distributions were observed from spinal microstimulation in chronically deafferented rats. In the deafferented animal shown in Figure 9A, we placed the ankle closer to the hip and observed a clearer separation between these two types of responses. This clearer separation was consistent with the force fields corresponding to these two responses (see Fig. 7 and below).

We examined whether there was evidence for multiple modes within these distributions of force directions using a statistical analysis described by Hsu et al. (Hsu et al., 1986; see Appendix). Applying this analysis to each individual rat showed that there was evidence ($p<.05$) for at least 2 modes in the majority of animals (8/11), for at least 3 modes in two animals, and only one mode in one animal. An example of the modes found for a distribution of data is shown in Figure 6E. The distribution of force directions observed in this animal is shown in Figure 6D, again showing evidence for at least two types of repsonses. The mixture of probability distributions found by the EM algorithm (see Appendix), is illustrated in Figure 6E, showing a correspondence with visual inspection of the data in Figure 6D. This analysis suggested the existence of at least two different classes of force directions which were observed from application of microstimulation throughout the rat spinal cord.

The force fields corresponding to the two classes indicated in the distributions are illustrated in Figure 7A and 7B. The first force field (Fig 7A) corresponds to the class pulling the limb forwards and toward the body. The second force field (Fig 7B) corresponds to the class pulling the limb backwards and also to the body. In Figure 7C, a third type of force field is also illustrated, which drives the limb away from the body. This third type of force field was observed in only 14% (6/42) of the force fields we measured and we did not observe it consistently in each animal, as evidenced by the analysis described above. When we did observe it, it was quite distinct, and we therefore include it here as a third type of response. Because of the rarity of this extension type of response, however, the rest of the analyses in this paper focus on the two main types of responses.

Anatomical organization of microstimulation evoked responses

In several animals (n=8: 5 afferents intact, 3 deafferented), we explored many different sites throughout the rostrocaudal and mediolateral extent of the spinal cord. In the animal shown in Figure 8, the direction of force evoked from microstimulation is plotted against the rostrocaudal

location of the stimulation site. In this animal, sites in caudal and middle lumbar segments evoked responses driving the limb dorsally and rostrally while sites in the rostral segments evoked responses driving the limb dorsally and caudally. We examined the significance of these relationships by a regression analysis, fitting the direction of evoked force to the rostrocaudal and mediolateral coordinates of the stimulation site. Four of the five animals showed a significant relationship to the rostrocaudal coordinate of the simulation site, and one of these animals showed a significant relationship to the mediolateral location of the simulation site. Figure 9B illustrates data from a similar experiment with a chronically deafferented animal. Regression analyses for two of three chronically deafferented animals showed a dependence on the rostrocaudal coordinate of the stimulation site, and one deafferented animal showed a dependence on the mediolateral coordinate.

Relationship between microstimulation responses and cutaneous receptive fields

This relationship between the direction of force and the anatomical location of the stimulation site seemed to roughly correspond to the somatotopic organization of the spinal cord shown in previous studies (Brown, 1981). By a combination of receptive field mapping and microstimulation, we examined this correspondence in more detail in several rats (n=4: afferents intact). Spinal receptive fields found in one animal in response to brushing of hair or light skin contact are illustrated in Figure 10. As has been shown in previous studies, we found that receptive fields systematically varied with the rostrocaudal location of the site. This relationship between receptive field and rostrocaudal location was paralleled by the variation of the direction of the response evoked by microstimulation, such that sites which had receptive fields on the front of the leg produced caudally directed responses consisting of a hip extension and knee flexion, sites with receptive fields on the foot produced responses directed dorsal and rostrally consisting of hip flexion and knee flexion, while in this rat sites with receptive fields on the back of the leg produced caudally directed responses. These caudally directed responses from the back of the cord were not observed in most other animals (see below). Some of the penetrations (2/13) in this animal were inconsistent with this general pattern, such as the sites at the rostral and caudal boundaries of the middle region which produced responses characteristic of this middle region but which did not have receptive fields on the foot.

To quantify this dependence of the response evoked by spinal microstimulation on the receptive field of a site in the spinal cord, we divided sites into three groups: those with receptive fields on the front of the leg and abdomen, those with receptive fields on the foot or responding to foot movement, and those with receptive fields on the back of the leg. As indicated in Figure 10, these divisions appeared to correspond to the main distinctions of force responses we observed. The mean force directions for sites divided into these categories for the 4 rats examined are shown in Figure 11 (rt65, rt66, rt73, rt74). It can be seen that in each rat examined, sites with receptive fields on the front of the leg tended to produce responses directed more caudally than sites with receptive fields on the foot. Figure 11 also illustrates that responses from sites with receptive fields on the back of the leg tended to drive the limb forwards. The effect of receptive field location on the response produced from microstimulation was found to be significant in each of these rats (bootstrap Y statistic , $p<.05$).

We also examined this correspondence between the somatotopical organization of the spinal cord and the responses evoked by microstimulation in animals with chronic unilateral deafferentation (n=2). We examined the relationship between the response from microstimulation of a site in the deafferented side of the spinal cord and the receptive field of the site in the contralateral, afferented, side at the same rostrocaudal and mediolateral location. Data from these two rats are illustrated in Figure 11 (rt72 and rt75). It can be seen that these two rats also showed the relationship between receptive field location and force direction shown by animals with intact

afferents. In particular, sites with receptive fields on the front of the leg produced different responses than those with receptive fields on the foot. This difference was significant for rt75 (bootstrap Y statistic, $p<.05$) but did not reach significance for rt72 ($p>.05$). This correspondence was supported in a third deafferented rat in which stimulation sites roughly paralleled the spinal cutaneous somatotopy in the contralateral spinal cord.

Further evidence that this relationship between the somatotopy of the spinal cord and the responses evoked by microstimulation was not due to the activation of sensory afferents was observed in an animal with a chronic partial deafferentation. In this animal, the dorsal roots supplying the front of the leg and all of the foot were cut, leaving intact the innervation of the back of the leg. After a period of 19 days following the deafferentation and spinalization, we found that sites in regions of the spinal cord which usually had receptive fields on the foot, as determined from recording receptive fields on the contralateral spinal cord, now were either unresponsive to cutaneous stimulation or had weak receptive fields on the back of the leg, consistent with previous physiological and anatomical studies (Pubols and Goldberger, 1980; Koerber and Mirnics, 1995; Wilson et al., 1996; Wall and Bennet, 1994). When these sites with aberrant receptive fields were stimulated, however, they produced responses significantly different (Y statistic bootstrap, $p<.05$) than those responses evoked from stimulation of sites with receptive fields on the back of the leg. This result, although from a single animal, further demonstrated that the response from microstimulation is not due to the activation of sensory afferents.

In Figure 12, we show the force fields measured from applying microstimulation to two different sites, one with a receptive field on the third most medial digit and the other with a receptive field on the shin. As expected from the results shown in Figure 11, these two sites produced different responses: stimulation of the site with the receptive field on the digit produced a rostrally directed force field (Fig. 12A) similar to that of Figure 7A, while stimulation of the site with the receptive field on the shin produced a caudally directed force field(Fig. 12C) similar to that of Figure 7B. For comparison, we show the force fields obtained in the same animal from electrical stimulation of the region of skin where the receptive fields of these two sites were located (Figs. 12B and 12C).

DISCUSSION

Summary

We have described several features of the responses evoked by microstimulation of the spinal cord of the chronically spinalized rat. Microstimulation of the interneuronal regions of the spinal cord produced force fields that were well structured across the workspace of the hindlimb. There only appeared to be a few types of responses which could be observed from spinal microstimulation. Force fields other than those few types, however, could in principle be produced by microstimulating multiple sites simultaneously and the response from such costimulation could be predicted simply. We further found that the different types of force fields were located in distinct regions of the spinal cord. This localization appeared to correspond to the cutaneous somatotopy of the spinal cord. Finally, these features were largely preserved following chronic deafferentation and so reflect the action of interneuronal systems in the spinal cord.

Before discussing these features in more detail, we address concerns about some of the methods used in the present study.

Methodological considerations

MICROSTIMULATION. The primary method used in this study was focal microstimulation of the spinal cord. This method has been used previously to address issues of the organization of

movements by the frog spinal cord (Bizzi et al., 1991; Giszter et al., 1993; Mussa-Ivaldi et al. 1994) as well as in other experiments examining the organization of motor systems throughout the nervous system (e.g. Asanuma et al., 1968; Drew and Rossignol, 1990; Yamaguchi, 1986). Microstimulation excites nervous structures within a distance of the stimulating electrode, activating axons, cell bodies, and dendrites. The strongest stimulation parameters used in this study would be expected to directly activate a volume of tissue of approximately 200μm radius (Gustafsson and Jankowska, 1976; Ranck Jr., 1975). This volume of tissue in the rat spinal cord should contain on order 100 neurons. However, the trains of microstimulation we used would be expected to indirectly activate a much larger region of the spinal cord (Gustafsson and Jankowska, 1976; Jankowska et al., 1975; Giszter et al. 1993) propagated through the connections of the directly activated site. Because of this nonspecificity, the results from microstimulation must be interpreted cautiously.

The basic assumption in using microstimulation is that neural elements within a distance of the electrode have a similar function. In the spinal cord there is reason to believe that this basic assumption is true. Several different systems in the spinal cord appear to have a topograhic organization, especially in the regions of the spinal cord which we examined in the present study. Both cutaneous and proprioceptive systems are topographically organized and withdrawal reflexes have similarly have been shown to activate neurons in localized regions of the spinal cord. (Brown, 1981; Riddel and Hadian, 1995; Rivero-Melian, 1996; Woolf and Fitzgerald, 1986; Light and Durkovic, 1981; Bullit, 1991; Menetrey et al., 1989). The observation in the present study of the relationship between the responses from spinal microstimulation and the spinal cutaneous somatotopy implies that microstimulation was reflecting this topography.

Other observations in the present study also suggest that the responses from microstimulation were reflecting the activation of particular sites in the spinal cord. First, microstimulation did not produce responses from every region of the spinal cord: regions which are well known to contain axons exciting motoneurons and which receive many projections from other spinal systems usually did not produce measurable responses. Second, the experiment in the partially deafferented animal suggests that the antidromic activation of afferents did not contribute significantly to the responses from microstimulation . Finally, the results from microstimulation were replicable in different animals, suggesting that the nonspecific nature of microstimulation did not obscure general aspects of the organization of the spinal cord. Also, recent work in the frog has demonstrated that responses similar to those observed from spinal microstimulation can also be evoked by focal iontophoresis of NMDA (Saltiel et al., 1996), again suggesting that the responses from spinal microstimulation are not dependent on the activation of axonal systems. However, even given these observations, the results obtained from microstimulation must be interpreted cautiously and, at best, can only reveal crude aspects of the organization of the spinal cord.

CHRONIC DEGENERATION. Another method used in the present set of experiments was chronic degeneration of descending and sensory afferent fibers. We performed these procedures first to isolate the spinal cord from the rest of the nervous system and second to remove the contribution of extraspinal fibers to the responses evoked from microstimulation. The survival times used in these experiments are sufficient to eliminate the ability of cut axons to conduct action potentials (Boyes and Veronesi, 1988; Tsao et al., 1994). We verified this loss of conduction in the present set of experiments by stimulating the proximal end of cut dorsal roots and observing no measurable response.

We therefore feel confident that the degeneration periods used in the present study were sufficient to eliminate the contribution of descending and sensory afferent systems to the responses observed from spinal microstimulation.

These chronic degeneration procedures are also known to induce large scale changes, both anatomical and physiological, in the organization of the spinal cord (e.g. Goldberger and Murray, 1975; Belanger et al., 1996). We initially attempted to perform these experiments in acutely spinalized animals but found that the reduced excitability of the spinal cord following transection required the use of very high stimulation currents in order to produce measurable responses, making their interpretation difficult. This observation suggests that the responses we observed in the present study performed in animals with chronic degeneration were dependent on the recovery of spinal excitability following transection. These results, therefore, might be considerably different than those found in normal, intact animals.

Comparision to results in the frog

The results described here for the rat spinal cord are similar to the results obtained from spinal stimulation of the frog spinal cord (Bizzi et al., 1991; Giszter et al., 1993; Mussa-Ivaldi et al., 1994). The convergence of force fields, the property of linear summation, the modularity of the responses, the anatomical segregation of different responses, and the similarity to cutaneous systems have all been previously shown for responses evoked from microstimulation of the frog spinal cord. Even the particular classes of force fields in the two animals are similar. These similarities suggest that, at the level of description examined here, both rat and frog spinal cords have a similar organization, suggesting the validity of generalizing these results to different vertebrate preparations.

Localization of microstimulation evoked responses

We focussed on the responses evoked from microstimulation of the superficial regions of the spinal cord which were clearly not due to direct motoneuronal activation. Responses from these regions were observed following chronic deafferentation and chronic spinalization and so were not dependent on the activation of either afferent, ascending, or descending fibers. And since the level of spinal transection in all of these animals was in the lower thoracic spinal cord, the responses were not dependent on long range propriospinal systems. These observations suggest that the responses evoked from microstimulation of this region of the spinal cord are due to the activation of spinal interneuronal systems intrinsic to the lumbar spinal cord.

It is not clear why we were unable to readily evoke responses from intermediate regions of the spinal cord, given the many neural elements in these regions which should activate motoneurons. While we were not able to determine the reason for this inability, it is possible that there might be substantial differences in the effect of microstimulation of these regions, such as a predominance of inhibitory effects, a difference in the anatomical organization (Scheibel and Scheibel, 1969) or in the excitability of these regions (e.g. Jankowska et al., 1967).

Convergent force fields

The force fields obtained from microstimulation of spinal interneuronal sites were found to drive the ankle toward a particular region of the workspace: the force fields evoked by spinal microstimulation were convergent. This type of convergent force field was not a necessary feature of force fields evoked from microstimulation of the spinal cord since microstimulation of very ventral structures was observed to produce force fields which were more incoherent. However, the pattern of convergence was not a unique feature of movements evoked from spinal microstimulation, since direct activation of individual hindlimb muscles often evoked very similar types of force fields (Tresch et al., 1994). Also, not every type of force field observed from spinal microstimulation could be characterized as convergent: the extension response shown in Figure 7C, although well organized across the workspace, would be best characterized as divergent or parallel. We therefore do not claim here that convergence, in of itself, differentiates the force fields evoked

from microstimulation of interneuronal systems of the rat spinal cord. In preliminary studies recording the patterns of muscle activations underlying these resposnes, we have found that they are produced by the activation of a group of muscles and not individual muscles. It is likely, therefore, that there are other features of these force fields which do distinguish them from those produced from single muscles.

Costimulation

The observation that the responses evoked from different sites in the spinal cord combine linearly is suprising. This observation implies that the different sites in the spinal cord produce movements in parallel and independent of one another. This implied independence is in conflict with the well known widespread connectivity of the spinal cord (Alstermark et al., 1990; Bras et al., 1989; Brink et al., 1983; Hultborn et al., 1976) and with the many nonlinearities in neural processing (e.g. Lundberg and Voorhoeve, 1962). The physiological relevance of summation is not clear at this point and will be the topic of future experiments.

Modularity of the spinal cord and relationship to cutaneous systems

The distributions of force directions observable from microstimulation of the rat spinal cord had two main characteristics: the distributions appeared to be both nonuniform and multimodal. The nonuniformity of the distributions was clear in all cases studied: microstimulation of the spinal cord tended to evoke responses which pulled the limb toward the body. The multimodality was seen in the majority of animals we examined in these experiments, as determined both by visual inspection and by a statistical analysis.

NONUNIFORMITY. The observation of a nonuniform distribution of force directions is suprising if one considers the responses evoked from microstimulation to reflect the activation of individual spinal interneurons. Experiments examining individual spinal interneurons have generally found that, even within a functionally homogenous group of neurons, the outputs and inputs of these neurons can be quite varied (Brink et al., 1983; Cavallari et al., 1987). Also, many interneuronal systems have multiple output pathways to motoneurons, the particular pathway expressed depending on the state of the preparation considered (e.g. Degtyarenko et al., 1996; LaBella et al., 1992; Pearson and Collins, 1993; Perreault et al., 1995; Pratt, 1995). Given the range of movements possible from activation of different motoneurons, one might expect many different types of movements to be observed from spinal microstimulation.

However, it is unlikely that the movements evoked from spinal microstimulation described here reflect the action of individual interneurons. As described previously, it is likely that the observed responses are due in large part to the indirect activation of a large population of neurons distributed throughout the spinal cord. The particular population which is activated depends on the propagation of activation through the patterns of interconnectivity of the spinal cord. And since the pathways of interneuronal systems in the spinal cord are dependent on the state of the preparation used, the particular neural population recruited by microstimulation will likely also be dependent on the state of the animal.

In this context, the nonuniform distribution of force directions is perhaps not quite so suprising. By far the most common response observable in the preparation used here, the quiescent chronically spinalized animal, is the classic flexion reflex (Eccles and Lundberg, 1959; Sherrington, 1910). Stimulation over large regions of the hindlimb skin surface as well as stimulation of some muscle afferent systems can readily evoke a flexion withdrawal of the stimulated limb, drawing the limb in toward the body. In the present study the vast majority of responses from stimulation of the spinal cord similarly moved the limb toward the body. The regions of the spinal cord from which

we obtained responses have previously been implicated in the processing of cutaneous information (Kitazawa et al. 1993; Moschovakis et al. 1992; Schouenborg et al. 1995) and there was a relationship between the organization of the responses evoked by microstimulation and the cutaneous somatotopy of the spinal cord. All of these observations suggest a relationship between the responses from microstimulation and spinal cutaneous systems.

MULTIMODALITY. Although this similarity between microstimulation and cutaneous systems might help explain the nonuniform range of forces observed in the present experiments, this similarity makes the multimodality of these forces we observed difficult to understand. Although the flexion reflex was initially characterized by Sherrington as a "type reflex" (Sherrington, 1910), he later described that withdrawal reflexes could vary depending on the stimulation (Creed and Sherrington, 1926). A more recent set of experiments has shown that the tuning of individual muscles in withdrawal reflexes can be very precise, such that the region of the skin surface which maximally activates a muscle is that region of skin which is most effectively removed by contraction of that muscle (Schouenborg et al., 1992; Schouenborg and Kalliomaki, 1990; Schouenborg and Weng, 1994).

In the present set of experiments, we observed a similar, albeit crude, relationship between the responses evoked from microstimulation and the cutaneous receptive field of a site in the spinal cord. On a gross level, this relationship was organized so that the response moved the limb away from a stimulus which would activate the receptive field.

Observing this principle of organization in the responses evoked by spinal microstimulation makes it difficult to understand the evidence for a few discrete directions of forces produced by microstimulation. Given the precise tuning of muscle activations in withdrawal reflexes, one would expect the pattern of muscle activations to change systematically as cutaneous stimulation was applied to different regions of the skin surface. This systematic change in muscle activations should also produce a systematic change in the forces observed, and one would consequently expect to observe a continuous distribution of different force directions evoked from activation of cutaneous systems. We see several possible explanations for this discrepancy.

One explanation for this discrepancy is that it results from the fact that our study was biased to the actions of the proximal musculature, since we were measuring forces produced at the ankle, while other experiments have tended to focus on the movement of the limb as a whole or of the foot. If this difference is responsible for the observed discrepancy, the observation that cutaneous systems controlling proximal musculature had an apparently modular organization would still be an interesting finding.

The modularity of microstimulation responses observed here might also be related to the well known existence of private pathways for cutaneous afferents. Perhaps most relevant to the results described here, Hagbarth (Hagbarth, 1952) demonstrated that stimulation of the skin on the front of the femur was capable of activating knee extensor muscles. This finding is reminiscient of the present observation of a different response from stimulation of sites with receptive fields on the front of the leg.

The final explanation we suggest here is that this discrepancy arises from a difference between preparations examined. Our study has examined responses produced by the chronically spinalized rat while the precise tuning of withdrawal reflexes was described for intact adult rats. In fact, the spinal cord acting on its own appears to be incapable of either developing or expressing this precise tuning (Schouenborg et al., 1992; Holmberg and Schouenborg, 1996; Schouenborg et al., 1996). In the absence of descending inputs, hindlimb withdrawal reflexes appear to be crudely tuned to the region of the skin stimulated. The results of the present study imply that the interneuronal systems underlying these behaviors in the chronically spinalized rat are modularly

organized. It seems possible that these two different observations, one of a crude tuning of cutaneous reflexes and the other of a modularity of microstimulation responses, might reflect the same underlying spinal organization. Further experiments will be required to examine this relationship more directly.

Conclusions

The results of the present study have demonstrated that, at the level of description examined here, the organization of movements evoked from microstimulation of the rat spinal cord is similar to that found for the frog. In both species, force fields obtained from microstimulation were well organized across the workspace, only a few types of responses were observed, and responses other than these few types could be obtained from simultaneous stimulation of multiple sites in the spinal cord. We have further provided evidence that these microstimulation evoked responses are related to cutaneous systems of the spinal cord. Establishing this relationship to cutaneous systems makes the similarity of the responses from microstimulation of rat and frog spinal cords we observed more understandable. Given that the hindlimbs of both rats and frogs are similar and that the two animals face a similar set of behavioral demands, especially for protective behaviors, one might have expected to observe similarities between the responses evoked by activation of cutaneous spinal systems in these two animals. Establishing this relationship to cutaneous systems also helps to place the responses evoked from spinal microstimulation in a physiological context where their possible involvement in the production of behaviors can be evaluated.

APPENDIX

EM applied to circular statistics

We wanted to fit a mixture of probability densities to the distribution of force distributions found from microstimulation. For circular data, the standard probability distribution is the von Mises distribution (Fisher, 1993; Mardia, 1972) where the probability of observing an angle θ is given by:

$$p(\theta) = \frac{1}{2\pi I_0(\kappa)} exp(-\kappa cos(\theta - \mu))$$

where μ and $\varkappa$ are the mean and concentration parameters describing the distribution, analagous to the mean and variance of the normal distribution and I_0 is the modified zero order Bessel function of the first kind. A mixture model consists of a number of such distributions, along with a set of mixing parameters which give the relative contributions of each distribution to the model.

We found the best fit mixture of a number of von Mises distributions to a particular set of data using the Expectation Maximization algorithm (EM) as described by Dempster et al. (Dempster et al., 1977). The explanation given here follows that of Jordan and Jacobs (Jordan and Jacobs, 1994). EM assumes that the set of observed data is an incomplete description of the system under consideration: the complete description of the system consists of the observed data plus the values of a number of other hidden variables. In the case considered here, the observed data is the force direction. The unobserved data is the probability distribution from which the data was generated, or the cluster to which the data point belongs. If we knew this complete data, consisting of both observed and unobserved data, fitting a mixture model would be straightforward: form the likelihood of the data given our probability model and maximize it with respect to the parameters of the model. Intuitively, maximizing this likelihood is equivalent to taking all the data belonging to each distribution and finding the means and concentration parameters that best describe each

distribution, the mixing parameters given by the amount of data that each distribution explains. This complete data log likelihood is given by:

$$\lambda = \sum_n \sum_j z_{nj}\left[\ln \pi_j - \ln I_0(\kappa_j) - \kappa_j \cos(\theta^n - \mu_j)\right]$$

where n indexes each individual data point; j indexes the different modes of the mixture model; π_j, μ_j, and κ_j are the mixing parameter, mean, and concentration parameter of the jth mode; and $\vec{z}$ is a vector of ones and zeros with only a single one indicating which mode produced the data. This $\vec{z}$ vector is the unobserved data. We wish to find the parameters of the mixture model which maximize this likelihood. This likelihood cannot be calculated, however, since we don't know $\vec{z}$. We therefore have to estimate the complete data likelihood by taking its expected value. This is the E-step of the EM algorithm. We find the expected value of the complete data likelihood:

$$Q = \sum_n \sum_j h_{nj}\left[\ln \pi_j - \ln I_0(\kappa_j) - \kappa_j \cos(\theta^n - \mu_j)\right]$$

where h_{nj} is the posterior probabilty given by:

$$h_{nj} = \frac{\pi_j p(\theta^n | j)}{\sum_j \pi_j p(\theta^n | j)}$$

where $p(\theta^n | j)$ is probability of observing the angle θ^n from the distribution j of the mixture model. The M-step of the EM algorithm maximizes this expected complete data log likelihood with respect the parameters of the mixture model. Using simple calculus we obtain the update equations for the parameters of the mixture model:

$$\frac{\partial Q}{\partial \mu_j} = 0 \rightarrow \mu_j^{new} = \tan^{-1} \frac{\sum_n h_{nj} \sin\theta^n}{\sum_n h_{nj} \cos\theta^n}$$

$$\frac{\partial Q}{\partial \kappa_j} = 0 \rightarrow \kappa_j^{new} = \frac{I_1(\kappa_j)}{I_0(\kappa_j)} = \frac{\sum_n h_{nj} \cos(\theta^n - \mu_j^{new})}{\sum_n h_{nj}}$$

$$\frac{\partial Q}{\partial \pi_j} = 0 \rightarrow \pi_j^{new} = \frac{1}{N} \sum_n h_{nj}$$

The update equation for κ_j is not straightforward to apply since it is expressed as a function of two modified Bessel functions. This ratio of modified Bessel functions of the first kind, however, is the same as the mean resultant length of a circular distribution, $R(\kappa)$, and there are numerical approximations for κ as a function of R (Fisher, 1993; Mardia, 1972). Although these update equations maximize the complete data log likelihood, Dempster et al. (Dempster et al., 1977) proved that these update equations will always increase the likelihood of the incomplete (i.e. the observed) data as well. Thus, by applying these update equations iteratively, the algorithm converges on a set of model parameters which maximizes the likelihood of our observed data. Note that there are no learning rates or other values to set for the convergence to occur.

Bootstrap procedure for evaluating number of modes

Once the optimal fit of a particular number of von Mises distributions was found using EM, the goodness of fit of this mixture was calculated using the U^2 statistic for circular data (Mardia,

1972). The significance of this statistic for a particular fit to the data was evaluated using a bootstrap procedure. This U^2 statistic measures the goodness of fit of a given mixture to the data set. If this statistic is significantly bad, we can reject the null hypothesis that the data was generated by a mixture of n modes, allowing us conclude that there are at least n+1 modes in the data. But since we don't know the distribution of this U^2 statistic for an arbitrary mixture model, we cannot evaluate the significance of a particular U^2 value directly. Instead, we must estimate its significance using a bootstrap procedure (Hsu et al., 1986). We wish to estimate the distribution of U^2 values one would expect if the observed data were actually generated by a mixture of n modes, our null hypothesis, and determine if the particular U^2 value is significantly unlikely with respect to this distribution. First, a set of data was randomly generated using the probabilities determined by the best fit mixture model of n modes to the original data set. This new data set had the same number of data points as the original data set. Second, the best fit mixture of n modes to this new data set was found using EM. Third, the U^2 statistic for the goodness of fit of this mixture model to the new data set was calculated. These three steps were then repeated 1000 times, giving the distribution of U^2 values expected for a set of data which was produced by a mixture of n modes. The fraction of U^2 values in this distribution which were larger than the observed U^2 value is the probability that the observed distribution of data was generated by a mixture of n modes. A significantly low probability indicates that there is at least one more mode in the data. It should be mentioned here that this statistical analysis is dependent on the data in our experiments being well fit by the probability distribution used. Applying this statistical test to data which are not well described by this probability distribution will give an overestimate of the number of modes in the data distribution. As mentioned in the Methods section, we found that most data sets could be well described by this distribution, but there were a few data sets which appeared not to be.

REFERENCES

Alstermark, B., Kummel, H., Pinter, M. J., and Tantisira, B. (1990). Integration in descending motor pathways controlling the forelimb in the cat. 17. Axonal projection and termination of C3-C4 propriospinal neurones in the C6-Th1 segments. Exp. Brain Res. *81*, 447-461.

Alstermark, B., and Lundberg, A. (1992). The C3-C4 propriospinal system: target-reaching and food-taking. In Muscle Afferents and Spinal Control of Movement (Oxford, New York: Pergamon Press).

Asanuma, H., Stoney, S. D. J., and Abzug, C. (1968). Relationship between afferent input and motor outflow in cat motorsensory cortex. J. Neurophys. *31*, 670-681.

Behrman, D. L., Bresnahan, J. C., and Beattie, M. S. (1994). Modeling of acute spinal cord injury in the rat: neuroprotection and enhanced recovery with methylprednisolone, U-74006F and YM-14673. Exp. Neurol. *126*, 61-75.

Belanger, M., Drew, T., Provencher, J., and Rossignol, S. (1996). A comparison of treadmill locomotion in adult cats before and after spinal transection. J. Neurophys. *76*, 471-491.

Bizzi, E., Mussa-Ivaldi, F. A., and Giszter, S. F. (1991). Computations underlying the execution of movement: a biological perspective. Science *253*, 287-291.

Boyes, W. K., and Veronesi, B. (1988). Electrophysiological and morphological evidence for a diameter-based pattern of the superior colliculus. Exp. Neurol. *101*, 190-207..

Bras, H., Cavallari, P., Jankowska, E., and Kubin, L. (1989). Morphology of midlumbar interneruons relaying information from group II muscle afferents in the cat spinal cord. J. Comp. Neurol. *290*, 1-15.

Brink, E., Jankowska, E., McCrea, D. A., and Skoog, B. (1983). Inhibitory interactions between interneurones in reflex pathways from group Ia and group Ib afferents in the cat. J. Physiol. *343*, 361-373.

Brink, E., Jankowska, E., McCrea, D. A., and Skoog, B. (1983). Post-synaptic potentials in a population of motoneurones following activity of single interneurones in the cat. J. Physiol. *343*, 341-359.

Brown, A. G. (1981). Organization in the Spinal Cord (Berlin, Heidelberg, New York: Springer-Verlag).

Bullit, E. (1991). Somatotopy of spinal nociceptive processing. J. Comp. Neurol. *312*, 279-290.

Cavallari, P., Edgley, S. A., and Jankowska, E. (1987). Post-synaptic actions of midlumbar interneurones on motoneurones of hind-limb muscles in the cat. J. Physiol. *389*, 675-689.

Creed, R. S., and Sherrington, C. S. (1926). Observations on concurrent contraction of flexor muscles in the flexion reflex. Proc. Royal Soc. Lond. *100B*, 258-267.

Degtyarenko, A. M., Simon, E. S., and Burke, R. E. (1996). Differential modulation of disynaptic cutaneous inhibition and excitation in ankle flexor motoneurones during fictive locomotion. J. Neurophys. *76*, 2972-2985.

Dempster, A. P., Laird, N. M., and Rubin, D. B. (1977). Maximum likelihood from incomplete data via the EM algorithm. J. Royal. Stat. Soc. Series B *39*, 1-38.

Drew, T., and Rossignol, S. (1990). Functional organization wihtin the medullary reticular formation of intact unanesthetized cat. I. Movements evoked by microstimulation. J. Neurophys. *64*, 767-781.

Dum, R.P. and Strick P.L. (1996). Spinal cord terminations of the medial wall motor areas in macaque monkeys. J. Neurosci. *16*, 6513-6525.

Eccles, R. M., and Lundberg, A. (1959). Synaptic actions in motoneurones by afferents which may evoke the flexion reflex. Arch. Ital. Biol. *97*, 199-221.

Fisher, N. I. (1993). Statistical Analysis of Circular Data (Cambridge: Cambridge University Press).

Giszter, S. F., Mussa-Ivaldi, F. A., and Bizzi, E. (1993). Convergent force fields organized in the frog's spinal cord. J. Neurosci. *13*, 467-491.

Goldberger, M.E., and Murray, M. (1974). Restituition of function and collateral sprouting in the cat spinal cord: the deafferented animal. J. Comp. Nuerol. *158*, 37-54.

Grillner S. (1981). Control of locomotion in bipeds, tetrapods, and fish. Handbook of Physiology: Sect I. The Nervous System Vol. 2: Motor Control, V.B. Brooks, ed. (Bethesda: American Physiological Society), pp. 1179-1236.

Gustafsson, B., and Jankowska, E. (1976). Direct and indirect activation of nerve cells by electrical pulses applied extracellularly. J. Physiol. *258*, 33-61.

Hagbarth, K.-E. (1952). Excitatory and inhibitory skin areas for flexor and extensor motoneurones. Acta Physiol. Scand. *26: Suppl.*, 1-58.

Holmberg, H., and Schouenborg, J. (1996). Postnatal development of the nociceptive withdrawal reflexes in the rat: a behavioral and electromyographic study. J. Physiol. *493*, 239-252.

Hsu, Y.-S., Walker, J. J., and Ogren, D. E. (1986). A step-wise method for determining the number of component distributions in a mixture. Math. Geol. *18*, 153-160.

Hultborn, H., Illert, M., and Santini, M. (1976). Convergence on interneurons mediating the reciprocal inhibition of motoneurons: Disynaptic inhibition of Ia inhibitory interneurons. Acta Physiol. Scand. *96*, 193-201.

Jankowska, E., Jukes, M. G. M., Lund, S., and Lundberg, A. (1967). The effect of DOPA on the spinal cord. 5. Reciprocal organizatino of pathways transmitting excitatory action to alpha motoneurons of flexors and extensors. Acta Physiol. Scand. *70*, 369-388.

Jankowska, E., Jukes, M. G. M., Lund, S., and Lundberg, A. (1967). The effect of DOPA on the spinal cord. 6. Half-centre organization of interneurones transmitting effects from the flexor reflex afferents. Acta Physiol. Scand. *70*, 389-402.

Jankowska, E., Padel, Y., and Tanaka, R. (1975). The mode of activation of pyramidal cells by intracortical microstimulation. J. Physiol. *249*, 617-636.

Jordan, M., and Jacobs, R. (1994). Hierarchical mixtures of experts and the EM algorithm. Neural Comp. *6*, 181-214.

Kitazawa, S., Ohki, Y., Sasaki, M., and Xi, M. (1993). Candidate premotor neurons of skin reflex pathways to T1 forelimb motoneurons of the cat. Exp. Brain Res. *95*, 291-307.

Koerber, H. R., and Mirnics, K. (1995). Morphology of functional long-ranging primary afferent projections in the cat spinal cord. J. Neurophys. *74*, 2336-2348.

LaBella, L. A., Niechaj, A., and Rossignol, S. (1992). Low-threshold, short-latency cutaneous reflexes during fictive locomotion in the "semi-chronic " spinal cat. Exp. Brain Res. *91*, 236-248.

Light, A. R., and Durkovic, R. G. (1984). Features of laminar and somatotopic organization of lumbar spinal cord units receiving cutaneous inputs from hindlimb receptive fields. J. Neurophys. *52*, 449-458.

Loeb, G.E. (1985). Motoneurone task groups: coping with kinematic heterogeneity. J. Exp. Biol. *115*, 137-146.

Lukashin A.V., Amirikian B.R., and Georgopoulos A.P. (1996). Neural computations underlying the exertion of force: a model. Biol. Cybern. *74*, 469-478.

Lundberg, A., and Voorhoeve, P. (1962). Effects from the pyramidal tract on spinal reflex arcs. Acta Physiol. Scand. *56*, 201-219.

Lundberg, A., Malmgren K., and Schomburg E.D. (1987) Reflex pathways from group II muscle afferents. 3. Secondary spindle afferents and the FRA: a new hypothesis. Exp. Brain. Res. *65*, 294-306.

Mardia, K. V. (1972). Statistics of Directional Data (London: Academic Press).

Menetrey, D., Gannon, A., Levine, J. D., and Basbaum, A. I. (1989). Expression of c-*fos* protein in interneurons and projection neurons of the rat spinal cord in response to noxious somatic, articular, and visceral stimulation. J. Comp. Neurol. *285*, 177-195.

Moschovakis, A. K., Solodkin, M., and Burke, R. E. (1992). Anatomical and physiological study of interneurons in an oligosynaptic cutaneous reflex pathway in the cat hindlimb. Brain Res. *586*, 311-318.

Mussa-Ivaldi, F. A., Giszter, S. F., and Bizzi, E. (1994). Linear combinations of primitives in vertebrate motor control. Proc. Nat. Acad. Sci. *91*, 7534-7538.

Pearson, K. G., and Collins, D. F. (1993). Reversal of the influence of group Ib afferents from plantaris on activity in medial gastrocnemius muscle during locomotor activity. J. Neurophys. *70*, 1009-1017.

Perreault, M.-C., Angel, M. J., Guertin, P., and McCrea, D. A. (1995). Effects of stimulation of hindlimb flexor group II afferents during fictive locomotion in the cat. J. Physiol. *487*, 211-220.

Pratt, C. A. (1995). Evidence of positive force feedback among hindlimb extensors in the intact standing cat. J. Neurophys. *73*, 2578-2583.

Pubols, L.M. and Goldberger M.E. (1980). Recovery of function in dorsal horn following partial deafferentation. J. Neurophys. *43*, 102-117.

Ranck Jr., J. B. (1975). Which elements are excited in electrical stimulation of mammalian central nervous system: a review. Brain Res. *98*, 417-440.

Riddel, J. S., and Hadian, M. R. (1995). Topographic organization of spinal neurons in group II reflex pathways of the anesthetized rat. J. Physiol. *483P*, 37.

Rivero-Melian, C. (1996). Organization of hindlimb nerve projections to the rat spinal cord: a choleragenoid horseradish peroxidase study. J. Comp. Neurol. *364*, 651-663.

Saltiel, P., Tresch M.C., and Bizzi E. (1996). Modularity of the frog spinal cord motor system. Soc. Neurosci. Abstr. *23*, Wash. D.C.

Scheibel, M. E., and Scheibel, A. B. (1969). A structural analysis of spinal interneurons and Renshaw cells. In The Interneuron, M. A. B. Brazier, ed. (Berkeley: University of California Press), pp. 159-208.

Schouenborg, J., Holmberg, H., Luo, X.-L., and Levinsson, A. (1996). Adult rats spinalized at birth exhibit inappropriate withdrawal reflexes. Soc. Neurosci. Abstr. *22*.

Schouenborg, J., Holmberg, H., and Weng, H.-R. (1992). Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. Exp. Brain Res. *90*, 469-478.

Schouenborg, J., and Kalliomaki, J. (1990). Functional organization of the nociceptive withdrawal reflexes. I. Activations of hindlimb muscles in the rat. Exp. Brain Res. *83*, 67-78.

Schouenborg, J., and Weng, H.-R. (1994). Sensorimotor transformations in a spinal motor system. Exp. Brain Res. *100*, 170-174.

Schouenborg, J., Weng, H.-R., Kalliomaki, J., and Holmberg, H. (1995). A survey of spinal dorsal horn neurones encoding the spatial organization of withdrawal reflexes in the rat. Exp. Brain Res. *106*, 19-27.

Sherrington, C. S. (1910). Flexion-reflex of the limb, crossed extension reflex and reflex stepping and standing. J. Physiol. *40*, 28-121.

Shinoda, Y., Arnold, A.P., and Asanuma, H. (1976). Spinal branching of corticospinal axons in the cat. Exp. Brain Res. *26*, 215-234.

Simpson, J. I. (1976). Functional synaptology of the spinal cord. In Frog Neurobiology, R. Llinas and W. Precht, eds. (Berlin: Springer), pp. 679-706.

Stein P.S.G. (1983). The vertebrate scratch reflex. Symp. Soc. Exp. Biol. *37*, 383-403.

Tresch, M. C., and Bizzi, E. (1995). Convergent force fields in the rat spinal cord. Soc. Neurosci. Abstr. *22*.

Tresch, M. C., and Bizzi, E. (1996). Relationship of movements from spinal microstimulation to natural behaviors in the rat. Neural Control of Movement *1*.

Tresch, M. C., Loeb, E., and Bizzi, E. (1994). Hindlimb muscles of the rat: force fields and redundant summation. Soc. Neurosci. Abstr. *20*.

Tsao, J. W., Brown, M. C., Carden, M. J., McLean, W. G., and Perry, V. H. (1994). Loss of the compund action potential: an electrophysiological, biochemical and morphological study of early events in axonal degeneration in the C57BL/Ola mouse. Eur. J. Neurosci. *6*, 516-524.

Wall, P. D., and Bennet, D. L. H. (1994). Postsynaptic effects of long-range afferents in distant segments caudal to their entry point in rat spinal cord under the influence of picrotoxin or strychnine. J. Neurophys. *72*, 2703-2713.

Wilson, P., Kitchener, P. D., and Snow, P. J. (1996). Intraaxonal injection of neurobiotin reveals the long-ranging projections of Ab-hair follicle afferent fibers to the cat dorsal horn. J. Neurophys. *76*, 242-254.

Woolf, C. J., and Fitzgerald, M. (1986). Somatotopic organization of cutaneous terminals and dorsal horn neuronal receptive fields in the superficial and deep laminae of the rat lumbar spinal cord. J. Comp. Neurol. *251*, 517-531.

Yamaguchi, T. (1986). Descending pathways eliciting forelimb stepping in the lateral funiculus: experimental studies with stimulation and lesion of the cervical cord in decerebrate cats. Brain Res. *379*, 125-136.

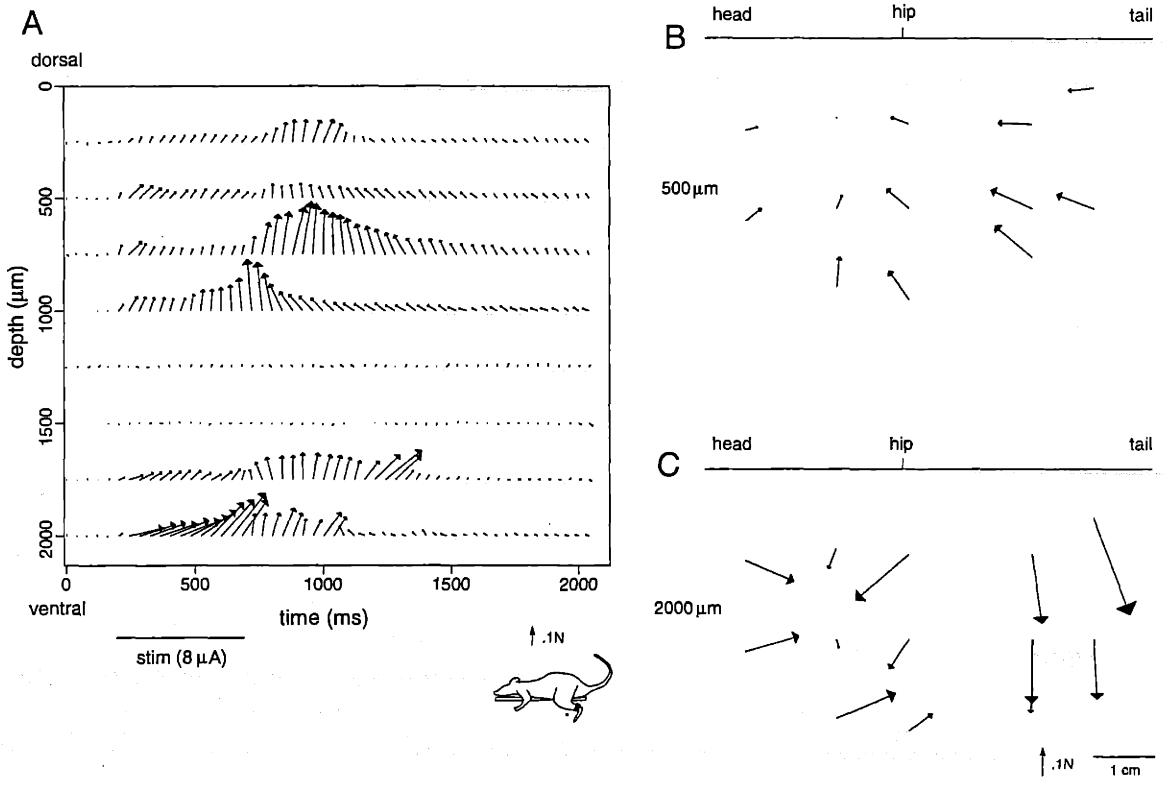

Figure 1: A) An example of the responses evoked from microstimulation along a single electrode penetration in L3. The temporal evolution of the evoked force is shown for different depths along this penetration. The force at each time is indicated by the arrows in the figure: the direction indicating the direction of the evoked force and the length indicating the magnitude of the force. The orientation of the forces relative to the rat is shown in the lower right of the figure, with the dot indicating the position of the ankle at which the forces illustrated here were collected. Depths are indicated on the y axis with 0 as the dorsal surface of the cord. Stimulation of 8μA, 70Hz, .3ms, was applied for 500ms, indicated by the solid line below the figure. In (B) and (C) are shown force fields evoked from two sites along the same electrode penetration. The force field shown in (B) was taken at 500μm from the dorsal surface of the cord with a current of 8μA in L6. Stimulating at a site 1500μm deeper along the same penetration at the same current strength produced the force field shown in (C).

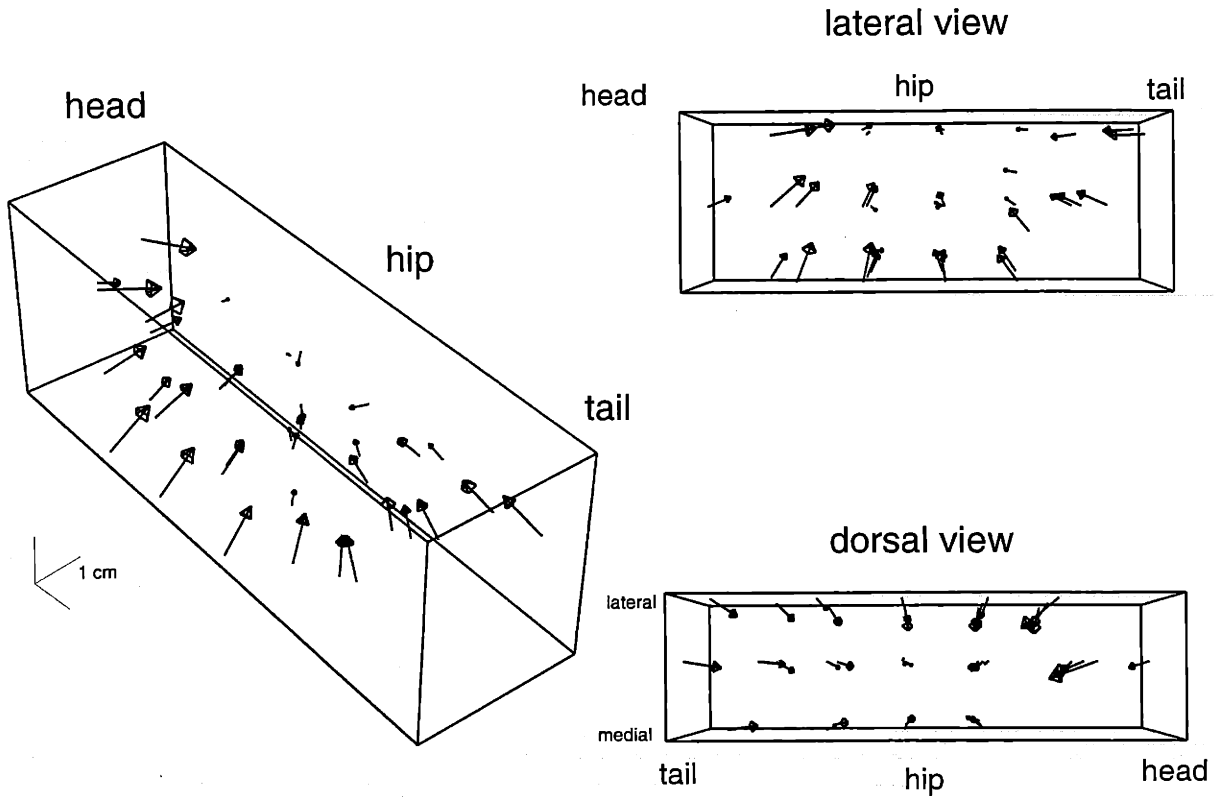

Figure 2: An example of a force field measured in the three dimensional workspace of the rat hindlimb. Forces evoked from microstimulation in the spinal cord were measured at ankle positions in three sagittal planes: one directly below the hip, one 1cm lateral to the hip, and one 1cm medial to the hip. Three views of the force field are shown here. In addition to the perspective view shown on the left, a lateral view (in the top right), and a dorsal view (in the bottom right) are shown. This response was obtained from micros timulation of a site at a depth of 750μm with a current of 8μA in L5.

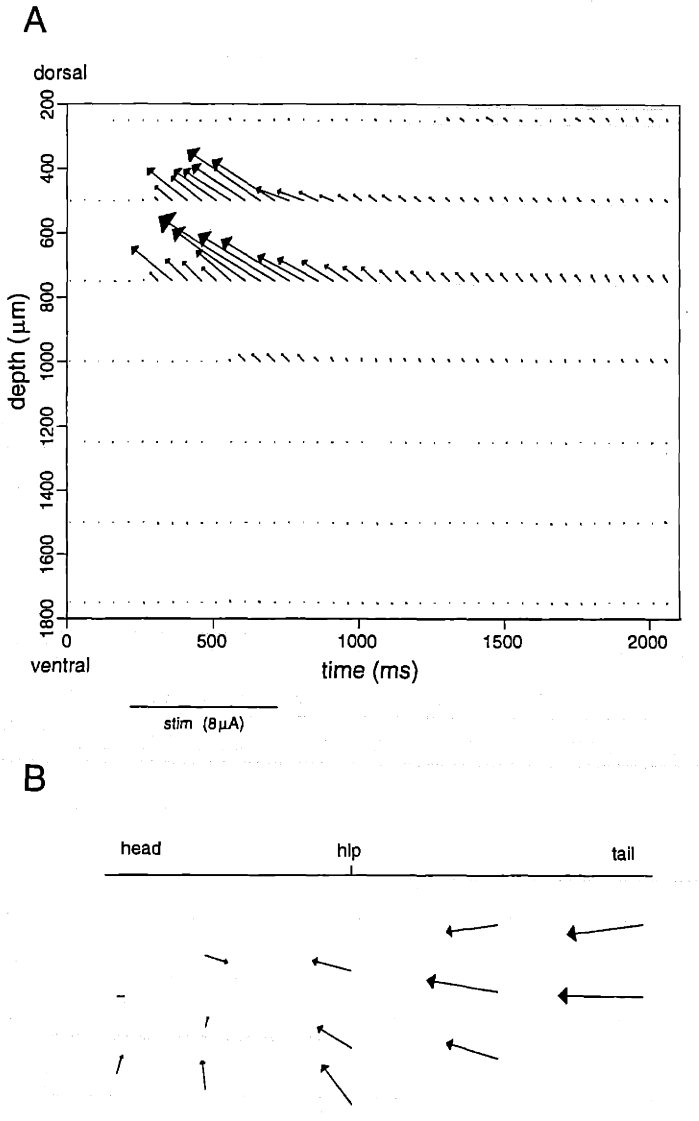

Figure 3: Responses evoked from microstimulation of a chronically deafferented rat. (A) Responses evoked by microstimulation of different depths along an electrode penetration (conventions the same as in Figure 1). (B) Aforce field obtained at a depth of 750µm with 8µA current in L5.

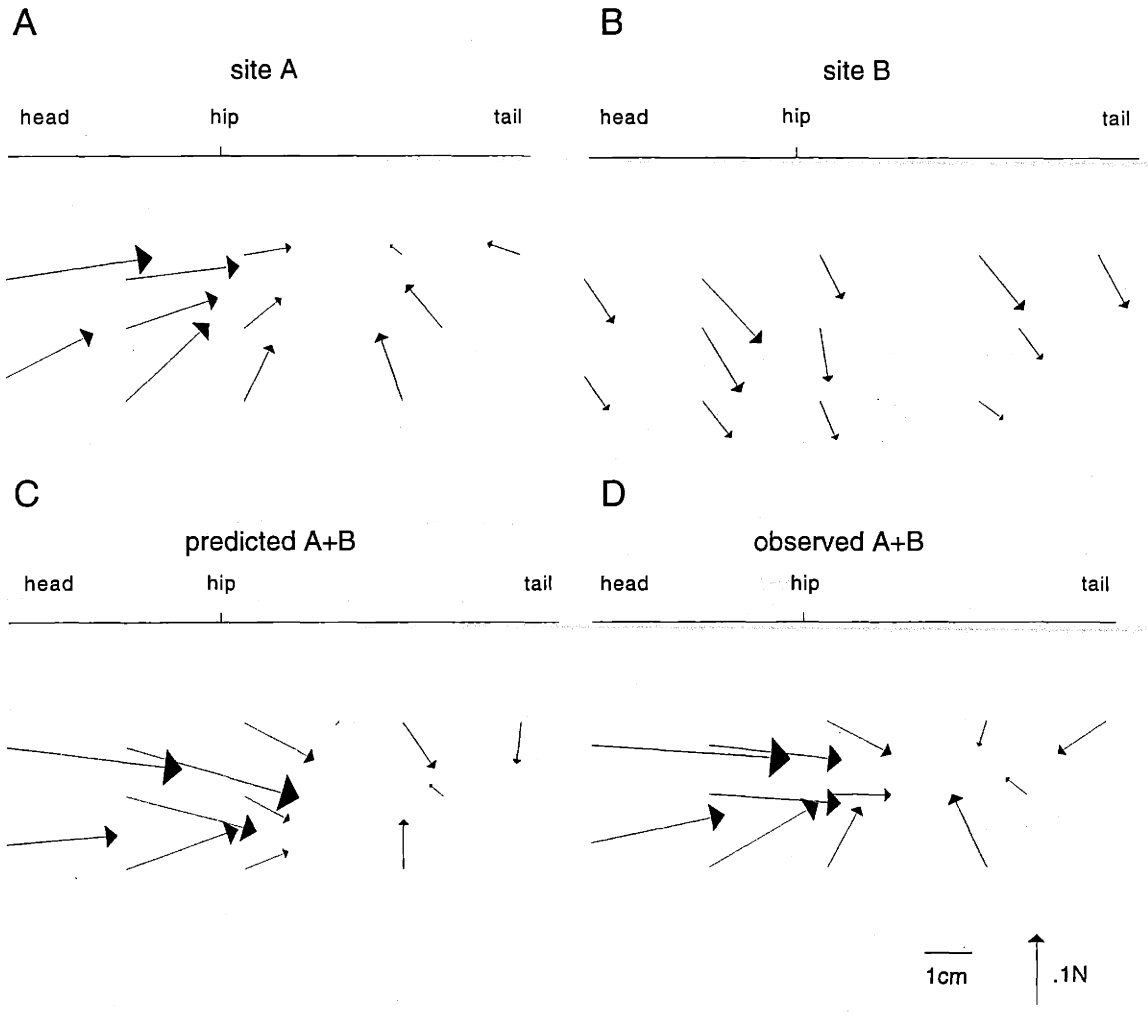

Figure 4: An example of the linear combination of force fields evoked by costimulation. The force fields evoked by separate microstimulation of site A and of site B are shown in (A) and (B). Site A was obtained from stimulation of a site 1000μm deep with a current of 6μA in L2. Site B was obtained from stimulation of a site 1000μm deep with a current of 5μA in L5. (C) shows the force field predicted if the two force fields combined linearly. (D) shows the force field produced from simultaneous stimulation of the two sites.

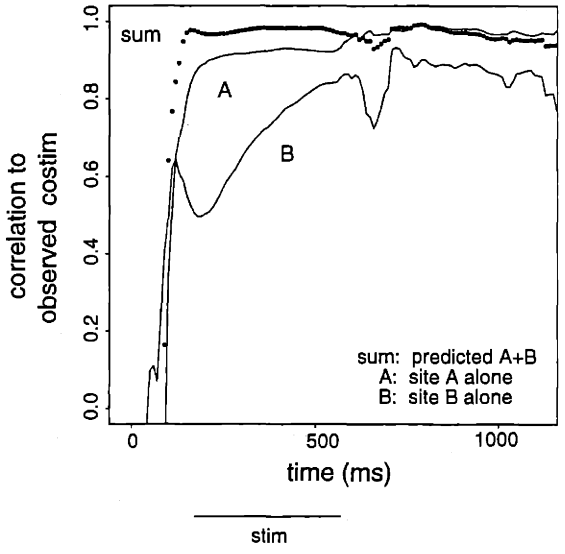

Figure 5: The correlation (see Methods) of the observed costimulation force field of Figure 7 to the separate force fields from sites A and B (A), (B) of Figure 4, and to the force field predicted from the linear summation of the two separate force fields. Identical force fields are indicated by a correlation of 1, directly opposite force fields are indicated by a correlation of -1.

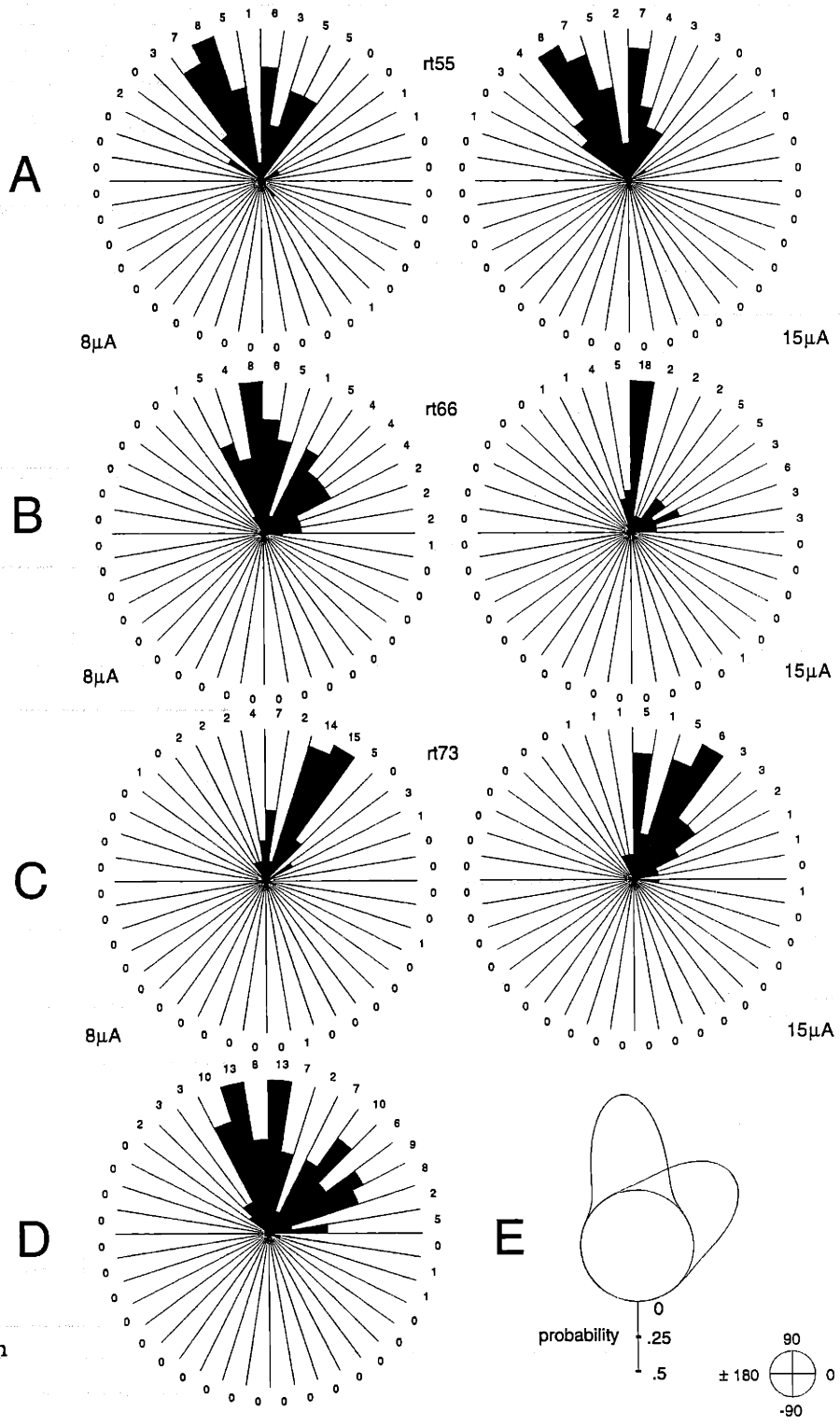

Figure 6: Angular histograms of the force directions evoked from spinal microstimulation in three different rats, shown for two different levels of current. Angular histograms of 9 degree bins are shown. Force data are measured for a single position of the ankle in the workspace. In the animals illustrated here, this position was directly below the hip and with the knee extended. Only responses from sites between 250μm and 1250μm from the dorsal surface of the spinal cord and with magnitude greater than .022N are included. (E) shows the mixture of von Mises probability distributions fit to the data shown in (D) (see Appendix). The data in (D) are combined for all responses obtained from currents of 2, 8, and 15μm as there was no apparent change in directions at the three intensities. Directions of the histograms are shown with reference to the orientation of the animal illustrated in Figure 1.

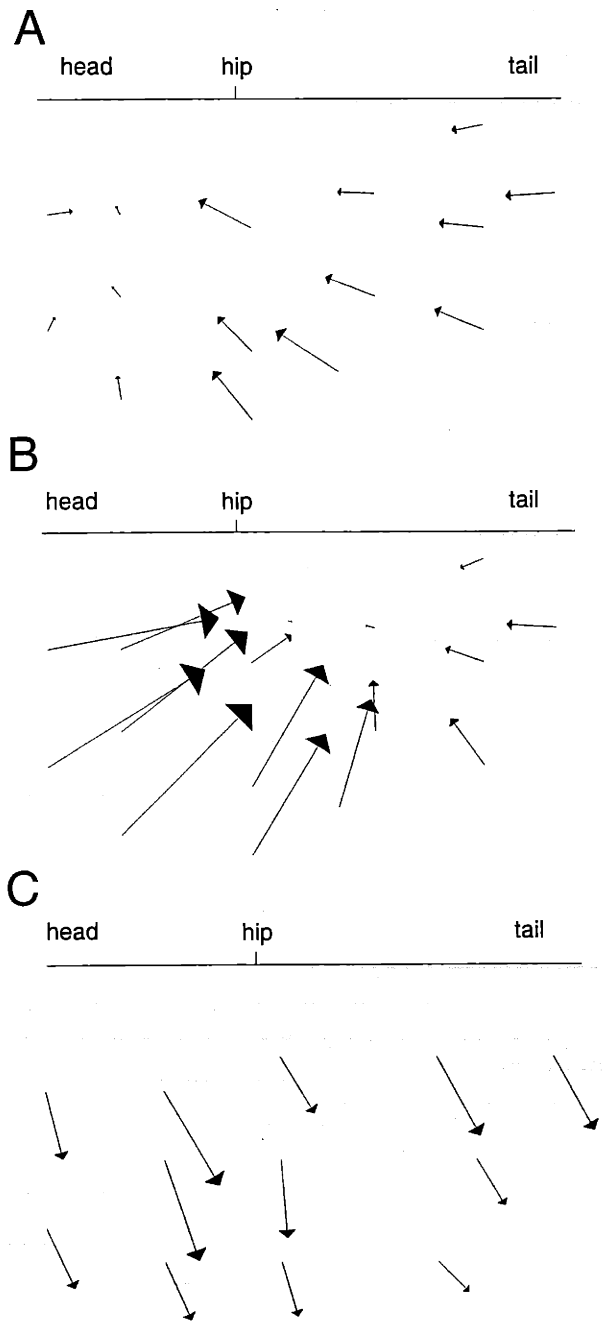

Figure 7: Three main types of force fields observed from spinal microstimulation. (A) shows a force field drawing the limb dorsally and rostrally. (B) shows a force field drawing the limb dorsally and caudally. In (C) is shown a force field not as commonly observed, which drove the limb away from the body.

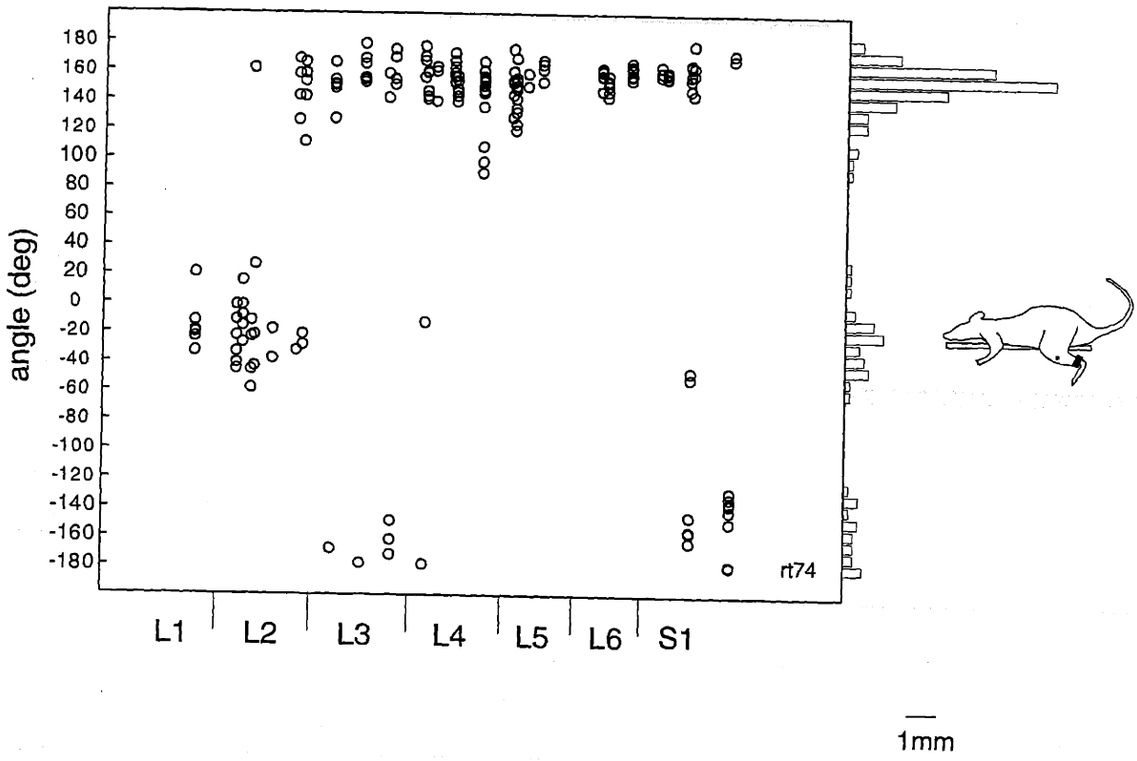

Figure 8: The variation of force responses with rostrocaudal location. Responses measured at a single ankle position from stimulation of sites dorsal to 1500μm with currents of 2, 8, or 15μA are shown. The histograms on the right show the frequencies of the different force directions. The ankle positions at which the set of data were taken is indicated by the dot in the schematic of the rat shown at right.

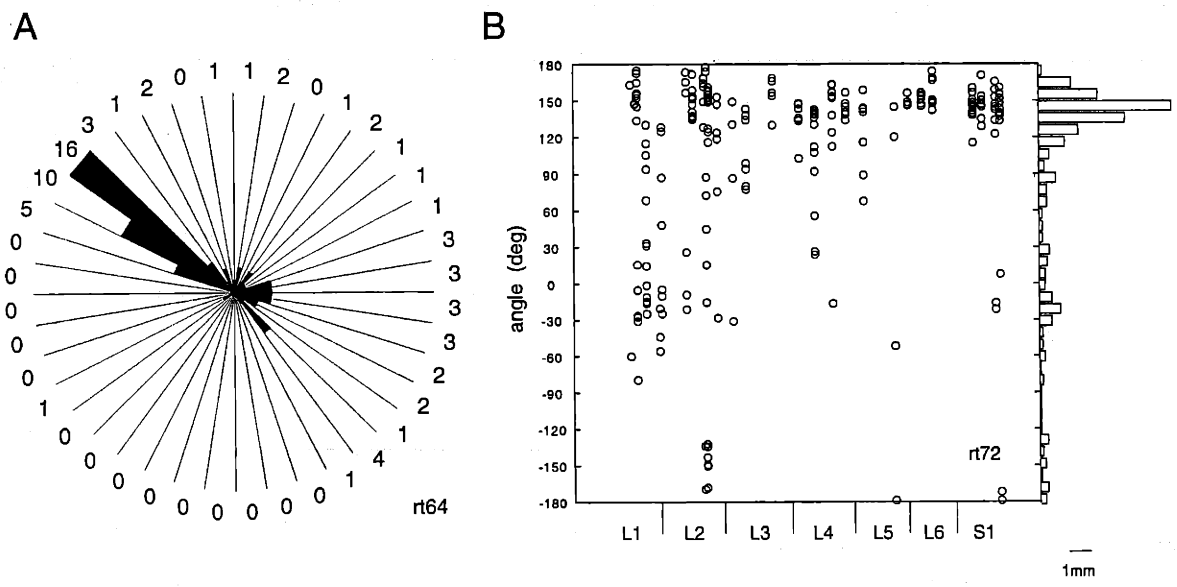

Figure 9: Responses obtained from a chronically deafferented animal. (A) An example of the distribution of force directions evoked from microstimulation of a chronically deafferented rat. Conventions same as Figure 6. (B) The variation of force responses with rostrocaudal location in a chronically deafferented animal. Conventions same as in Figure 8.

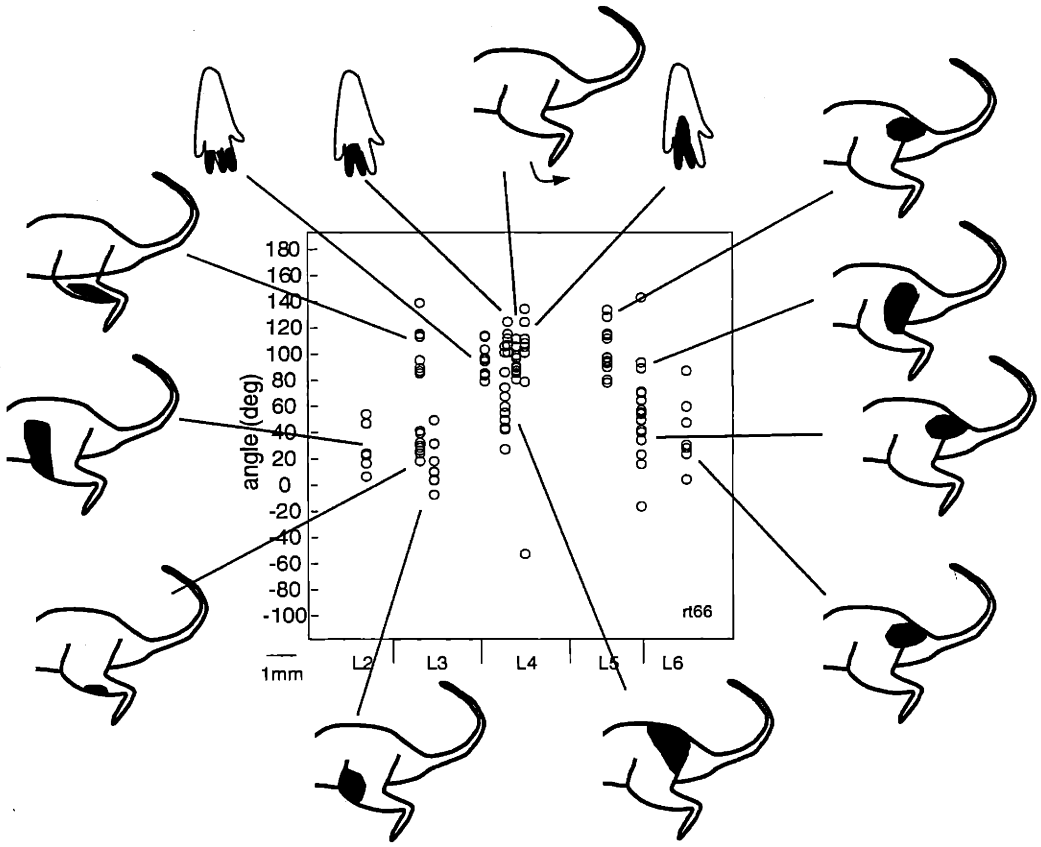

Figure 10: The relationship between force response, rostrocaudal location, and receptive field of sites throughout the spinal cord. Composite receptive fields obtained from each penetration are illustrated: each vertical column of responses at a given rostrocaudal level was taken from a single penetration. Receptive fields of multiple units and occasional single units were mapped using light brushing of the hair or gentle pressure which did not produce a measurable reflex. Other conventions same as Figure 8.

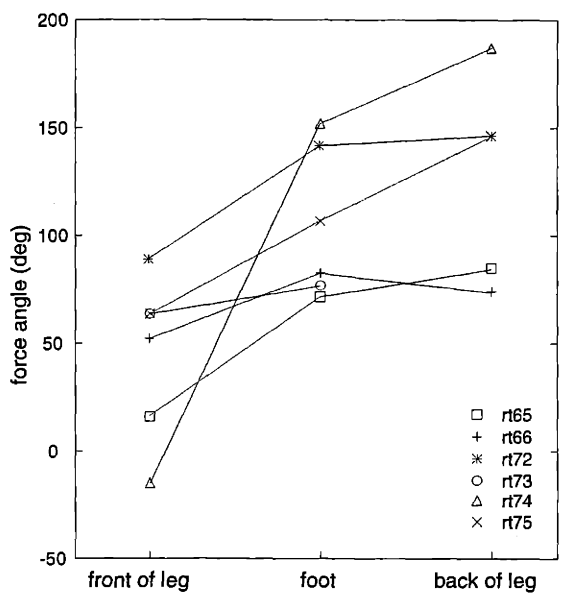

Figure 11: Mean force directions evoked from spinal microstimulation of sites with receptive fields on the front of the leg, on the foot, or on the back of the leg are shown for six animals for which this relationship was examined. Note for rt73 we did not measure any responses from sites with receptive fields on the back of the leg. Note also that rt72 and rt75 were both chronically deafferented and sensory receptive fields were measured in the contralateral spinal cord at the same rostrocaudal and mediolateral location as the
stimulation site in the deafferented cord.

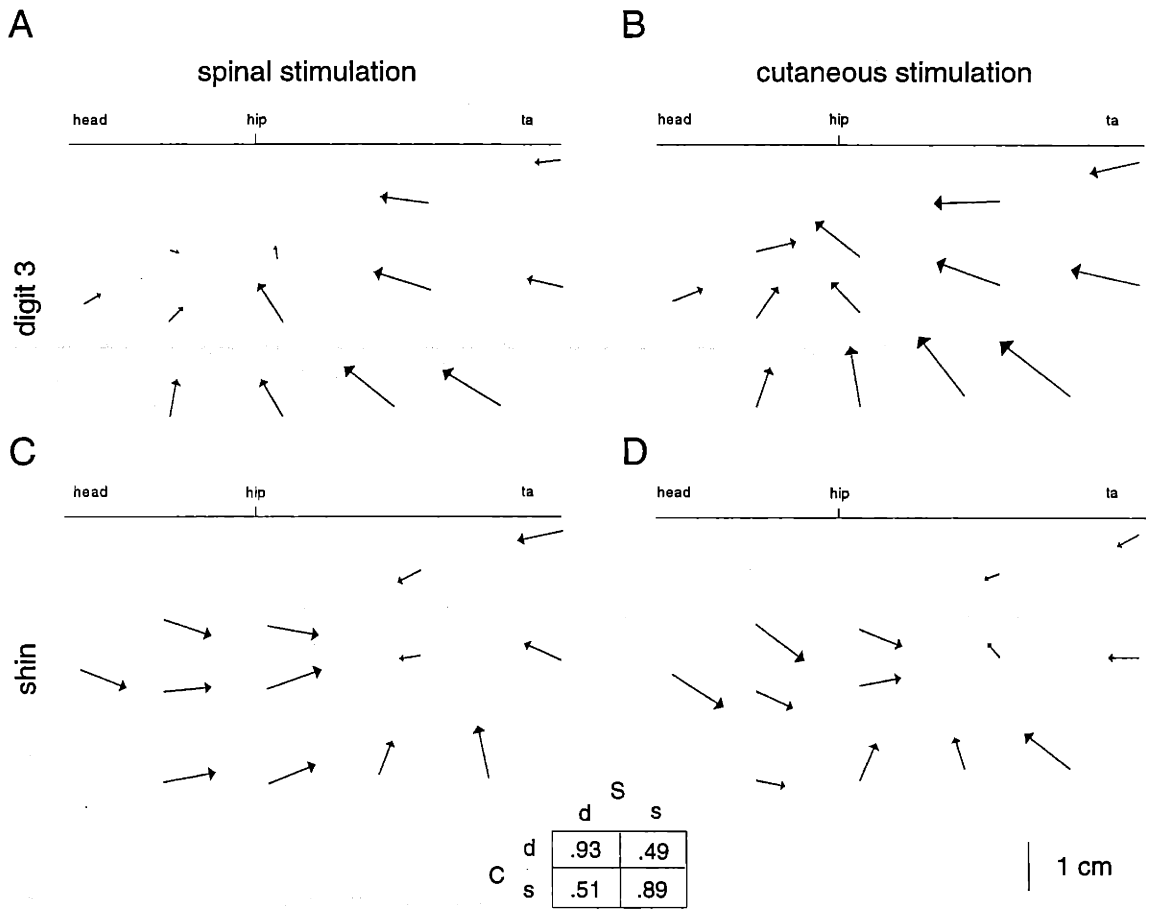

Figure 12: Force fields obtained from spinal microstimulation and from cutaneous stimulation. The two force fields on the left were obtained from applying microstimulation to sites in the spinal cord with receptive fields on the third most medial digit (top left) and on the shin (bottom left). The response from the site with a receptive field on the digit was obtained from 750μm deep in caudal L4 with a current of 8μA. The response from the site with a receptive field on the shin was obtained from 500μm deep in mid L3 with a current of 8μA. The two force fields on the right were measured after electrical stimulation of the skin at these same two locations. The box on the bottom shows the correlations between the fields obtained from microstimulation and from cutaneous stimulation. S indicates spinal microstimulation, C indicates cutaneous stimulation, d indicates digit 3, and s indicates shin.

Chapter 3: Organization of withdrawal reflexes in the spinalized frog

Introduction

The results of the previous chapter suggested that, in the rat, the organization of responses from spinal stimulation paralleled the organization of spinal cutaneous systems. It was further suggested that the responses from spinal microstimulation were related to withdrawal reflexes. This suggestion was supported by the observation that the responses from microstimulation tended to move the leg away from the receptive field represented at that site and from anecdotal descriptions of the similarity between the responses from spinal and noxious cutaneous stimulation. Previous work in the frog has also described that the force fields from spinal stimulation are similar to those produced from cutaneous stimulation (Giszter et al., 1993).

However, the evidence for the relationship between spinal responses and withdrawal reflexes was only circumstantial and by no means definitive. It seems possible that the relationship between cutaneous receptive field and spinal response might be better interpreted in terms of a behavior other than withdrawal reflexes. For instance, this relationship might be better described in terms of the organization of scratch reflexes. Similar to withdrawal reflexes, scratch reflexes would be expected to have a systematic relationship to different parts of the skin. Without a direct comparison between the responses from spinal stimulation and a candidate behavior, it is not possible to make definitive claims about such a relationship.

The work described in the rest of this thesis will directly address this issue. We will first describe the organization of withdrawal reflexes in detail, examining whether the modularity of responses described for spinal stimulation can be observed in this behavior as well. We will then directly compare the responses from spinal stimulation to withdrawal reflexes measured in the same animal. This comparison will allow us to assess the degree to which spinal stimulation is activating the neural systems underlying the production of withdrawal reflexes.

We have chosen to examine these issues in the frog as opposed to the rat. There were several reasons for this decision. The main reason is that frogs are much easier to work with than rats. In particular, the procedures used in the following experiments could take as long as 8 or 9 hours before data collection began. If we performed such procedures in the rat, we could collect data for a limited amount of time. Another reason is that the original work performed with spinal stimulation was performed in the frog and the organization of these responses is more fully characterized in the frog than in the rat (Bizzi et al., 1991; Giszter et al., 1993).

The first issue we examine is the organization of withdrawal reflexes produced by the spinalized frog. The particular question we ask here is whether the withdrawal reflexes of the frog are organized at the level of individual muscles or at the level of muscle synergies. If withdrawal reflexes are in fact specified at the level of individual muscles, then it would be difficult to imagine that the responses from spinal stimulation, which appear to be organized across groups of muscles, are related to the withdrawal reflexes. On the other hand, finding evidence for muscle synergies in the withdrawal reflexes would provide a basis from which to compare spinal responses and withdrawal reflexes.

There is reason to expect that the responses evoked from noxious cutaneous stimulation would be organized at the level of each individual muscle. The work done by Schouenborg et al. in the rat has demonstrated that the activation of each muscle can be related to its mechanical action, such that the region of skin which most activates a given muscle is that region of the skin which that muscle most effectively removes (Schouenborg et al., 1992; Schouenborg and Kalliomaki, 1990; Schouenborg and Weng, 1994). These results suggest that the withdrawal reflexes in the rat are the result of a number of parallel and independent decisions made for each muscle. The observed pattern of muscle activations across the hindlimb is just the final result of each of these independent decisions.

However, the results found by Schouenborg et al. might be also explained, at least in part, by the activation of muscle synergies. If withdrawal reflexes were organized in terms of muscle synergies, one would also expect to find particular regions of the skin from which each muscle was activated, but that the region would be the same for each muscle within the synergy. In fact, a number of the proximal muscles described by Schouenborg et al. appear to be activated from similar regions of the skin surface (Schouenborg et al., 1992; Schouenborg and Kalliomaki, 1990). Further, there appear to be only a few different regions from which different muscle groups are activated. This similarity appears to be even clearer after the spinal cord is isolated from the rest of the nervous system (Schouenborg et al., 1992). For many of the distal muscles controlling the foot and digits, however, the organization is much more precise and difficult to reconcile with the idea of muscle synergies.

These considerations suggest that the control of the proximal musculature by the spinal cord isolated from the rest of the nervous system might be organized by muscle synergies. Since the responses from spinal stimulation have been characterized in terms of the forces measured at the ankle, the results from spinal stimulation are likely to have been biased to the actions of the proximal musculature. It seems possible, therefore, that the responses from spinal stimulation and the withdrawal reflexes are in fact related in this manner. The experiments described in this chapter will examine these issues by simultaneously recording the activity patterns in a large number of muscles underlying withdrawal reflexes in the frog.

Methods

Preparation

We used 7 adult bullfrogs (*Rana catesbiana*) in these experiments. All procedures were approved by the M.I.T. Committee on Animal Care. Frogs were anesthetized with tricaine (.6cc of 5% solution) and submerged in ice until they stopped breathing and were unresponsive to cutaneous stimulation. The spinal cord was transected by aspiration at the base of the fourth ventricle and rostral neural tissues were destroyed by heat cautery. Completeness of the spinalization was determined visually. The wound was packed with gelfoam and closed.

We then placed electromyographic (EMG) recording electrodes in a number of hindlimb muscles. Bipolar electrodes (multistranded stainless steel, insulated with Teflon, ~1mm exposure, backed with a small ball of wax) were threaded through muscles prependicular to the orientation of the muscle. Electrodes within the same muscle were separated by ~2mm. We placed electrodes in the following muscles: semitendinosus (ST), sartorius (SA), rectus internus (RI), adductor magnus (AM), semimembranosus (SM), vastus internus (VI), vastus externus (VE), biceps femoris (BI), iliopsoas (IP), gastrocnemius (GA), tibialis anterior (TA), ankle flexor (AF), ankle extensor (AE). In one animal we recorded from rectus anterior (RA) in the place of IP.

We addressed concerns about crosstalk between adjacent muscles in a number of ways. First, we intentionally placed EMG electrodes in adjacent muscles close to one another in order to be able to observe crosstalk if it were present. Second, in 4/7 of these animals we sutured small pieces of parafilm insulation in between adjacent muscles and compared the results obtained in these animals to those animals without insulation. There did not appear to be substantial differences in the overall patterns in these animals. Third, we explicitly examined the amount of crosstalk in these experiments by cutting nerves to individual muscles. These last procedures confirmed that without insulation there was crosstalk in these experiments of, in the worst case for a small muscle adjacent to a large muscle, 10-20% of the maximal EMG of the small muscle. This crosstalk was reduced to 1-5% when the insulation was in place.

After the electrodes and, in most animals, insulation were inserted, we placed several bone screws in the ventral surface of the distal tibia. In the frog, the tibia can be exposed from the ventral

aspect with a minimum of surgical intervention. Wire was then wrapped around these screws and a threaded attachment was cemented to the framework. A bone screw was also placed in the metatarsus and a metal rod was cemented between this screw and the framework on the tibia. This rod fixed the ankle joint at a 90° angle.

In some of these animals, an additional surgery was performed in order to expose the spinal cord and observe the responses to spinal microstimulation. This procedure is described in detail in the following chapter. In these animals, the responses from cutaneous stimulation were examined extensively prior to applying microstimulation to the spinal cord.

Following these surgical procedures, animals were refrigerated and allowed to recover overnight. Responses to cutaneous stimulation were measured on several experimental days, typically 2 or 3 consecutive days.

Data collection

Animals were placed on a horizontal stand and their pelvis clamped securely with a pair of vises. The attachment on the tibia was connected to a multiaxis force transducer mounted on a positioning device. The ankle was the placed so that the hip was at 0° and the knee at 90°. The hindlimb was suspended securely in this position with the limb in a horizontal plane. Only the most proximal part of the leg near the hip was in contact with any surface. The bone screws and their attachment maintained the leg in this configuration even when the animal produced a maximal contraction, so that responses produced no movement of the hindlimb.

We then stimulated different regions of the skin surface. We initially stimulated the skin by pinching different regions with a mousetooth forceps ($1mm^2$) but found that responses often habituated quickly, especially from proximal regions on the calf and femur. Weak acid (.2M, $4mm^2$ surface) appeared to habituate even faster. We therefore switched to scratching the skin surface with a small piece of wood ($1mm^2$ tip, .01 to .1N force, 1-4Hz, over a range of 1 to 3mm), always along the distal-proximal orientation of the hindlimb. This stimulation did not habituate as quickly as the other methods of stimulation and we found that the responses from pinching and from scratching were very similar. We stimulated at strengths sufficient to produce measurable responses but not intense enough to produce a scratch reflex.

EMG activity was high pass filtered (50Hz) and amplified (25k) before being sampled at 1kHz and recorded for offline analysis. We also recorded the isometric force with the force transducer attached to the ankle (1000Hz, 6 axes). In most responses, however, the mechanical stimulation produced forces which were measured by the force transducer at the ankle, making the evoked force frequently difficult to identify with clarity. We have therefore not systematically examined the forces evoked from cutaneous stimulation. The site of each stimulation trial was recorded on sketches of the frog hindlimb surface.

Data analysis

All data were analyzed using routines written in Matlab software. The onsets and offsets of responses from stimulation were identified using an interactive program. The EMG activity between these onsets and offsets was rectified and averaged, giving a vector of 9 positive numbers indicating the activation level of the nine muscles examined here. We then divided the activations of each separate muscle by the maximal value observed in that muscle for any trial of stimulation. This scaling helped to eliminate differences between electrode placements in different frogs, as well as differences between the size of different muscles. The set of activations for each muscle now ranged between 0 and 1.

We were interested mainly in the balance of muscles within a given response and not in the overall strength of the response. In the vector representation used here, responses which had the same balance of muscles should point in the same direction in this nine dimensional space. Differences in overall strength would be reflected in differences in the length of each vector. In order to eliminate this difference in strength between different responses, we therefore normalized each response to be of unit magnitude. This normalization procedure scaled down the largest responses and scaled up the weakest responses. These weak responses, because they were closer to the noise level, were more variable than the larger responses. This variability was greatly amplified following the normalization described here, thereby obscuring subsequent analyses. We therefore excluded these weak responses by thresholding. Only responses with a vector magnitude greater than one standard deviation of the entire distribution of magnitudes, including wipe reflexes not included in these experiments, were examined further.

We divided stimulation sites across the hindlimb skin surface into several different regions. These regions are illustrated in Figure 1A and reflect the main divisions of the skin surface. These regions also appeared to reflect the main divisions of the responses from cutaneous stimulation (see Results). The activation of each muscle was examined with respect to these regions in each frog.

We attempted to extract distinct patterns of muscle activations in these responses by a clustering analysis. We used a k-means clustering algorithm (Hartigan and Wong, 1979). K-means attempts to place a specified number of cluster means such that the total sum of square distances between each data point and the nearest mean is minimal. For the data in this study, the appropriate measure of distance is the inner product of two vectors. After the normalization described above, all responses will be of unit magnitude and can therefore be thought of as lying on a nine dimensional sphere. Two responses which are close to one another will point in a similar direction. The similarity of two vectors is determined by their inner product. After k-means found the clusters in a set of data, each individual response was classified as belonging to the cluster with the nearest mean.

Results

Sample responses from cutaneous stimulation

In Figure 1 we show some typical examples of responses evoked from noxious cutaneous stimulation of the frog hindlimb. In Figure 1B we show a typical response evoked from stimulation of the back of the foot (site 8). This response was characterized by a large activation of ST, along with activation of BI, IP, and sometimes SA. The force produced by this response was a strong flexion of both hip and knee, drawing the ankle toward the hip. In Figure 1C we show a typical response evoked from stimulation of the back of the calf near the ankle (site 6). This response was characterized by a strong activation of VE along with weaker activation of BI and IP. This response typically produced a weak force that was usually difficult to distinguish from the force used to stimulate the skin. The response shown in this figure is an especially large one in which we were able to observe a sizable knee extension, as is indicated by the caudal and lateral force shown in the figure. In Figure 1D we show a response evoked from stimulation of the front of the leg near the knee (site 16). This response was characterized by activation of SA, AD, VI, and IP. This response typically produced a hip flexion, indicated by the rostral flexion force shown in the figure. Similar responses to those shown in Figure 1 were observed when the leg was allowed to move and in each case the response could be characterized as withdrawing the leg from the site of stimulation. Stimulation of the foot drew the foot in toward the body, stimulation of the back of the calf extended the knee away from the stimulus, and stimulation of the knee produced a hip flexion which served to pull the knee in toward the body. These responses were consistent with the evoked force measured at the ankle and shown in Figure 1.

These three types of responses were observed regularly in every animal (see below). Other types of responses were occasionally produced by a few animals. For instance, two of the animals studied here produced an extension of the limb in response to stimulation of either side of the toes. An example of this response is shown in Figure 2A. Stimulation of the back of the toes (site 10) initially evoked a response with strong ST, then added an activation of RI and SM to this response. These additional muscle activations resulted in a strong, medially directed force. An example of a response evoked from stimulation of the cloaca is shown in Figure 2B. In this response, activation of BI and IP resulted a force directed toward the hip. These responses, however, were not observed in each animal.

Figure 3 shows the frequency of responses observed from different parts of the skin for one animal. As indicated in this figure, responses were easiest to elicit from stimulation of the foot (8-13) and calf(4-7 and 14-18) whereas stimulation of more proximal regions produced fewer responses. We therefore limited our subsequent analyses to responses evoked from these more distal regions of the hindlimb skin surface. Restricting our analysis to these stimulation sites also ensured that the responses we observed were not due to irritating muscles underlying the skin or to mechanical manipulation of the EMG electrodes since we examined the activation of proximal muscles.

Variation of muscle activation with stimulation site

Figure 4 shows the entire set of data obtained from stimulation of these sites for one frog. Each of the nine muscles we examined is plotted with respect to the stimulation sites shown in Figure 1A. The responses shown here were first normalized to the maximal value observed for each muscle across any stimulation trial and then normalized so that each response was unit magnitude. The values shown in this plot therefore reflect how much each muscle contributes to the entire response observed: a value of one means that it was the only muscle activated in that response. Figure 4A shows that the activation of several muscles was clearly tuned to the site of stimulation on the skin in a manner consistent with the examples shown above. In particular, ST, BI, and IP were activated from regions of the skin on the foot (sites 8 through 13). Stimulation of regions on the front of the calf and knee (sites 14 through 18) produced responses with activation SA, AD, and VI with weaker activation of BI and IP. Stimulation of regions on the back of the calf (sites 4 through 7) produced activation of VE with some activation of BI and IP as well. Figure 4B shows the averaged normalized EMG for this same animal at each stimulation site. For each muscle shown here, there was a significant dependence of the level of activation as a function of stimulus location (ANOVA, $p<.05$). Data from another animal are shown in Figure 5A, in which the same general pattern of muscle activations can be observed. The activation levels of these muscles were also significantly dependent on the site of stimulation (ANOVA, $p<.05$). Every animal showed a significant variation in activation with different stimulation sites for the vast majority of muscles. Figure 5B also shows that stimulation on the dorsal surface of the calf produced a similar segregation of different types of responses. Thus, it appeared that stimulation of any region of the foot produced the flexion type of response shown in Figure 1B, while stimulation of the dorsal surface and back of the calf produced a knee extension type of response as shown in Figure 1C.

It was commonly observed that stimulation of a given site on the hindlimb was capable of producing more than one type of response. An example is shown in Figure 6A. In this response, stimulation of the back of the calf initially produced activation of VE with BI and IP. This activation was then replaced by activation of ST and BI, which was in turn replaced by a response similar to that produced initially. Note that even though each pattern was dominated by the activation of a single muscle with weaker activation of other muscles, the change from one pattern to another was exhibited by both strong and weak muscles. This switching phenomenon was

especially pronounced in the animal shown in Figure 6B. In this animal, it can be seen that stimulation of the back of the calf produced responses with activation of either ST or VE, but not with the two together.

Responses across transition zones

The exclusive nature of these two sets of muscle activations suggests that they are not produced by the specification of each individual muscle independently, but rather by the specification of the entire pattern of muscle activation patterns. This issue was examined further by considering the responses evoked from stimulation across the boundary of the skin regions that preferentially produce these responses. An example of such an experiment where the skin was stimulated from the foot to the ankle is shown in Figure 7. Stimulation of the back of the foot (Fig. 7A) produced a strong activation of ST along with a weaker activation of BI. As the stimulus was moved toward the heel, however, the first observed response became activation of VE and BI which was then followed by activation of ST and BI (Fig 7B, 7C). When the stimulus reached the heel, this second response of ST and BI was followed by another activation of VE (Fig. 7D). In each case, the two types of responses were not activated together but in exclusion. Thus, as the stimulus was moved across the transition zone between these two responses, there was not a smooth blending of the two responses. Instead, the two responses remained distinct.

The distinction between responses from the foot and the front of the calf, however, was not as clear. As stimulation was moved from the foot to the front of the calf, responses became similar to that shown in Figure 1B, but the distinction between the different types of responses was not dramatic. In the animal shown in Figure 4 in which the transition between different types of responses was sharp, sites near the front of the ankle appeared to produce responses intermediate to those produced from stimulation of the knee and from stimulation of the foot. In particular, stimulation of sites at location 15 produced responses in which both ST and AD were activated, as can be seen by their intermediate levels of activation. In the animal shown in Figure 5A these intermediate levels of activation are even more apparent. It therefore appeared that there was a more gradual transition between responses from these regions of the hindlimb. This gradual transition between the foot and the front of the ankle was in contrast to the sharp transition between the foot and the back of the ankle.

This gradual transition across the foot and front of calf was also supported by examining the correlation of the activation of each muscle to the site of stimulation. The correlation coefficient of each muscle's activation to stimulation sites on the foot and front of the calf is shown in Table 1. We show the average correlation coefficient of each muscle averaged across all animals in Figure 8A. A positive correlation indicates that a muscle contributed more as stimulation approached the front of the calf. As would be expected from the data shown in Figures 4 and 5, the activation of SA, AD, VI, VE, and IP were each positively correlated to the stimulus location. Conversely, ST and BI were negatively correlated to the stimulation site. In Figure 8B we show the slope of the regression of each muscle's activation to the site of stimulation. Similar to the results shown in Figure 8A, SA, AD, VI, VE, and IP each had a positive regression slope while ST and BI had a negative slope. These results show that there tended to be a gradual increase in the activation of these five muscles as stimulation sites approached the knee accompanied by a gradual decrease in the activation of other muscles. These results suggest that the activations of these muscles are related in a similar way to the site of stimulation.

K-means clustering

We attempted to separate these different types of responses from one another irrespective of the stimulation site from which they were evoked using a clustering algorithm. We extracted

clusters from the responses evoked from cutaneous stimulation using the k-means algorithm and then examined which part of the skin each cluster was evoked from. An example of the clusters found by k-means is shown in Figure 9. The first cluster shown consisted of activation of SA, AD, and IP. This pattern of muscle activations was evoked from stimulation of the front of the leg. The second pattern of muscles consisted of strong activation of ST and IP and was evoked from sites all over the foot, but preferentially from sites on the back of the foot. The third cluster shown consisted mainly of an activation of a strong activation of VE and was evoked from the back of the calf. The final cluster shown consisted of activation of SA, AD, and IP, similar to that of the first cluster, but there was additional activation of other muscles as well, notably ST and BI. This type of response was preferentially evoked from sites on the front of the foot, where the intermediate responses described above were observed. This pattern of responses is consistent with the pattern of muscle activations shown in Figure 4. The results from two other animals are shown in Figure 10. The first three clusters shown for each animal were similar. The fourth cluster found by k-means was also roughly similar, in that it usually consisted of activation of ST and several other muscles.

In each animal these three types of clusters were consistently observed. This consistency allowed us to compare the frequency of each type within the responses from a given animal. The frequency of each response type is shown in Table 2. For each animal, the responses typically evoked from stimulation of the foot were more numerous that the responses typically evoked from the calf.

These results suggest that the responses from cutaneous stimulation fell into three main types of responses, each preferentially evoked from different regions of the skin surface. The response from the back of the calf appeared to be distinct from the responses from the rest of the leg, in that it was usually activated in exclusion of the other types of responses. The separation between responses evoked from the foot and from the front of the calf, however, did not appear to be as distinct.

Discussion

Responses from noxious cutaneous stimulation

As described in the Introduction, the motor responses from cutaneous stimulation have been studied extensively in several species and several different classes of responses have been observed (Berkinblitt et al., 1986; Schouenborg and Kalliomaki, 1990; Stein, 1983). In the present study we restrained the hindlimb to avoid movement artifacts in the EMG recordings and therefore could not observe the free limb movements in order to clearly classify these behaviors. However, the same stimulation applied with the limb free produced roughly similar patterns of EMG activations and all responses could be interpreted as withdrawal reflexes. Stimulation of the foot produced the typical flexion withdrawal with hip and knee flexion. Stimulation of the back of the ankle produced a knee extension response. Stimulation of the front of the calf produced a hip flexion which pulled the calf medially due to the closing of the hip angle. These free limb behaviors are each consistent with the force directions of responses indicated in Figure 1. Wiping responses resulting from stimulation of these same sites were clearly distinct from the responses observed in these experiments. We therefore consider the responses observed in this study to be withdrawal reflexes both by their strength and by the movement that they tended to produce.

The results of these experiments suggest that the withdrawal reflexes in the frog can be described as a combination of a small number of muscle activation patterns. These activation patterns are preferentially evoked from cutaneous stimulation of either the back of the calf, the foot, or the front of the leg. The nature of these different muscle activation patterns, however, appears to be quite different.

The responses evoked from the back of the calf and from the foot appear to be recruited in exclusion of another. This finding was supported by examination of responses across transition zones (Fig. 7), demonstrating that each of the patterns of muscle activation underlying these responses was not activated simultaneously. Each muscle within the response demonstrated this exclusivity: when the activation of ST began in Figure 6A, the activation of both VE and IP ended. Similarly, the activity of BI, which was activated in each response, changed between the two responses. These observations suggest that these two patterns of muscle activations were distinct from one another. Further, these observations suggest that each pattern is organized across a group of muscles controlled as a unit. These responses can therefore be considered as muscle synergies.

The observation of distinct types of responses is difficult to reconcile with the hypothesis that the responses are organized primarily at the level of each individual muscle. In the hypothesis proposed by Schouenborg, the activation of an individual muscle across the hindlimb surface is determined by that muscle's mechanical action (Schouenborg and Weng, 1994). In this hypothesis, one would expect to observe muscles with similar tuning to be functional synergists. This similarity in receptive fields might produce the observation of distinct type of responses observed here. However, the responses we observed did not consist of only mechanical synergists. For instance, in the response from the front of the leg there was often coactivation of VI, a knee extensor, and SA, a knee flexor. Further, independent muscle control would imply a different result from stimulation across transition regions between different responses than that observed here. If each muscle were specified independently, one would also expect the variation in each muscle's activation level to be independent. Stimulation of a site at the transition between responses, therefore, should produce an particular average response with independent, uncorrelated variability for each muscle around this average. The observation in the present study that stimulation of a transition zone produced only one of a discrete set of responses means that the variation between muscles was not independent at these transition zones. These observations support the notion that withdrawal reflexes in the spinalized frog are organized by a small number of global control strategies, at least for stimulation of the back of the calf and the foot.

The responses evoked from the front of the calf, however, are more difficult to characterize. In most animals, stimulation of sites near the knee would, at least in some cases, evoke a response which consisted of activation of SA, AD and VI or IP or all four muscles. In particular, responses without activation of ST could be observed. A large number of responses, however, did not consist of the activation of these muscles alone. Instead, these muscles could be activated in combination with other muscles such as ST or BI. The contribution of these additional muscles appeared to be larger for stimulation sites closer to the foot, as indicated in the responses of the animals shown in Figures 4 and 5. As stimulation sites approached the knee, the contribution of these muscles decreased.

Because of this type of gradual transition, it is difficult to interpret the responses evoked from stimulation of the front of the leg. One possibility is that modulation of activation in this set of muscles reflects independent control strategies for each separate muscle. The activation of each muscle may be specified as a function of stimulation site, in parallel and independently of the other muscles. The shift from one set of muscles to another set of muscles as the stimulation site moves, along with the intermediate responses where both sets of muscles are activated, would then be due to the overlap in the activation rule for each separate muscle. In this possibility, responses from the foot and the front of the leg consist of the activation of a particular superset of muscles, but each muscle within that set is individually modulated to the site of stimulation.

Alternatively, the two sets of muscle activations might each be organized as a functional unit. In this possibility, each set of muscles is recruited by its own function of stimulation site. The intermediate responses result from the overlap between these recruitment functions exerted across

each set of muscles. In this possibility, responses from these regions of the leg can still be described in terms of muscle synergies, but synergies can be expressed simultaneously.

Although these possibilities can both account for the responses observed here, it is still possible to distinguish them from one another. In particular, the independent control of muscles considers that the covariation of a set of muscles is the result of the similar recruitment of these muscles to the same external variable. Instead, the control of a set of muscles as a functional unit considers that this covariation is due to a coupling between the activation patterns of the muscles within the set. The recruitment of this set of muscles might be related to an external variable, but not necessarily. Because of this difference, if a set of muscles is found to covary irrespective of an external variable, this observation suggests that the group of muscles is controlled as a functional unit. This distinction will be explored further in the final chapter of this thesis.

In either possibility, the results described here demonstrate that withdrawal reflexes can be described in terms of a small set of muscle activation patterns. This organization is similar to that described for the responses from spinal stimulation (Bizzi et al., 1991; Giszter et al., 1993), suggesting that the two types of responses might be related. The next chapter explores this possibility more systematically, by comparing the muscle activations evoked from spinal and cutaneous stimulation in the same animal.

References:

Berkinblitt, M. B., Feldman, A. G., and Fukson, O. I. (1986). Adaptability of innate motor patterns and motor control mechanisms. Behavioral and Brain Sciences *9*, 585-638.

Bizzi, E., Mussa-Ivaldi, F. A., and Giszter, S. F. (1991). Computations underlying the execution of movement: a biological perspective. Science *253*, 287-291.

Giszter, S. F., Mussa-Ivaldi, F. A., and Bizzi, E. (1993). Convergent force fields organized in the frog's spinal cord. J. Neurosci. *13*, 467-491.

Hartigan, J. A., and Wong, M. A. (1979). A k-means clustering algorithm. Applied Statistics *28*, 100-108.

Schouenborg, J., Holmberg, H., and Weng, H.-R. (1992). Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. Exp. Brain Res. *90*, 469-478.

Schouenborg, J., and Kalliomaki, J. (1990). Functional organization of the nociceptive withdrawal reflexes. I. Activations of hindlimb muscles in the rat. Exp. Brain Res. *83*, 67-78.

Schouenborg, J., and Weng, H.-R. (1994). Sensorimotor transformations in a spinal motor system. Exp. Brain Res. *100*, 170-174.

Stein, P. S. G. (1983). The vertebrate scratch reflex. Symp. Soc. Exp. Biol. *37*, 383-403.

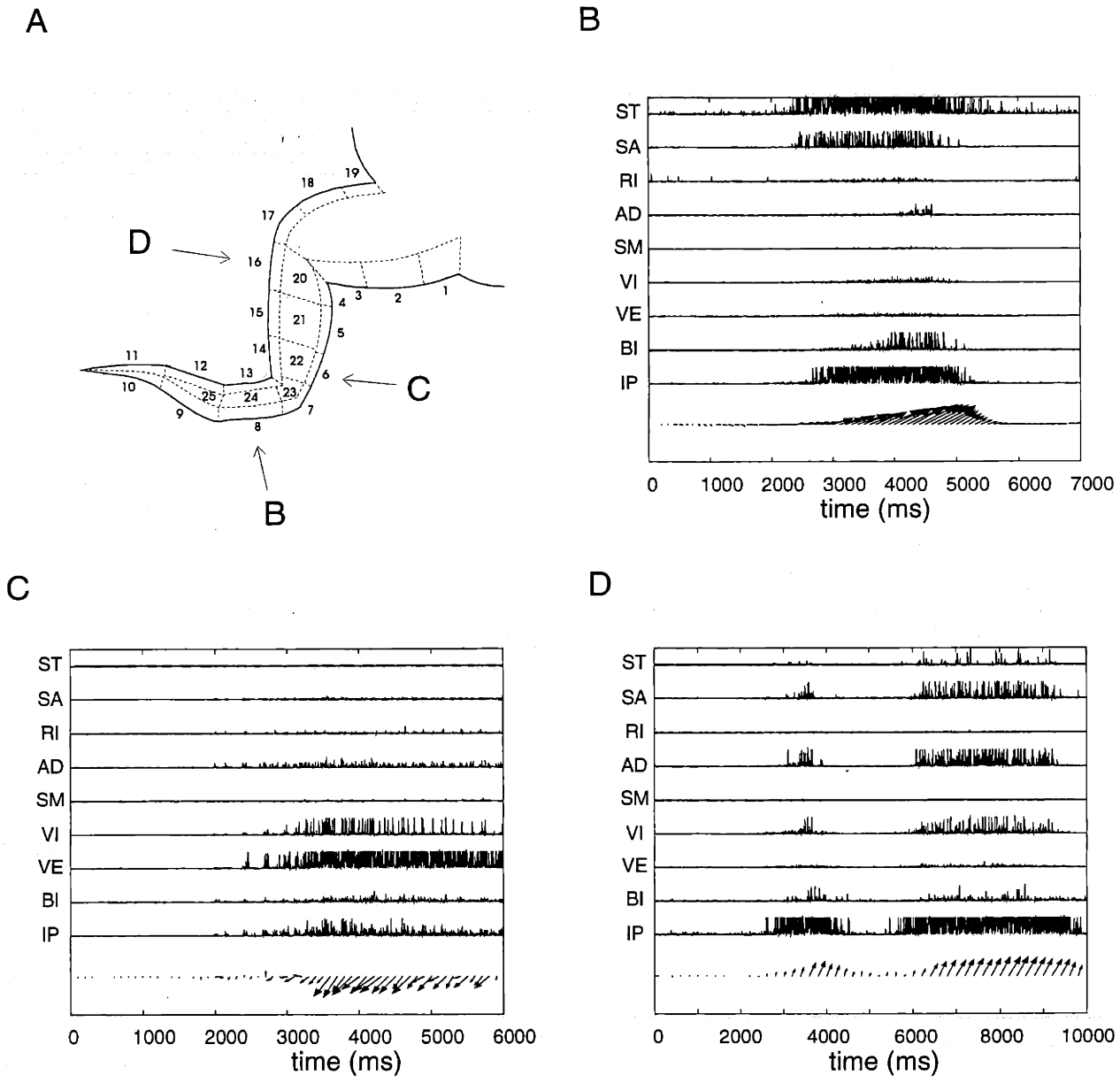

Figure 1. Typical responses from cutaneous stimulation. (A) shows the sites on the hindlimb which were stimulated to produce withdrawal reflexes. The left leg is shown from a dorsal perspective. The arrows indicate the stimulation sites which produced the responses shown in the other figures. (B) shows a response evoked from stimulation of the back of the metatarsus. Force direction is shown in the same frame of reference as in (A). (C) shows a responses from stimulation of the back of the calf near the ankle. (D) shows a responses from stimulation of the front of the calf near the knee.

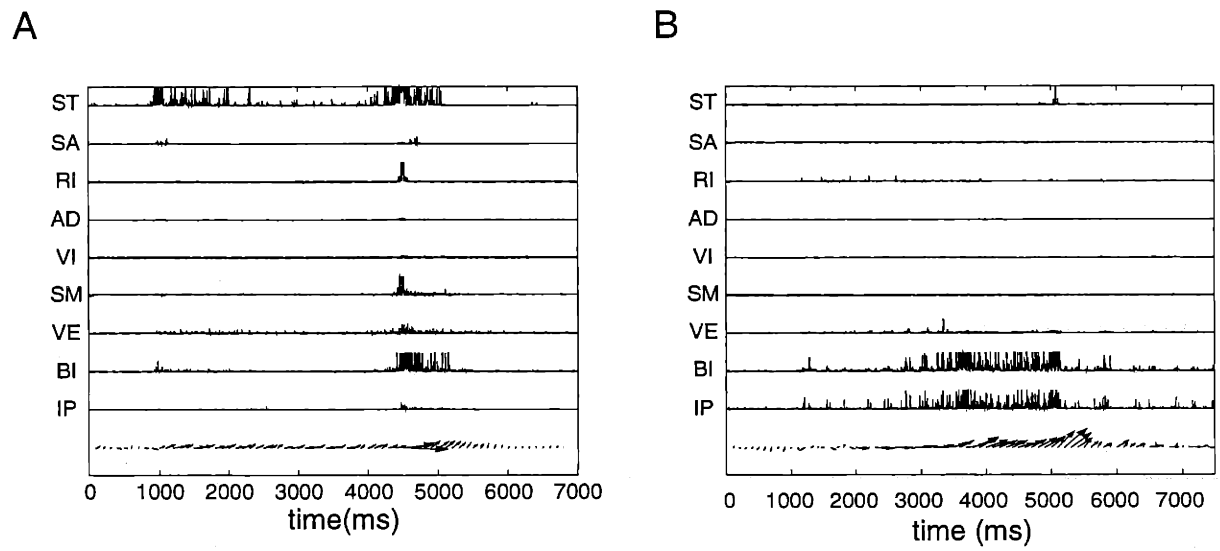

Figure 2. Two less frequently observed types of responses. (A) shows an extensor thrust response evoked from stimulation of the back of the toes (site 11). (B) shows a response evoked from the cloaca.

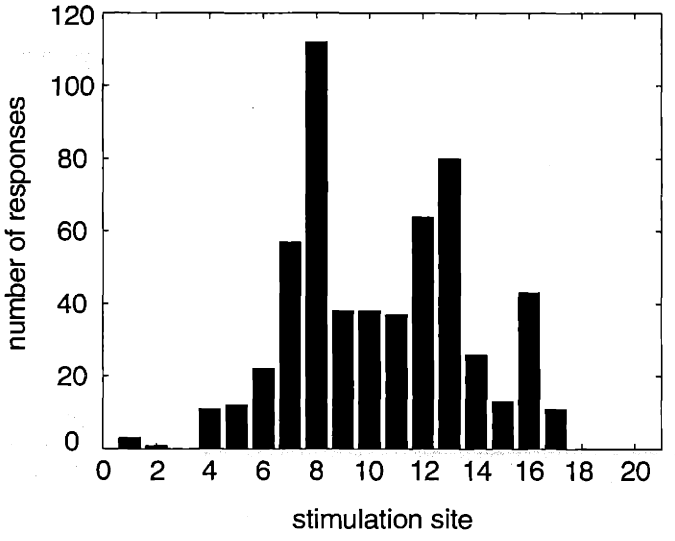

Figure 3. Frequency of responses observed from different regions of the hindlimb. The skin locations on the x-axis refer to the stimulation sites shown in Figure 1A.

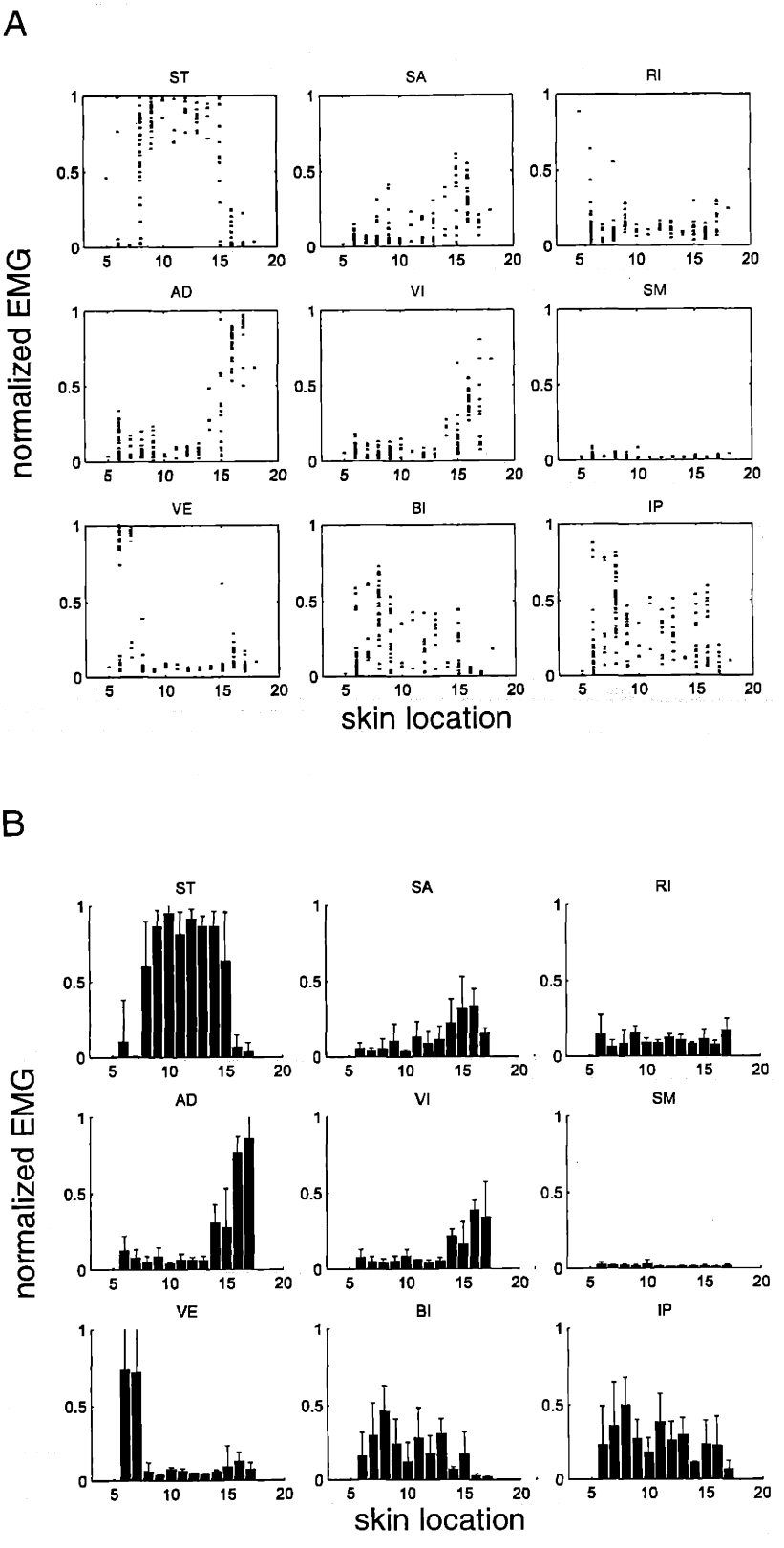

Figure 4. The variation of muscle activation with stimulation location. (A) shows the normalized muscle activation evoked from stimulation of sites on the foot and calf for nine different muscles. Each point in one of these plots represents the contribution of that muscle to one response. (B) shows the same data in (A), but averaged for each stimulation site. Error bars represent one standard deviation from the mean.

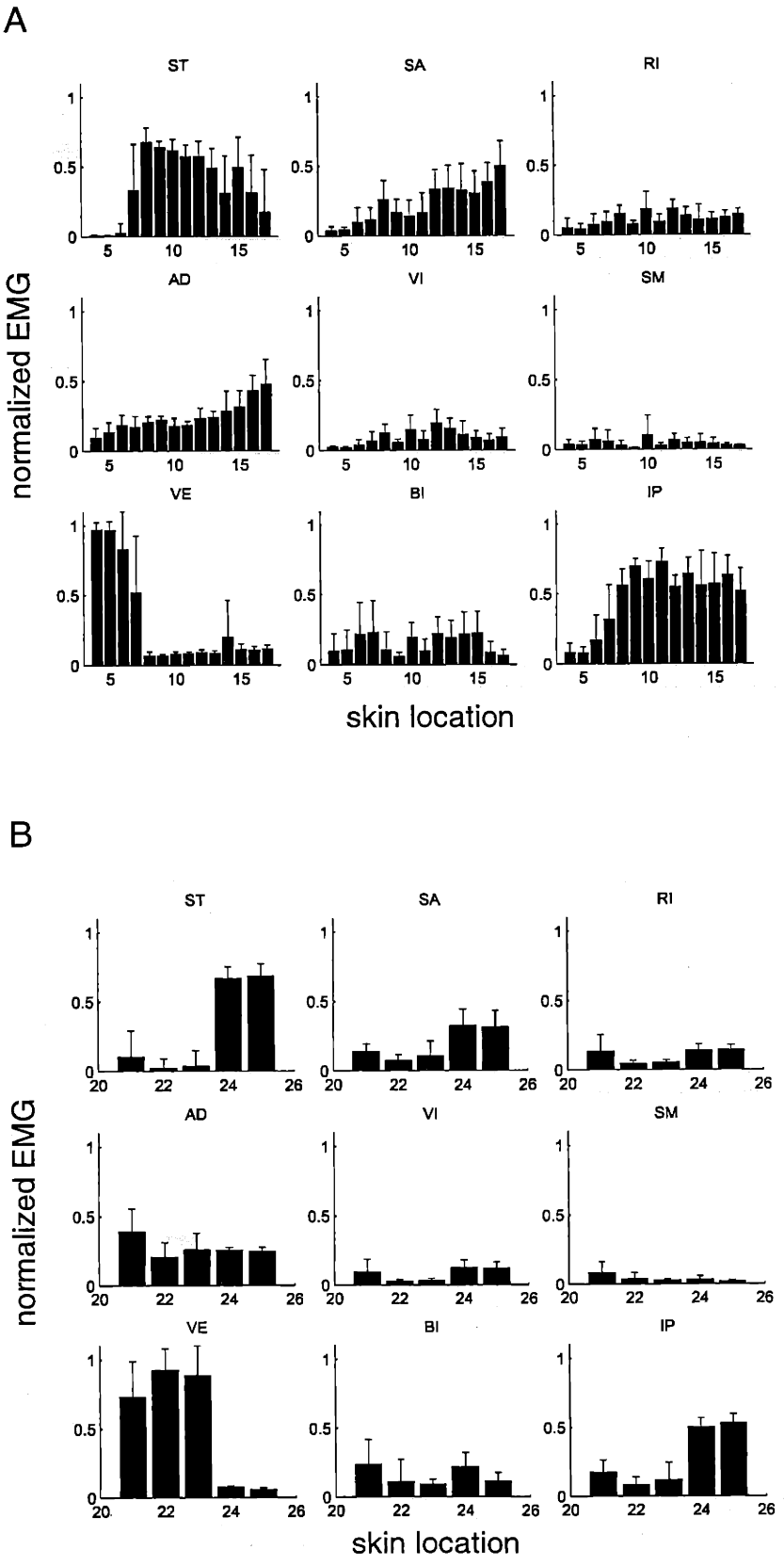

Figure 5. (A) shows the averaged normalized activation of each muscle from different regions of the skin. Conventions are the same as in Figure 4. (B) shows the averaged normalized activation of each muscle from sites along the dorsal surface of the calf and foot (sites 20-25 in Figure 1A).

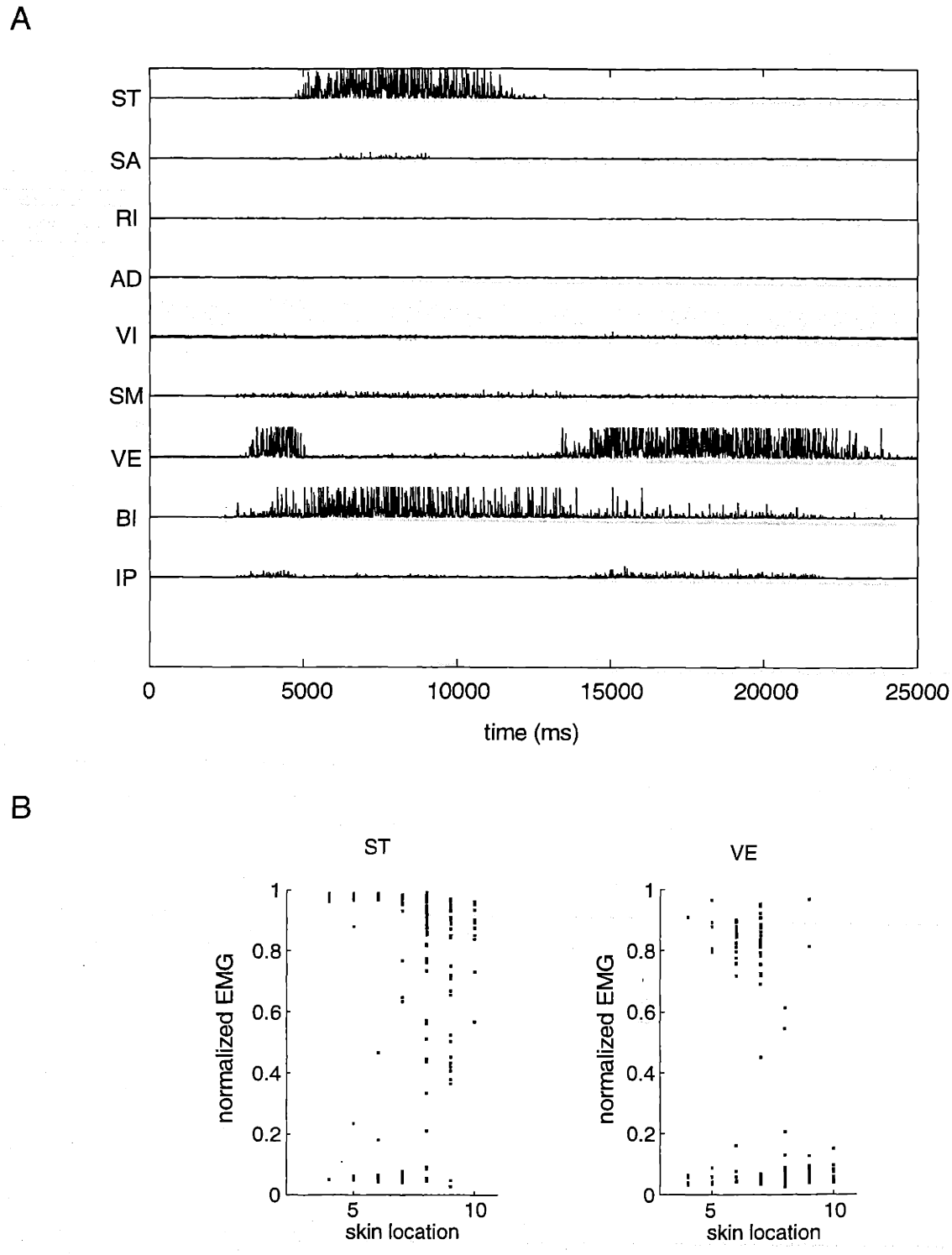

Figure 6. Exclusivity of different types of responses. (A) shows a response evoked from stimulation of the back of the calf (site 6). (B) shows the normalized activation of two muscles for stimulation sites on the back of the calf and on the foot.

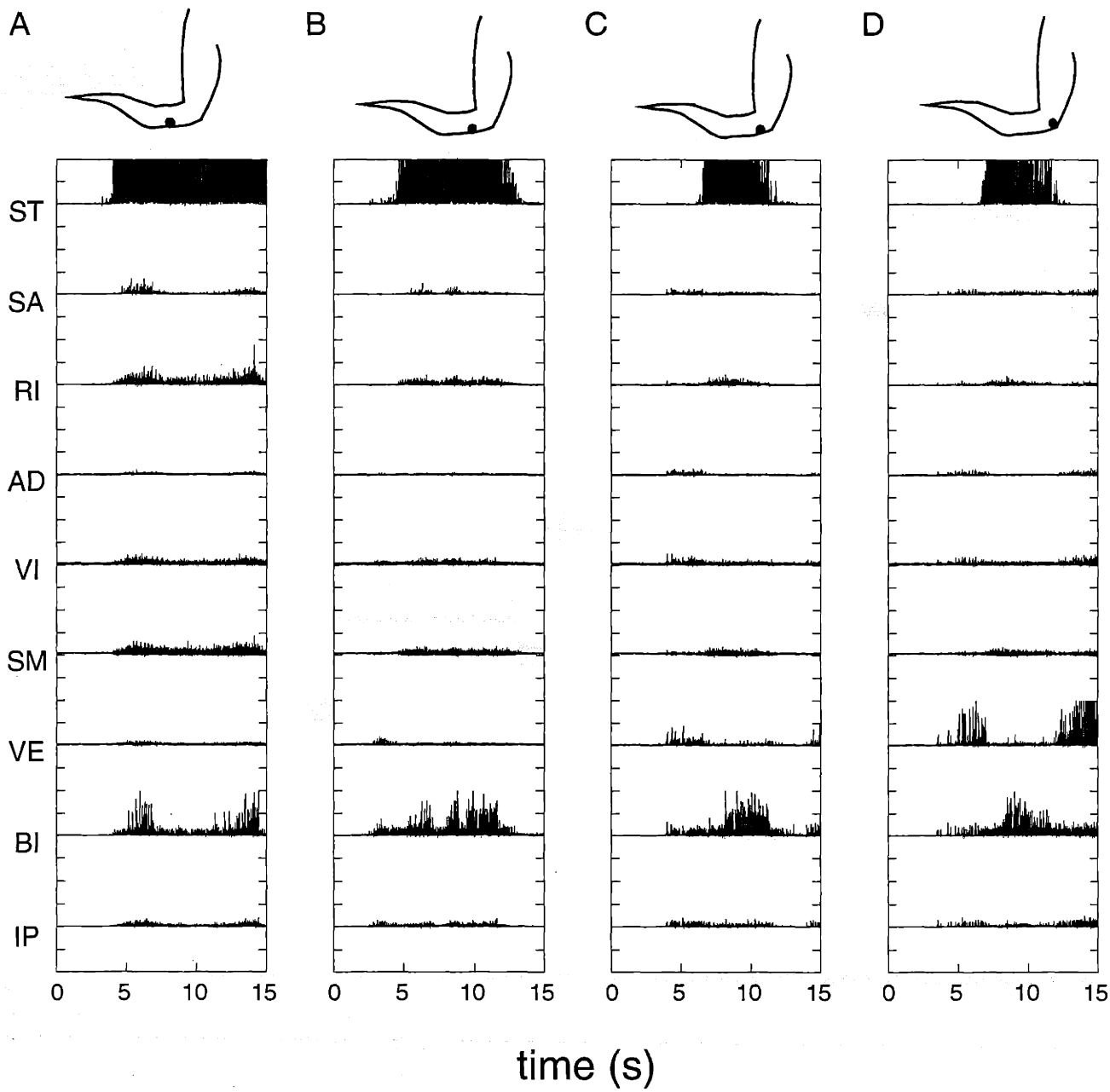

Figure 7. Responses across a transition zone. The stimulus location was changed progressively from the back of the foot (A) to the back of the ankle (D). (C) and (D) show responses evoked from intermediate sites.

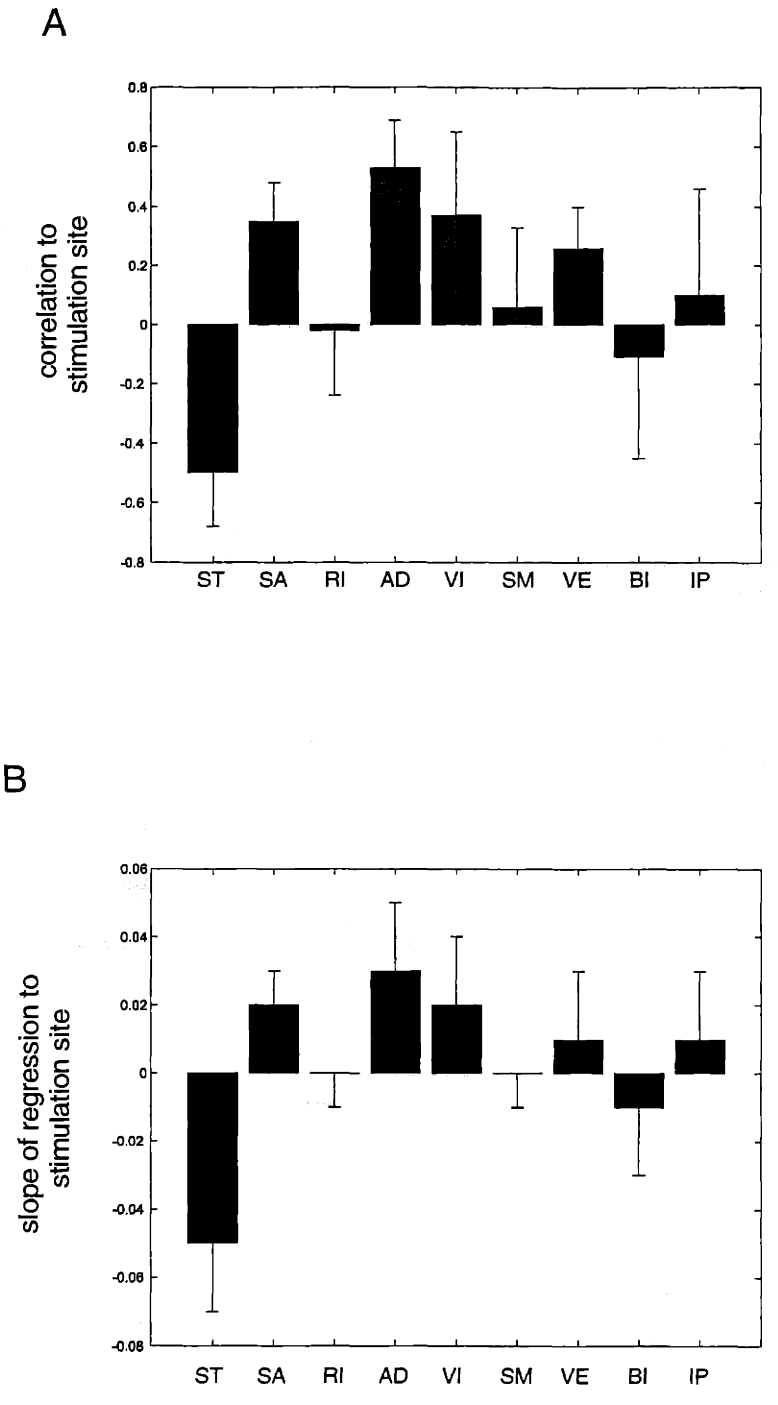

Figure 8. Relationship between muscle activation and stimulus location. (A) shows the correlation of the normalized activation of each muscle to the stimulation site. Each bar represents the correlation average for all animals. Error bars represent one standard deviation from the mean. Only sites from the foot to the front of the knee (sites 8-18) were used for this analysis. (B) Slope of the regression of muscle activation to stimulation site. Note that the small value of these slopes is due to the difference in the range of muscle activations (0-1) and stimulus locations (8-18).

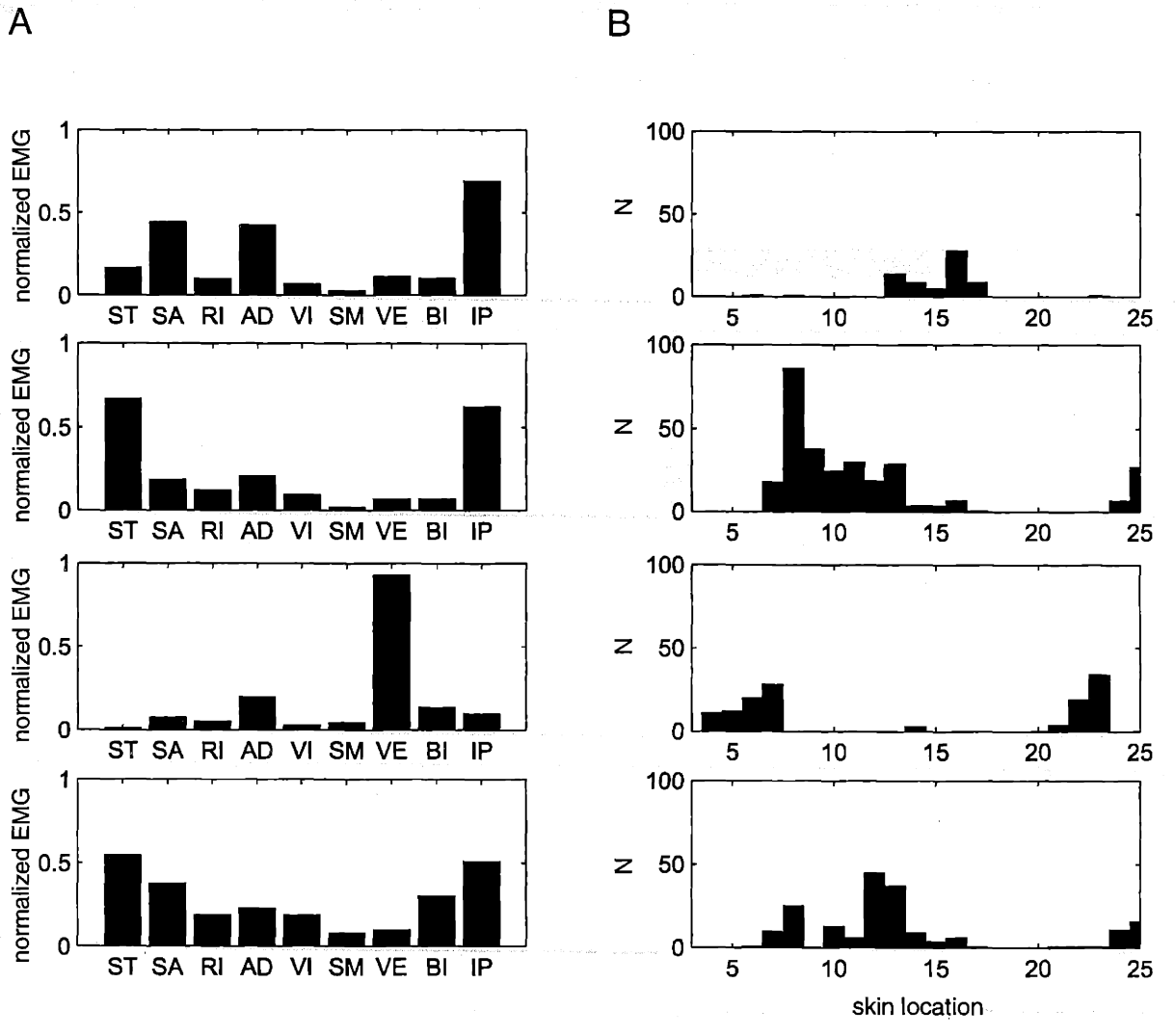

Figure 9. K-means clustering of cutaneous responses. (A) shows the clusters found by the k-means algorithm applied to one animal. The bar charts represent the means of each of the clusters found by k-means. The height of each bar reflects the mean normalized activation of each level for each muscle in a given cluster. (B) shows the frequency of each of these clusters from different parts of the skin surface.

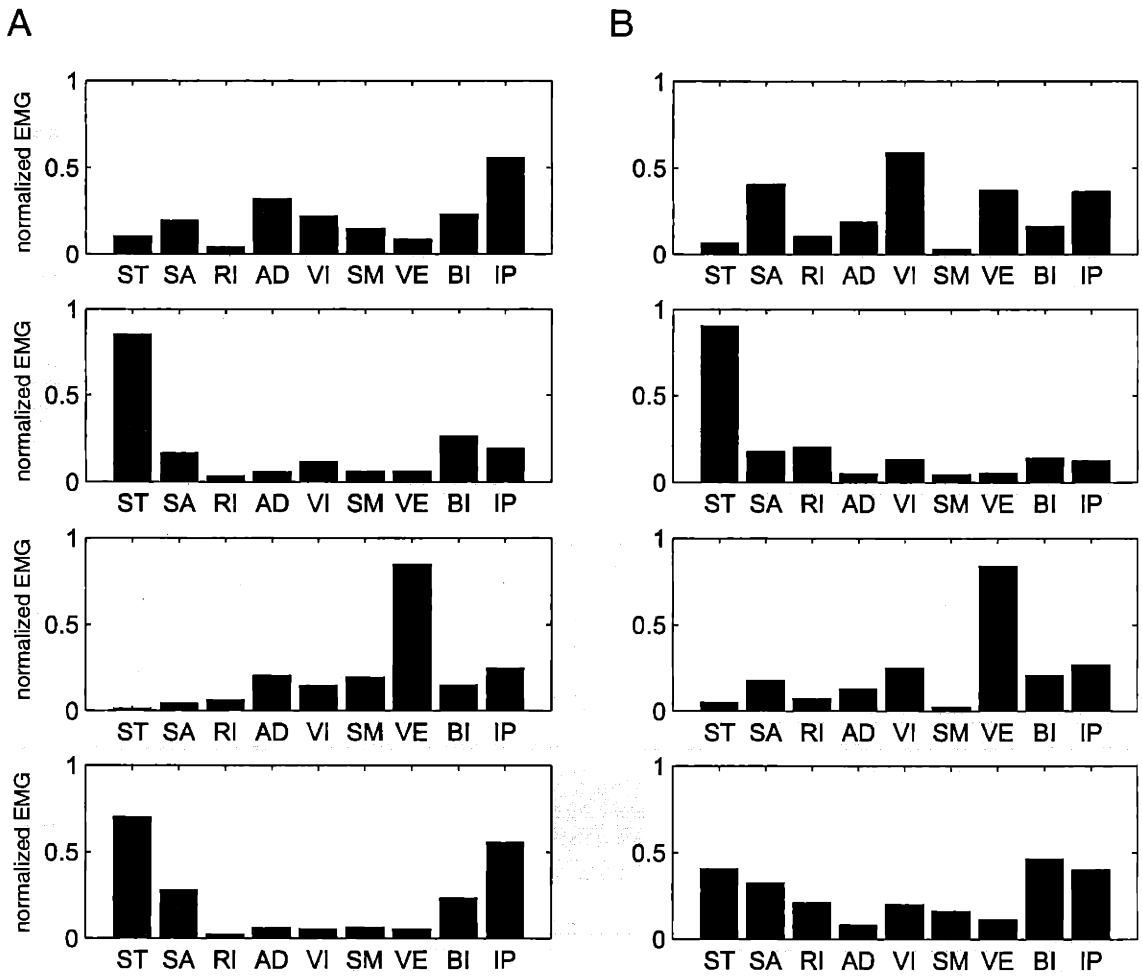

Figure 10. K-means clustering of cutaneous responses from two other animals. Conventions same as in Figure 8.

Correlation of normalized muscle activation to stimulation site (sites 8-18)									
F507	-0.33	0.38	-0.25	0.26	0.12	0.00	0.07	0.09	
F508	-0.55	0.20	-0.32	0.58	0.57	-0.01	0.45	-0.15	0.19
F509	-0.62	0.36	-0.05	0.58	-0.01	0.05	0.26	0.13	0.03
F511	-0.67	0.24	0.13	0.53	0.15	0.61	0.25	-0.02	0.11
F514	-0.55	0.58	0.07	0.79	0.72	-0.21	0.28	-0.67	-0.54
F515	-0.60	0.43	0.31	0.53	0.54	0.13	0.09	0.27	0.49
F516	-0.17	0.25	-0.07	0.43	0.48	-0.15	0.39	-0.45	0.34
average	-0.50	0.35	-0.02	0.53	0.37	0.06	0.26	-0.11	0.10
Slope of the regression of normalized muscle activation to stimulation site (sites 8-18)									
F507	-0.04	0.03	-0.01	0.01	0.01	0.00	0.00	0.01	
F508	-0.07	0.01	-0.01	0.01	0.04	0.00	0.04	-0.01	0.01
F509	-0.05	0.02	0.00	0.02	0.00	0.00	0.01	0.01	0.00
F511	-0.06	0.01	0.00	0.03	0.00	0.01	0.01	0.00	0.01
F514	-0.06	0.03	0.00	0.08	0.04	0.00	0.01	-0.04	-0.03
F515	-0.07	0.02	0.02	0.04	0.01	0.00	0.00	0.01	0.03
F516	-0.02	0.01	0.00	0.02	0.02	-0.01	0.02	-0.04	0.02
average	-0.05	0.02	0.00	0.03	0.02	0.00	0.01	-0.01	0.01

Table 1. The correlation coefficient (top) and slope of the regression (bottom) for the activation of each muscle for each animal to stimulation site. Only sites from the foot to the front of the knee (sites 8-18) were used for this analysis. Note that the activity in IP was not recorded in F507.

	Percent of observations							
Animal	ST,BI,IP		SA,AD,VI,IP		VE,BI,IP		Other	
F507	36%	(118/326)	8%	(27/326)	18%	(59/326)	37%	(122/326)
F508	49%	(180/370)	12%	(46/370)	32%	(120/370)	6%	(24/370)
F509	43%	(294/681)	10%	(71/681)	19%	(130/681)	27%	(186/681)
F511	30%	(206/678)	16%	(109/678)	8%	(54/678)	46%	(309/678)
F512	26%	(18/69)	26%	(18/69)	26%	(18/69)	22%	(15/69)
F513	15%	(19/128)	10%	(13/128)	30%	(38/128)	45%	(58/128)
F514	49%	(98/202)	21%	(43/202)	18%	(36/202)	12%	(25/202)
F515	48%	(168/353)	20%	(70/353)	21%	(75/353)	11%	(40/353)
F516	54%	(201/372)	4%	(14/372)	27%	(101/372)	15%	(56/372)
Average	38.89%		14.11%		22.11%		24.56%	

Table 2. Frequency of different k-means clusters. The clusters found by k-means applied to each animal were identified by inspection as one and the number of responses classified into each cluster was counted.

Chapter 4: Relationship between responses evoked from spinal and cutaneous stimulation in the spinalized frog

Introduction

The results of the previous chapter suggested that the withdrawal reflexes are organized in a small number of different types of muscle activation patterns. This organization is similar to that described previously for the responses from spinal stimulation in the frog and rat. In this chapter we examine directly this relationship between spinal responses and withdrawal reflexes.

There are several reasons to expect that the two types of responses might be related. First, the force fields produced by stimulation of the frog spinal cord appear similar to the force fields produced from cutaneous stimulation (Giszter et al., 1993). Second, the experiments presented in Chapter 2 of this thesis demonstrated a relationship between the anatomical organization of the responses evoked from spinal stimulation and the somatotopical organization of spinal cutaneous systems in the rat. Those experiments also gave some anecdotal evidence suggesting that the force fields produced from spinal stimulation were similar to those produced from cutaneous stimulation. Third, the movements evoked from focal iontophoresis of NMDA showed a topographical organization in the spinal cord which could be related to the cutaneous somatotopical organization of the spinal cord in a manner expected if NMDA were evoking withdrawal reflexes(Saltiel et al., 1996) All of these observations suggested that the responses from spinal stimulation were related to the withdrawal reflexes.

The specific hypothesis we test in these experiments is that the patterns of muscle activations produced by spinal stimulation are the same as the patterns produced by cutaneous stimulation. We assessed the degree of similarity by using a number of different quantitative methods. Each method attempted to find a concise description of one set of responses and then use this description to explain the other set of responses. The nature of the description used in each method was slightly different. By using these different descriptions, we were able to assess the degree of similarity between the different data sets, without relying on the results of only a single means of description.

Methods

Preparation

We used 6 adult bullfrogs (*Rana catesbiana*) in these experiments. All procedures were approved by the M.I.T. Committee on Animal Care. Frogs were anesthetized with tricaine (dose) and submerged in ice until they stopped breathing and were unresponsive to cutaneous stimulation. The spinal cord was transected by aspiration at the base of the fourth ventricle and rostral neural tissues were destroyed by heat cautery. Completeness of the spinalization was determined visually. The wound was packed with gelfoam and closed.

We then placed electromyographic (EMG) recording electrodes in a number of hindlimb muscles. Bipolar electrodes (multistranded stainless steel, insulated with Teflon, approximately 1mm exposure, backed with a small ball of wax) were threaded through muscles prependicular to the orientation of the muscle. Electrodes within the same muscle were separated by approximately 2mm. We placed electrodes in the following muscles: semitendinosus (ST), sartorius (SA), rectus internus (RI), adductor magnus (AM), semimembranosus (SM), vastus internus (VI), vastus externus (VE), biceps femoris (BI), iliopsoas (IP). In one animal we recorded from rectus anterior (RA) in the place of IP. In all

but one animal, pieces of insulation were inserted between muscles and sutured to the fascia in order to reduce crosstalk.

After the electrodes and insulation were inserted, we placed several bone screws in the ventral surface of the distal tibia. Wire was then wrapped around these screws and a threaded attachment was cemented to the framework. This attachment was used to fix the hindlimb during the experiments. A bone screw was also placed in the metatarsus and a metal rod was cemented between this screw and the framework on the tibia. This rod fixed the ankle joint at a 90° angle.

We then exposed the lumbar spinal cord by dorsal laminectomy. The dorsal surfaces of the vertebrae were first exposed. In the frog, the lumbar spinal cord lies underneath vertebrae 4, 5, and 6. Before removing these vertebrae, we placed several bone screws into the vertebrae. A single bone screw was placed in the right lateral part of dorsal vertebrae 4, 5 and 6 and two bone screws were placed in dorsal vertebrae 3 and 7. We then performed a hemilaminectomy of the left part of vertebrae 4, 5, and 6, and then opened and retracted the dura to expose the left half of the spinal cord. The exposed spinal cord was then completely covered with thin layers of gelfoam soaked in Ringers. We then wrapped wire around the bone screws in the vertebrae, threading it between the screws several times. A small metal post was then secured in the wire mesh and dental cement was applied over the entire frame, taking care that no cement encroached on the exposed cord. This post could later be attached to a restraining device which provided excellent spinal fixation during the experiment.

Following these surgical procedures, animals were refrigerated and allowed to recover overnight.

Data collection

Animals were placed on a horizontal stand and their pelvis clamped securely with a pair of vises. The attachment on the tibia was connected to a multiaxis force transducer mounted on a positioning device. The ankle was placed so that the hip was at 0° and the knee at 90°. The hindlimb was suspended securely in this position with the limb in a horizontal plane. The metal post cemented to the vertebrae was also attached and fixed in place. These attachments eliminated almost all visible movement of the spinal cord even during very vigorous hindlimb movements.

Responses to cutaneous stimulation were measured as described in the previous chapter. Small regions of the hindlimb skin surface were scratched by moving a piece of wood back and forth. We stimulated at strengths sufficient to produce measurable responses but not intense enough to produce a scratch reflex.

We also measured the responses evoked from electrical microstimulation of the frog spinal cord. Microelectrodes (stainless steel, .5-1um tip exposure, 5Mohm) were inserted into the spinal cord. Electrodes were advanced in steps of 250um from the first location at which neural activity could be recorded in response to sensory stimulation. At each location, brief trains of microstimulation were applied (400ms, 50Hz, .3ms) at currents from 1 to 8uA. Only sites within 1000um from the dorsal surface of the spinal cord are reported here.

EMG activity evoked by cutaneous and spinal stimulation was high pass filtered (50Hz) and amplified (25k) before being sampled at 1kHz and recorded for offline analysis.

At each site in the spinal cord where microstimulation was applied we also examined the sensory receptive field of neurons near the stimulating electrode. We usually examined the receptive field of multiple units near the electrode although single units were isolated

whenever possible. We recorded the regions of the skin surface at which light pressure evoked neural responses.

Data analysis

Because of potential differences between EMG electrode placement in different animals and between the behavioral strategies used by different animals, we examined results from each animal individually.

Analysis of cutaneous responses was performed as described in the previous chapter. Briefly, different responses were identified using interactive software using the Matlab software package and the EMG activity for each muscle averaged through the entire response. Individual muscles were then normalized to the maximum value observed across any trial. The normalization was to the maximum value observed for any cutaneous stimulation or any spinal stimulation response. Responses less than one standard deviation of the distribution of response magnitudes from spinal stimulation were removed. The remaining responses were normalized to be of unit magnitude.

We performed a similar analysis for the muscle activations produced from spinal microstimulation. The response from microstimulation was divided into 100ms bins following the onset of the microstimulation train. The muscle activity within each 100ms bin was then averaged for each muscle separately. Each muscle's activation level was then normalized to the maximum value observed for any trial of either cutaneous or spinal stimulation. Responses with magnitudes of less than one standard deviation of the observed distribution of magnitudes were removed. The remaining responses were then normalized to be unit magnitude.

Ability to cluster cutaneous and spinal responses

From previous research we expected that both cutaneous and spinal microstimulation would evoke a small number of distinct muscle patterns. We examined whether there was a better separation between these different muscle patterns in the responses from cutaneous or spinal stimulation. We first examined this issue using a principal components analysis (PCA). PCA attempts to find a set of axes which explain decreasing amounts of variance in the data. We used PCA primarily as a way to simplify the description of the high dimensional data examined here, in order to examine it qualitatively.

We also quantitatively examined this issue of the ability to cluster cutaneous and spinal responses. The basic idea of this analysis was to try to fit each data set to a small number of clusters, and then examine whether the variability within each cluster was smaller for one of the two different types of stimulation. If the variability of one of the stimulation types was in fact smaller, this would be evidence supporting the idea that one of the types of stimulation was better clustered than the other. We clustered each data set using the k-means algorithm. K-means attempts to find a set of EMG vectors such that the distance between each data point and the nearest vector is minimal. For the case of the nine dimensional EMG data considered here, each vector would consist of nine values corresponding to the mean value for each muscle. We applied the k-means algorithm to 90% of the data chosen randomly and at each iteration examined the error of the k-means clusters applied to the other 10% of the data. The algorithm was considered to have converged when this error increased for 20 consecutive iterations. We repeated this procedure for several different subsets of the data chosen randomly. This procedure produced slightly different solutions depending on the particular subset of data chosen and the initial conditions of the algorithm. For a given set of clusters obtained from k-means, we

assigned each data point to the cluster with the nearest mean. We then found the variance within each cluster. This procedure was then repeated for a new set of clusters found by k-means starting from different initial conditions and applied to a different subset of data for both cutaneous and spinal stimulation, giving two distributions of cluster variances. We then examined whether there was a significant difference between these two distributions of variances by performing a bootstrap statistic on the difference between the mean variances of these two populations (500 bootstrap steps).

Predicting spinal responses from cutaneous responses and vice versa: k-means classification

We attempted to directly assess the similarity between the responses from spinal and cutaneous stimulation. The basic idea of these analyses was to find a representation which described the responses from one set of data concisely and then determine how well the same representation described the other set of data.

The first method used the k-means algorithm described previously (Hartigan and Wong, 1979). We found a set of EMG weighting vectors using k-means applied to the spinal and to the cutaneous responses separately. We then used these two sets of vectors to classify one of the data sets, either the cutaneous or the spinal. This classification labeled each individual response as belonging to the cluster with the nearest vector. We then examined how often the two classifications, one derived from the data set being classified (e.g. the cutaneous responses) and the other derived from a different data set (e.g. the spinal responses), corresponded to one another. This correspondence was measured as the percentage of responses placed into the same category by each clustering method.

In order to interpret this correspondence, however, we must estimate the correspondence expected by chance. For instance, if the data were heavily biased to one type of response, one would expect a large degree of correspondence between two clustering methods only by chance. This chance correspondence represents the null hypothesis, that the classifications of the data by the two clustering methods were unrelated. We estimated the correspondence predicted if the two classifications were unrelated by permuting the data sets. By performing this permutation, the classification of an individual response using one method was compared to the classification of a different individual response using the other method. The correspondence found after this permutation reflects the correspondence predicted if the two classifications were unrelated.

We also estimated an upper bound of the correspondence for a data set. As described above, starting the k-means algorithm from different starting conditions and applied to different subsets of the data produced slightly different sets of EMG centers. One would expect that the best correspondence for a given data set would be found between the classifications produced by two different solutions found by k-means applied to the same data set. By comparing the correspondence between any two of these clusters found from k-means applied to the same data set, we estimated the correspondence between two classifications based on clustering the same data set. This correspondence is that expected if two sets of data were actually found from the same set of data. This estimated correspondence reflects how consistently a particular data set can be classified.

Predicting spinal responses from cutaneous responses and vice versa: k-means residual variance

The previous analysis examined the degree of correspondence between the classifications produced by each data set. The analysis described here examines how well a description of one set of data can describe the other set of data. The basic idea in this analysis and in the subsequent analyses is to first find a concise description of one of the

data sets, then use that description to explain the other data set. The quality of the description is assessed here by calculating the amount of variance that the description explains. We assessed the ability of several different description methods to explain the variance in the observed responses.

The first method we used to describe a set of data used the k-means clusters directly. We first found the clusters for one data set and then used these clusters to classify the other data set. We then found the squared difference between each data point and the nearest cluster center and summed each of these differences to obtain the sum squared error of this clustering. We also calculated the total sum squared difference for the data set by finding the difference between each data point and the grand mean of the data set. The ratio of these two sum of squares gives the amount of residual variance left unexplained by the clustering. The amount of explained variance is just one minus this residual variance. The residual variances produced from several different sets of clusters found by k-means were used to estimate the residual variance.

The explained variance expected from a random model was also calculated. The values of a random set of clusters were chosen randomly from 0 to 1. The explained variance found by such random models was then calculated as above.

We also calculated an upper bound on the ability of any model to explain a particular data set. As described for the k-means classification, we assumed that the best description of a data set would come from a set of clusters found directly from that data set. To estimate this upper bound for a data set, we fit a set of clusters using the k-means algorithm applied to 90% of the data set chosen randomly. We then calculated the explained variance produced by this set of clusters applied to the 10% of the data not originally used to fit the clusters. This procedure estimates the ability of a set of clusters to generalize to new data drawn from the same distribution used to fit the clusters originally.

Predicting spinal responses from cutaneous responses and vice versa: linear combination of k-means clusters

The second method of description we examined was a variant of this k-means classification. In this method we fit one set of data using the k-means algorithm, but then allowed the description of the other set of data to be a linear combination of these clusters. This model was based on previous work demonstrating that simultaneous stimulation of two sites in the spinal cord results in a response which is a simple linear combination of the response from stimulation of each site separately(Bizzi et al., 1991; Mussa-Ivaldi et al., 1994). This linear combination is observed in both the evoked forces and in the evoked EMGs. Thus, if stimulation at a site in the spinal cord activated elements of two different types of responses, the observed response would be expected to be the linear combination of these responses. In this model each response in one data set was fit as a nonnegative linear combination of the clusters obtained from k-means applied to the responses of the other data set. We used the nnls routine supplied by Matlab to perform this fit. The fit was again assessed by examining the amount of explained variance. We also estimated the explained variance expected by chance and an upper bound on the explained variance in the same way as described in the previous section.

Predicting spinal responses from cutaneous responses and vice versa: linear subspace

The final method we used to describe the data was an extension of this linear combination model. The model described in the previous section assumed that the responses in one data set were drawn from distinct clusters while the responses in the second data set could be produced as linear combinations of these clusters. In the model

described here, the responses in both data sets could be a linear combination of a set of EMG vectors. We found a set of these vectors using a nested gradient descent method. We first choose a set of random EMG vectors between 0 and 1. We then fit each response within a data set to a nonnegative linear combination of these vectors, using the nnls routine supplied by Matlab. We then used this error to update the EMG vectors using the least mean squares (LMS) learning rule:

$$\Delta w_i = -\mu \sum_j (m_j^{pre} - m_j^{obs}) c_{ij}$$

where Δw_i is the *ith* EMG vector, c_{ij} is the positive weighting of the *ith* EMG vector for the *jth* response, m_j^{pre} is the best fit response for the *jth* component, m_j^{obs} is the jth observed response, and μ is a step size which determines the algorithm's rate of convergence. The parameters of w and m are nine dimensional vectors while the c parameter is a single number. In essence, the LMS rule changes an EMG weighting vector in relation to the amount that the vector contributes to the prediction error, which is equivalent to performing gradient descent on the error with respect to the EMG vectors (Hertz et al., 1991). After the vectors were updated, each response was again fit to a combination of these new vectors. The errors of these predicted responses were again used to update the weights using the LMS rule. This algorithm was iterated in this manner until it converged on a solution. We determined that the algorithm had converged to a solution by assessing the ability of the solution to generalize to data which weren't used in the fitting procedure. We withheld 10% of the responses chosen randomly and fit the weighting vectors on the remaining 90% using the procedure described above. At each iteration of the algorithm, we measured the prediction error of the withheld 10% of the data fit to the weighting vectors. We considered the algorithm to have converged when the prediction error of this withheld data increased for twenty consecutive iterations. This procedure is a standard way of assessing when a learning algorithm is overfitting the training data and becomes unable to generalize to a new data set (Bishop, 1995). Note that if each response is fit as a linear weighting of the single EMG vector which predicts the response the best and the vectors are updated according to the LMS rule, the algorithm described here is equivalent to the k-means algorithm.

This algorithm is very similar to that described by Olshausen et al. (Olshausen, 1996; Olshausen and Field, 1996). The main difference between the two methods is that in Olshausen, the solution found by the algorithm was further constrained by making assumptions on the distribution weighting coefficients. In particular, this distribution was assumed to be sparse, by penalizing solutions resulting in a large number of large weighting coefficients. In the present use of this algorithm, we did not impose such a constraint. We did not explicitly enforce any particular distribution of weighting coefficients, allowing any arbitrary combination. This absence of explicit constraint, however, implicitly enforces that the distribution of weighting coefficients is uniform. This distribution seems to be the most appropriate, but it must be acknowledged that we might have found different solutions if different distributions were imposed.

Along with other methods such as PCA and factor analysis, this analysis essentially tries to find a linear subspace which describes a set of high dimensional data concisely. However, it avoids some of the assumptions inherent in those other methods. In particular, both PCA and factor analysis allow the EMG weightings to be negative and also for the linear combination of these weightings to be negative as well. Since the EMG data examined in the present study are all positive, these negative values are difficult to interpret.

Further, PCA assumes that each weighting vector must be mutually orthogonal, an assumption not clearly supported in the present case.

Once a set of EMG vectors was found using this method, it was used to describe the responses evoked from spinal microstimulation, and the quality of this description was assessed by examining the amount of explained variance as described before. We also estimated the explained variance expected by chance and an upper bound on the explained variance as described above.

Results

General observations

In Figure 1 we show some examples of good correspondence between patterns of muscle activations produced by cutaneous and spinal microstimulation. Figure 1A and 1B show a response with activation of ST and IP evoked by cutaneous stimulation of the back of the foot and by spinal stimulation with a receptive field at the same location. Figure 1C and 1D show examples of a response with activation of SA, AD, VI and IP with a weaker activation of ST from cutaneous stimulation of the front of the knee and from spinal stimulation at a site with a receptive field on the front of the calf. Figure 1E shows a response with activation of VE, BI and IP from cutaneous stimulation of the back of the calf and 1F shows a similar response from spinal stimulation of a site with the corresponding receptive field. In each of these cases, the two types of stimulation produced similar responses. This similarity is examined more systematically in later sections.

The mean EMG magnitude at different periods following the offset of the microstimulation train for one animal is plotted in Figure 2. As indicated for the animal shown in this figure, maximal EMG responses were observed between 300 and 400ms following the onset of the microstimulation train. The largest magnitude responses were observed in this time period for each animal. We chose to analyze the responses obtained from microstimulation during this period. The numbers of responses obtained for cutaneous and for spinal stimulation for each frog are given in Table 1. These are only the responses with a magnitude larger than one standard deviation of the entire population of responses for each frog.

The entire set of EMG responses obtained from spinal stimulation for one animal is shown in Figure 3. This figure shows the activation of each muscle evoked from sites at different rostrocaudal locations of the spinal cord. In comparison to the distribution of responses observed for cutaneous responses from the skin shown in Chapter 3 of this thesis (see Figures 4 and 5 of Chapter 3), the variation of muscle activations with rostrocaudal location of the stimulation site was not very systematic. In particular, sites throughout the spinal cord produced responses with activation of ST, SM, and BI. Other types of responses were also observed, such as the VE activation from the caudal spinal cord and SA from the rostral spinal cord, but they were less frequent (see below). Further, the locations in the spinal cord which produced these other types of responses also produced the ST, SM, and BI type of response.

Number of muscles activated in cutaneous and spinal responses

We first examined whether the responses from cutaneous and spinal stimulation activated a similar number of muscles. For each response, we found the number of muscles with an activation larger than a certain level. We then averaged the number of muscles above this level across all responses to obtain the average number of muscles above a particular activation level. The results obtained from performing this procedure for

activation levels between 0 and 1 are shown in Figure 4, for two representative animals. In the animal shown in Fig. 4A both cutaneous and spinal stimulation activated a similar number of muscles for all threshold levels of activation. In the animal shown in Fig. 4B, however, spinal microstimulation consistently activated a larger number of muscles than cutaneous stimulation. In general, all animals either showed a similar number of activated muscles from cutaneous and spinal stimulation (3/6) or spinal stimulation activated a larger number of muscles than cutaneous stimulation (3/6).

Ability to cluster cutaneous and spinal responses

We next compared the ability of the responses from cutaneous and spinal stimulation to be explained by a number of distinct clusters. We first examined this ability visually by performing a principal components analysis (PCA). Figure 5A shows the responses evoked from cutaneous stimulation projected onto the first two principal components. It can be seen in this animal that displaying the data in this way reveals three main clusters of responses, corresponding to the three types of responses described in Chapter 3 and illustrated in Figure 1 of this chapter. Figure 5B shows the results obtained when the same procedure was applied to the responses from spinal stimulation. The responses from spinal microstimulation are not as well segregated as those from cutaneous stimulation. Qualitatively similar results were obtained in the other animals as well.

We examined this ability to cluster responses quantitatively by performing a k-means clustering and examining the amount of variance within each cluster. We found that in the majority of animals (4/6) the variance within a cluster obtained from spinal responses was on average larger than the variability within a cluster obtained from cutaneous stimulation ($p<.001$). In the other two animals there was no difference between the variances ($p>.001$). This result was consistent with the observation that the responses from spinal stimulation were not as well clustered as the responses from cutaneous stimulation.

Comparison between cutaneous and spinal responses: k-means classification

We first examined the clusters obtained from applying the k-means algorithm to each set of responses. The cluster means found for the set of responses taken from the animal shown in Figure 3 are shown in Figure 6A and 6B. Figure 6A shows the clusters found by k-means applied to the cutaneous responses while Figure 6B shows the clusters found by k-means applied to the spinal responses. It can be seen that the two sets of clusters qualitatively corresponded well to one another. This correspondence was especially true for the first three sets of clusters. These three clusters corresponded to the clusters described in the previous chapter as representing the main types of responses from cutaneous stimulation (see Figures 9 and 10 of Chapter 3). The clusters found from k-means applied to the data set from another animal are shown in Figure 6C and 6D. These clusters are not as similar as for the animal shown in Figure 6A and 6B, but it is still possible to relate the clusters found from each set of data.

We then used the results obtained from the k-means analysis to examine the similarity between cutaneous and spinal responses. We first examined the correspondence of classification of a data set between the results of k-means applied to either cutaneous or spinal stimulation. For this analysis, we first classified the cutaneous responses according to the k-means centers found from clustering the cutaneous responses themselves. We then classified the cutaneous responses according to the k-means centers found from clustering the microstimulation responses. We then asked how often the two classification patterns

corresponded. This correspondence was compared to the correspondence expected if the two classifications were randomly related and to the correspondence found from comparing the classification of the cutaneous responses from two different sets of k-means centers found from clustering the cutaneous responses themselves. Results from such an analysis are shown in Figures 7A and 7B for each of the different animals. It can be seen that the correspondence between the classification of cutaneous responses by the centers found by k-means was higher than that expected by chance for all animals ($p<.05$). This correspondence was almost as high as that expected for the classifications produced by two different sets of k-means centers found from the cutaneous responses themselves. In three animals this latter difference was not significant ($p>.05$). This result suggests that the clustering of spinal responses classified the responses from cutaneous stimulation in a similar way as the clustering of cutaneous responses themselves.

Figures 7C and 7D show the results of the complementary analysis examining the classification of spinal responses by the k-means centers from cutaneous responses. For five of the six animals, there was a higher correspondence between the classification of spinal responses by the k-means from cutaneous responses and by the k-means from spinal responses than expected by chance ($p<.05$). This correspondence was similar to the correspondence between the classification by different sets of k-means centers found from the spinal responses themselves. In three animals this latter difference was not significant ($p>.05$).

Examining the results shown in Figures 7 suggests that there was a higher correspondence of the classifications of cutaneous responses produced from k-means applied to the cutaneous responses themselves then from the classifications of spinal responses produced from k-means applied to the spinal responses themselves. This difference in correspondences was significantly better for clusters from cutaneous than from spinal stimulation for all animals ($p<.05$). This difference results from the fact that the EMG clusters found by k-means applied to the cutaneous responses were generally similar to one another, and therefore classified the data in a similar manner. The clusters found by k-means applied to spinal responses, on the other hand, were more variable, and this variability lead to lower correspondence in their classification of the data set. This variability is consistent with the observation of the previous section which suggested that the cutaneous responses were better clustered than the spinal responses.

Comparison between cutaneous and spinal responses: k-means explained variance

In the second comparison of spinal and cutaneous responses, we examined how well the responses from spinal stimulation could be described by the clusters found by k-means applied to cutaneous responses in the same animal. This description was evaluated by the amount of variance explained by the description, referred to as R^2. We compared this R^2 value to the R^2 value obtained from clusters chosen randomly and to the R^2 value obtained from clusters derived from k-means applied to the microstimulation responses directly. The results obtained for all animals are shown in Figures 8C and 8D. It can be seen that the k-means clusters obtained from cutaneous responses explained more variability than would be expected by chance (5/6 animals, $p<.05$) but not as much as the clusters found when k-means was applied to the responses from microstimulation itself (6/6 animals, $p<.05$). These differences were significant for all of the animals except for F514, for which the k-means from cutaneous responses actually predicted a smaller amount of variability in spinal responses than expected by chance.

The complementary analysis from using the clusters found from spinal stimulation to explain the cutaneous responses is shown in Figures 8A and 8B. These results were similar to those presented in Figures 8C and 8D. The R^2 values produced by this description were in general higher than expected by chance (4/6 animals, $p<.05$) but not as high as those produced by directly clustering the cutaneous responses (6/6, $p<.05$).

Comparison between cutaneous and spinal responses: k-means linear combination

In the third method of comparison, we examined how well the spinal responses could be described as a nonnegative linear combination of the k-means clusters obtained from cutaneous responses. The R^2 for this combination was then compared to the R^2 expected for a linear combination of random clusters and to the R^2 obtained from a linear combination of the k-means clusters obtained from spinal stimulation responses. The results for all animals are shown in Figures 9C and 9D. Similar to what was shown in Figure 8, it can be seen that a linear combination of cutaneous clusters predicts the spinal stimulation responses better than chance (5/6, $p<.05$) but not as well as the clusters obtained directly from spinal stimulation (6/6, $p<.05$).

Figures 9A and 9B show the complementary analysis in which the cutaneous responses are described as a linear combination of the clusters found from k-means of the spinal responses. Again, the cutaneous responses are described by a linear combination of spinal clusters better than expected by chance (4/6, $p<.05$), but not as well as a linear combination of clusters obtained directly from the cutaneous responses (6/6, $p<.05$).

Comparison between cutaneous and spinal responses: linear subspace

We examined this same issue of the similarity of cutaneous and spinal responses by performing a fourth analysis. In this analysis we described one set of responses as a linear combination of EMG vectors and then described the other set of responses as a linear combination of these same EMG vectors. The set of EMG vectors, or 'factors', which could best describe a set responses as a nonnegative linear combination was found using gradient descent (see Methods). The results obtained from applying this method for one animal are shown in Figure 10. Figure 10A shows the EMG factors found from applying this method to the responses from cutaneous stimulation. Figure 10B shows the EMG factors found from applying this method to the responses from spinal stimulation. The first three vectors found from cutaneous stimulation were very similar to those found from spinal stimulation.

We then examined how well the responses from spinal stimulation could be described by the factors obtained from cutaneous responses. We fit the spinal responses as a nonnegative linear combination of the factors from cutaneous responses and assessed this fit by the R^2 value. This R^2 value was compared to the R^2 value obtained from a linear combination of random factors and to the fit by EMG factors obtained from applying this method to the microstimulation data set itself. The results from this analysis are presented in Figure 11C and 11D for each animal. Again, the fit of spinal responses by the factors found from cutaneous responses was better than that expected by chance (6/6, $p<.05$). This fit was not as good as the fit of spinal responses by the vectors found from spinal responses themselves (6/6, $p<.05$).

The results found by applying this analysis to the responses from spinal stimulation and trying to explain the variance in the cutaneous responses are shown in Figure 11A and 11B. The responses from cutaneous could be described better than expected by chance

(6/6, $p<.05$) but not as well as if they were obtained from applying this algorithm directly to the cutaneous responses themselves (6/6, $p<.05$).

Comparison between cutaneous and spinal responses: summary of the different methods

In each method, one set of data was able describe the other set better than that expected by chance suggesting a similarity between the responses in each set of data. However, this description was not as good as that obtained from considering each data set separately, suggesting that there were also differences between one another. Comparison of Figures 7 through 11 also suggests that the different methods each explained different amounts of the variance in the data set. These differences are summarized in Figure 12. For each animal, we examined how much of the variance above chance which was possible for a model to explain was actually explained by the model. The average amount of variance explained by chance for one set of responses was subtracted from both the variance explained by models from the other set of responses and from the variance explained by models from the same set of responses. In other words:

$$f = \frac{R^2_{MC} - R^2_{Crand}}{R^2_{CC} - R^2_{Crand}}$$

in which f is the relevant ratio, R^2_{MC} is the amount of variance explained in the cutaneous responses by models from spinal responses, R^2_{Crand} is the amount of variance explained by chance, and R^2_{CC} is the amount of variance explained in the cutaneous responses by models from cutaneous responses themselves. The same ratio were calculated for the responses from spinal stimulation. This ratio allows the degree of similarity between the two sets of models to be assessed: a ratio of 1 implies that the two models are indistinguishable while a ratio of 0 implies that the two models are unrelated. This ratio is plotted in Figure 12 for both sets of data described by each type of model considered here. For both sets of data, the linear combination of arbitrary factors by the gradient descent method explained the most of the possible variance. Even this method, however, only explained approximately half of the possible variance in both cutaneous and spinal responses. This result is consistent with the observations indicated that each method explained some, but not all, of the variance in each set of responses.

Distributions of cutaneous and spinal responses

We compared the distribution of cutaneous responses across the skin surface to the distribution of spinal responses across the rostrocaudal extent of the spinal cord. Based on the results described for the rat in Chapter 2 of this thesis, we expected to find a rostrocaudal organization in the responses from spinal stimulation. In particular, we predicted that those regions of the spinal cord which were responsive to sensory stimulation from a particular part of the skin surface should tend to produce responses similar to those produced from cutaneous stimulation of the same part of the skin.

In Figure 13 we show the rostrocaudal distribution of responses from microstimulation of the spinal cord for one animal. Similar to the data shown in Figure 3, the rostrocaudal organization of responses within the spinal cord was not very systematic, with responses with strong activation of ST, SA, BI, and IP arising from all regions of the spinal cord. However, at the rostral and caudal extremes of the spinal cord, responses with the activation of other muscles were also observed. In particular, both AD and VE which were not strongly activated from midlumbar regions contributed more to responses from

these rostral and caudal regions. VI shows a similar trend and SA appears to increase its contribution in responses from more rostral sites. The absence of activation of these muscles from midlumbar regions of the spinal cord where the foot is represented is consistent with the organization of cutaneous responses described in Chapter 3, but their increased activation from both rostral and caudal regions of the cord appears to be in conflict. However, it appeared that cutaneous responses in this animal displayed an atypical organization. The responses from cutaneous stimulation for this animal are shown in Figure 14. Responses from cutaneous stimulation were not examined for every site of the hindlimb in this animal and so we have divided stimulation sites into three categories: back of the calf, foot, and front of the calf. These categories reflect the main subdivisions of responses found in Chapter 3. In this animal stimulation of the foot evoked responses with activation of ST, BI and IP, a response similar to that observed from midlumbar regions of the spinal cord in this animal. In this animal AD was activated in response to cutaneous stimulation of either the front or the back of the calf, a pattern not typical of most animals as indicated in Chapter 3. This atypical pattern, however, was reflected in the responses from spinal stimulation, so that AD was activated from both rostral and caudal regions of the spinal cord. Similar atypical patterns during cutaneous responses could also be observed for the activation of SA, VI and VE and these patterns were reflected to a partial degree in their activation patterns within the spinal cord.

This relationship could also be observed using a clustering analysis. Figure 15 shows such an analysis applied to the responses from the animal shown in Figure 3. Figure 15A shows the centers of the k-means clusters found from the cutaneous responses and Figure 15B shows the frequency of each cluster from different regions of the hindlimb. In general, this animal shows the pattern of responses described in Chapter 3, in which responses from the back of the calf activated VE, responses from the foot activated ST and/or BI, and responses from the front of the leg activated SA, AD, VI. However, this animal also occasionally produced activation of VE in responses from the transition of the foot and the front of the calf and stimulation of the front of the foot in this animal also occasionally produced responses with activation of SA, AD and VI. Figure 15C shows the frequency of responses from spinal stimulation classified into each of these same clusters at different rostrocaudal locations. Responses classified within the cluster with strong activation of VE were found both from the caudal part of the spinal cord and from the rostral midlumbar regions of the spinal cord. These spinal regions represented the back of the leg and the skin at the border of the foot and the front of the calf, respectively. Analogously, responses classified within the cluster with activation of SA, AD, and VI were found from the rostral part of the spinal cord and from regions through the midlumbar spinal cord. These spinal regions represented the front of the leg and the foot, respectively. Thus, in both animals, the individual differences in the organization of cutaneous responses were related to the organization of spinal responses. This relationship could be made through consideration of the spinal cutaneous somatotopy.

Discussion

Relationship between spinal responses and cutaneous systems

The results presented here provide evidence that the responses from microstimulation of the spinal cord are related to the withdrawal reflexes evoked by cutaneous stimulation. This conclusion was supported by the results of several different analyses. In each method, the responses from spinal stimulation could be well described by the responses from cutaneous stimulation. The reverse claim, that the responses from

cutaneous stimulation could be well described by the responses from spinal stimulation, was also supported. The relationship between cutaneous and spinal responses was also supported by the distribution of responses evoked from different regions of the spinal cord. This distribution appeared to follow the somatotopy of spinal cutaneous systems, such that the response produced from stimulating a site in the spinal cord tended to be similar to the response produced from stimulating that region of the skin surface represented at that site. These observations are consistent with the hypothesis that the responses from spinal stimulation were related to the responses from cutaneous stimulation.

This similarity helps place the results observed from spinal stimulation in a physiological context. One of the main concerns with using microstimulation anywhere in the nervous system is the extent to which the effects of such stimulation reflect the normal physiological function of the stimulated substrate. By demonstrating the similarity between the responses from spinal microstimulation and cutaneous stimulation, we can conclude that responses from spinal microstimulation are physiologically relevant. Given the nonspecific nature of microstimulation, this observation is important.

Despite this similarity, the two sets of responses did not appear to be identical. In each analysis examined here, the responses from spinal stimulation did not describe the cutaneous responses as well as would be expected if the spinal responses were identical to the cutaneous responses. The converse inability of cutaneous responses to explain the spinal responses was also observed for each method. These observations suggest that there are differences in the responses evoked by the two methods of stimulation. It is not clear how to interpret this partial similarity between cutaneous and spinal responses. This difference cannot be explained simply by the observation that the responses from spinal stimulation appeared to be less clusterable and therefore had more noise than those from cutaneous stimulation: the variance in a set of responses not explained by the other set could be explained by a different model. There therefore appeared to be systematic differences in the responses from spinal and cutaneous stimulation. It is possible that this difference arises because the responses from spinal stimulation reflect the organization of both cutaneous spinal systems and some other, currently uncharacterized, spinal system, such those activated by proprioceptive systems. This other system could contribute to the spinal responses in a manner which cannot be described by the cutaneous responses. Similarly, the difference could result from the fact the only cutaneous behavior we examined was withdrawal: cutaneous systems in the spinal cord might also be expected to be related to other behaviors evoked from cutaneous stimulation, such as scratch reflexes, which were not characterized here. However, the fact that cutaneous responses could explain a significant portion of the variance within spinal responses does suggest that microstimulation of the spinal cord is reflecting the organization of spinal cutaneous systems to a large degree.

It also appeared that the responses from microstimulation did not appear to be more simple or fundamental to the production of behavior than the withdrawal reflexes themselves. In both types of responses, there was a similar number of muscles activated. If anything, the responses from microstimulation appeared to activate a larger number of muscles than cutaneous stimulation. This observation suggests that the spinal responses are similar to cutaneous responses as a whole, and do not represent components of the cutaneous behaviors. It is still possible, however, that more complex behaviors might be constructed from the combination of the responses described here.

Does spinal stimulation reflect the sensory or the motor organization of the spinal cord?

Although this similarity between spinal and cutaneous responses helps make the responses from spinal stimulation more physiologically relevant, it also raises some obvious questions about these spinal responses. The main question is whether the responses we have observed reflect the motor organization of the spinal cord or the sensory organization of the spinal cord.

It is possible that the responses from spinal microstimulation are motor in nature. This is the perhaps the most natural way of interpreting the responses: a region of the spinal cord is activated and a motor reponse is observed and we therefore conclude that that region of the spinal cord is involved in the production of that motor act. The fact that the regions of the spinal cord of both frogs and rats stimulated in these experiments contain neurons which project directly to the motoneurons is consistent with this interpretation(Kitazawa et al., 1993; Moschovakis et al., 1992). From this point of view, microstimulation of the spinal cord allows us to examine the organization of the spinal cord in a manner which is not addressable from producing behaviors directly. Microstimulation, therefore, might allow us to study how such complex behaviors are organized at intermediate levels of representation within the spinal cord.

It is also possible that the responses produced from microstimulation of the spinal cord are sensory in nature. Previous work has demonstrated that responses from microstimulation are not critically dependent on the activation of either descending or afferent fibers (Giszter et al., 1993). Further, similar sets of responses can be observed by focal iontophoresis of NMDA into the spinal cord(Saltiel et al., 1996). These results suggest that the responses from spinal microstimulation are not trivially due to the direct activation of sensory afferents. However, spinal stimulation might still be activating the spinal cord in the same way as a sensory stimulation of the skin. In the spinal cord, there is not a clear segregation between neurons representing sensory information and neurons actually involved in producing the motor act (Brown, 1981). There is the obvious dorsal ventral gradient from sensory afferents in the dorsal horn to motoneurons in the ventral horn, but neurons in between these two extremes are difficult to classify. The high degree of interconnectivity of the spinal cord makes this distinction even more difficult (Alstermark et al., 1990; Bras et al., 1989; Hultborn et al., 1976). It is possible, therefore, that microstimulation of the spinal cord might be producing by an activation that is 'interpreted' by the spinal cord in a manner to similar to how a natural sensory stimulation is interpreted. From this perspective, the similarity between responses evoked from spinal and cutaneous stimulation is not surprising.

If this latter possibility is true, it is not clear what additional information is gained by examining the responses from spinal microstimulation. The method provides another way in which to access the modularity of responses observed within the withdrawal reflexes, but apparently obscures some of this modularity. This obscuring was reflected in the observation that spinal responses appeared to be more poorly clustered than the cutaneous responses. From this point of view, the information found from microstimulation of the spinal cord could be obtained just as easily, if not easier, from cutaneous stimulation of the skin surface.

Are there any observations which allow us to distinguish whether or not the responses from spinal microstimulation are sensory or motor in nature? The only suggestion that the responses observed from spinal microstimulation reflect the organization of motor and not sensory systems of the spinal cord comes from research using focal iontophoresis of NMDA. Those experiments found that there was a topographic organization of responses evoked from stimulation of the spinal cord. However, preliminary examinations suggest that

this topography does not follow the cutaneous somatotopy in the way described here for the responses evoked from electrical stimulation of the spinal cord. NMDA, therefore, might be reflecting the organization of motor structures in the spinal cord, while electrical stimulation might be more predominantly reflecting the organization of sensory structures. Such a distinction between the topographic organization of the sensory and motor interneuronal systems underlying the withdrawal reflexes has been made from previous research. However, this observation is preliminary at this point and must be examined more systematically.

In general, however, there do not appear to be any obvious distinctions between the responses observed from spinal and cutaneous stimulation. The utility of spinal microstimulation for the study of the organization of the spinal cord, therefore, is currently unresolved. While spinal microstimulation appears to be producing responses which are physiologically relevant, it is not clear that it is giving us any information that we could not have obtained from examining behaviors directly.

Modularity of spinally generated responses

It is striking that both methods of activating the spinal cord, cutaneous and intraspinal stimulation, produced only a few types of distinct responses. Similar conclusions were made based on the responses produced by focal iontophoresis of NMDA in the frog spinal cord (Saltiel et al., 1996). These observations have been previously characterized as reflecting a 'modular' organization of motor systems in the spinal cord. Based on the evidence from withdrawal reflexes, these responses appear to be organized across a group of muscles. These responses are therefore consistent with the idea that movements produced by the spinal cord are organized in terms of motor synergies. Combined with the experiments described in the Chapter 1 which supported the idea of muscle synergies produced by the spinal cord, the present results imply that at least some of the movements produced by the spinal cord are modularly organized.

This modularity of spinally generated movements is surprising given the potential capability of the nervous system to produce arbitrary movements. As described in Chapter 1, the complexity of movements produced by the nervous system is potentially only limited by the number of motor units in the musculature. The finding that the spinal cord organizes movements into only a small number of motor responses is surprising and begs the question of why this modularity arises.

One reason for a modular organization of spinal systems might be to reduce the number of degrees of freedom of the motor system in order to simplify the control of movements (Bizzi et al., 1991). By organizing the spinal cord into a small number of distinct muscle synergies, the nervous system can produce movements by specifying the states of only a handful of variables. In this context, the modularity of the spinal cord reflects an elegant solution by which the nervous system can produce a wide range of movements in a simple and flexible way.

On the other hand, the modularity of the spinal cord observed here might reflect more basic evolutionary pressures. In particular, the modularity of withdrawal reflexes described here might have arisen naturally from the demands of that behavior. The obvious goal of withdrawal reflexes is to remove the skin from the source of a noxious stimulation. In order to meet this demand, the nervous system clearly needs to produce different types of responses depending on the site of stimulation: there is no single motor response which will remove the leg from any arbitrary stimulus. For stimulation sites on a particular limb segment, however, the behavioral strategy required for withdrawal might be similar. One

might therefore expect that the responses evoked from a particular limb segment, such as the foot or the calf, would be similar to one another but different from those produced by stimulation of a different limb segment. This explanation is consistent with the finding in the present set of experiments that the each different type of response appeared to be preferentially evoked from a different segment of the hindlimb. This consistency of the behavioral demand across each limb segment might have lead naturally to the development of a few distinct motor strategies underlying withdrawal reflexes.

These two explanations for the modularity of the spinal cord are not mutually exclusive. It might be that, phylogenetically, this modularity arose because of the behavioral demands of the withdrawal reflexes. When faced with different tasks, however, the nervous system might have taken advantage of this modularity in order to produce more complex movements. The simplification of the control afforded by a modular organization of the spinal cord might have considerable facilitated the production of these more complicated behaviors. Such a subsumption hypothesis of the production of new behaviors, in which new behaviors are produced utilizing preexisting and simpler systems, has been proposed as an efficient method of robot design(Brooks, 1991).

This subsumption hypothesis implies that the basic organization of the spinal cord might be based on the systems underlying phylogenetically older behaviors. If we consider the lamprey as a prototypical vertebrate, the phylogentically oldest behaviors might be locomotion and withdrawal reflexes. Because of their importance in every animal and their need to be as adaptive as possible, it seems reasonable that the organization of the spinal cord is in someway based around the organization of withdrawal reflexes. Understanding the organization of the neural systems underlying withdrawal reflexes might therefore provide insights into how other systems utilize these preexisting systems in order to produce other types of behaviors.

Creating complex movements based on a modular organization of the spinal cord

This last point raises the question of how more complex movements might be created based on these more basic responses. Perhaps the most straightforward means to produce complex movements is by sequencing together these distinct responses. In this scheme, a complex behavior is divided into a number of discrete and separate phases, each of which corresponds to one of the movements produced by a 'module' of the spinal cord. Such a scheme was proposed by Berkinblitt et al. as a way that the complex behavior of scratch reflexes might be created (Berkinblitt et al., 1986; Berkinblitt et al., 1989). They proposed that the scratch reflexes of the frog were composed of the sequential combination of a number of separate, distinct movements. They further identified one of these distinct movements with the flexion withdrawal. A later study demonstrated that this identification was not correct; i.e. that the phase of the scratch reflex was not the same as the flexion withdrawal (Schotland and Rymer, 1993). This scheme is similar to that of Sherrington's proposal that locomotion might be created by the sequential alternation between flexion withdrawal and crossed extension(Sherrington, 1910).

It is important to note that although we observed evidence for a set of distinct spinally generated responses, within each of these responses there was evidence of modulation of individual muscles. This potential for modulation might be exploited by the nervous system in order to adapt more specifically the response with respect to its role in the more complex behavior. For instance, the differences between the muscle activations underlying the placing phase of the frog scratch reflex and those underlying the flexion withdrawal might reflect two different modulations of the same underlying response.

Similarly, the systematic variation in foot position observed for the aiming phase of the scratch reflex might reflect a systematic modulation of an underlying motor response (Giszter et al., 1989; Sergio and Ostry, 1993). This modulation might give the nervous system the potential for more flexibility than that afforded by a set of fixed muscle synergies.

Another way by which more complex movements might be created based on the modularity of the spinal cord is through the simultaneous combination of these distinct responses. This hypothesis is essentially the same as the summation hypothesis described for the frog and rat (Bizzi et al., 1991; Mussa-Ivaldi et al., 1994)and is also similar to Grillner's unit burster hypothesis (Grillner, 1981). In these hypotheses, complex movements are not created by the sequential activation of distinct responses, but by their coactivation. By coactivating multiple responses simultaneously, at different temporal offsets and at different strengths, the nervous system can produce movements other than the few movements actually encoded . Combined with the potential modulation of each response as described above, linear combination can allow the nervous system to produce a wide repertoire of movements.

In this context, it is interesting that the best description of the responses from cutaneous or spinal stimulation allowed for the simultaneous combination of the distinct responses. This model was simply used as a means to describe the responses from either spinal or cutaneous stimulation in a concise representation, but there were some cases observed for which a linear combination of distinct responses appeared to be a good description of the data observed here. It is, of course, difficult to interpret the results of a statistical model in terms of physiology but these results suggest that this type of analysis might provide a way in which to examine whether linear summation does in fact occur during natural behaviors. This possibility is explored in the final chapter of this thesis.

At some point, however, it would seem that the nervous system would need to use systems other than these distinct movements in order to produce a desired movement. These distinct responses might simplify the control of movements, but they also place a limit on the range of movements which the nervous system can produce. By only having a few types of responses available, the range of movements capable of being produced by the nervous system is limited to the space spanned by these few types of responses. If there is no movement which drives the limb to a particular region of the workspace, the nervous system would never be able to reach that region. In order to produce an arbitrary movement, which the nervous system of primates at least is capable of doing, it would appear that the nervous system would need to produce responses which were not a simple linear combination or modulation of only a few types of responses.

Some studies examining the interaction between descending systems and the spinal cord are interesting in this context. First, as was described in the Chapter 1, the work of Schouenborg has described that the regions of the skin from which a muscle is activated are precisely tuned, so that a muscle is activated most from the region of skin that it most effectively removes (Schouenborg and Weng, 1994). This precise tuning, however, is considerably reduced following acute spinalization (Schouenborg et al., 1992) and is not expressed in animals spinalized from birth (Schouenborg et al., 1996). These results suggest that it is only in the presence of descending inputs that the spinal cord produces responses which are specifically adapted to the desired goal. In the absence of these descending inputs, the spinal cord might revert to the production of fixed and nonadaptive responses.

The work of Holmqvist and Lundberg described the effects of a series of CNS lesions on the responses evoked from stimulation of the flexion reflex afferent (FRA) system (Holmqvist and Lundberg, 1961). They found that the weak responses from the FRA

observed in decerebrate animals could change to complete inhibition, or to the excitation of flexors and inhibition of extensor observed in the spinal animal, depending on the site of the lesion. These results suggest that descending systems exert a large control over the state of spinal systems. In particular, it appeared from these experiments that reticulospinal systems exert a tonic inhibitory control over the FRA spinal system. Lundberg also showed that the responses observed from stimulation of the cortex or the red nucleus produce responses which are generally similar to those produced by the FRA when the spinal cord is removed from the rest of the nervous system (Lundberg and Voorhoeve, 1962). These same studies showed that corticospinal or rubrospinal systems can also cause FRA systems in the spinal cord to produce slightly different responses than those produced in the isolated spinal cord. While these studies are difficult to interpret in a behavioral context, they do suggest that descending systems might dramatically mold the organization of the spinal cord in order to produce behavior which is specifically adapted to the demands of the situation. This increasingly detailed control of movements appears to be a trend in the organization of nervous systems through phylogeny (Loeb, 1985). As the behavioral demands of an animal become more complex, there is an increasing need for the motor system to produce behavior which is more specifically adapted to these demands.

References:

Alstermark, B., Kummel, H., Pinter, M. J., and Tantisira, B. (1990). Integration in descending motor pathways controlling the forelimb in the cat. 17. Axonal projection and termination of C3-C4 propriospinal neurones in the C6-Th1 segments. Exp. Brain Res. *81*, 447-461.

Berkinblitt, M. B., Feldman, A. G., and Fukson, O. I. (1986). Adaptability of innate motor patterns and motor control mechanisms. Behavioral and Brain Sciences *9*, 585-638.

Berkinblitt, M. B., Feldman, A. G., and Fukson, O. I. (1989). Wiping reflex in the frog: movement patterns, receptive fields, and blends. In Visuomotor Coordination, J. P. Ewert and M. A. Arbib, eds. (New York: Plenum Press), pp. 615-629.

Bishop, C. M. (1995). Neural Networks for Pattern Recognition (Oxford: Clarendon Press).

Bizzi, E., Mussa-Ivaldi, F. A., and Giszter, S. F. (1991). Computations underlying the execution of movement: a biological perspective. Science *253*, 287-291.

Bras, H., Cavallari, P., Jankowska, E., and Kubin, L. (1989). Morphology of midlumbar interneruons relaying information from group II muscle afferents in the cat spinal cord. J. Comp. Neurol. *290*, 1-15.

Brooks, R. A. (1991). Intelligence without representation. Arificial Intelligence *47*, 139-159.

Brown, A. G. (1981). Organization in the Spinal Cord (Berlin, Heidelberg, New York: Springer-Verlag).

Giszter, S. F., McIntyre, J., and Bizzi, E. (1989). Kinematic strategies and sensorimotor transformations in the wiping movements of frogs. J. Neurophys. *62*, 750-767.

Giszter, S. F., Mussa-Ivaldi, F. A., and Bizzi, E. (1993). Convergent force fields organized in the frog's spinal cord. J. Neurosci. *13*, 467-491.

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods, and fish. In Handbook of Physiology: Sect I. The Nervous System, V. B. Brooks, ed. (Bethesda, MD: American Physiological Society), pp. 1179-1236.

Hartigan, J. A., and Wong, M. A. (1979). A k-means clustering algorithm. Applied Statistics *28*, 100-108.

Hertz, J., Krogh, A., and Palmer, R. G. (1991). Introduction to the Theory of Neural Computation (Redwood City, CA: Addison-Wesley).

Holmqvist, B., and Lundberg, A. (1961). Differential supraspinal control of synaptic actions evoked by volleys in the flexion reflex. Acta Phys. Scand. *54: Suppl.*

Hultborn, H., Illert, M., and Santini, M. (1976). Convergence on interneurons mediating the reciprocal inhibition of motoneurons: Disynaptic inhibition of Ia inhibitory interneurons. Acta Phys. Scand. *96*, 193-201.

Kitazawa, S., Ohki, Y., Sasaki, M., and Xi, M. (1993). Candidate premotor neurons of skin reflex pathways to T1 forelimb motoneurons of the cat. Exp. Brain Res. *95*, 291-307.

Loeb, G. E. (1985). Motoneurone task groups: coping with kinematic heterogeneity. Journal of Experimental Biology *115*, 137-146.

Lundberg, A., and Voorhoeve, P. (1962). Effects from the pyramidal tract on spinal reflex arcs. Acta Phys. Scand. *56*, 201-219.

Moschovakis, A. K., Solodkin, M., and Burke, R. E. (1992). Anatomical and physiological study of interneurons in an oligosynaptic cutaneous reflex pathway in the cat hindlimb. Brain Res. *586*, 311-318.

Mussa-Ivaldi, F. A., Giszter, S. F., and Bizzi, E. (1994). Linear combinations of primitives in vertebrate motor control. Proc. Nat. Acad. Sci. *91*, 7534-7538.

Olshausen, B. A. (1996). Learning linear, sparse, factorial codes. Massachusetts Institute of Technology Artificial Intelligence Laboratory.

Olshausen, B. A., and Field, D. J. (1996). Emergence of simple-cell recpetive field properties by learning a sparse code for natural images. Nature *381*, 607-609.

Saltiel, P., Tresch, M. C., and Bizzi, E. (1996). Modularity of the frog spinal cord motor system. In Society for Neuroscience Abstracts (Washington D.C.

Schotland, J. L., and Rymer, W. Z. (1993). Wipe and flexion reflexes of the frog. I. Kinematic and EMG patterns. J. Neurophys. *69*, 1725-1735.

Schouenborg, J., Holmberg, H., Luo, X.-L., and Levinsson, A. (1996). Adult rats spinalized at birth exhibit inappropriate withdrawal reflexes. Soc. Neurosci. Abstr. *22*.

Schouenborg, J., Holmberg, H., and Weng, H.-R. (1992). Functional organization of the nociceptive withdrawal reflexes. II. Changes of excitability and receptive fields after spinalization in the rat. Exp. Brain Res. *90*, 469-478.

Schouenborg, J., and Weng, H.-R. (1994). Sensorimotor transformations in a spinal motor system. Exp. Brain Res. *100*, 170-174.

Sergio, L. E., and Ostry, D. J. (1993). Three-dimensional kinematic analysis of frog hindlimb movement in reflex wiping. Exp. Brain Res. *94*, 53-64.

Sherrington, C. S. (1910). Flexion-reflex of the limb, crossed extension reflex and reflex stepping and standing. J. Physiol. *40*, 28-121.

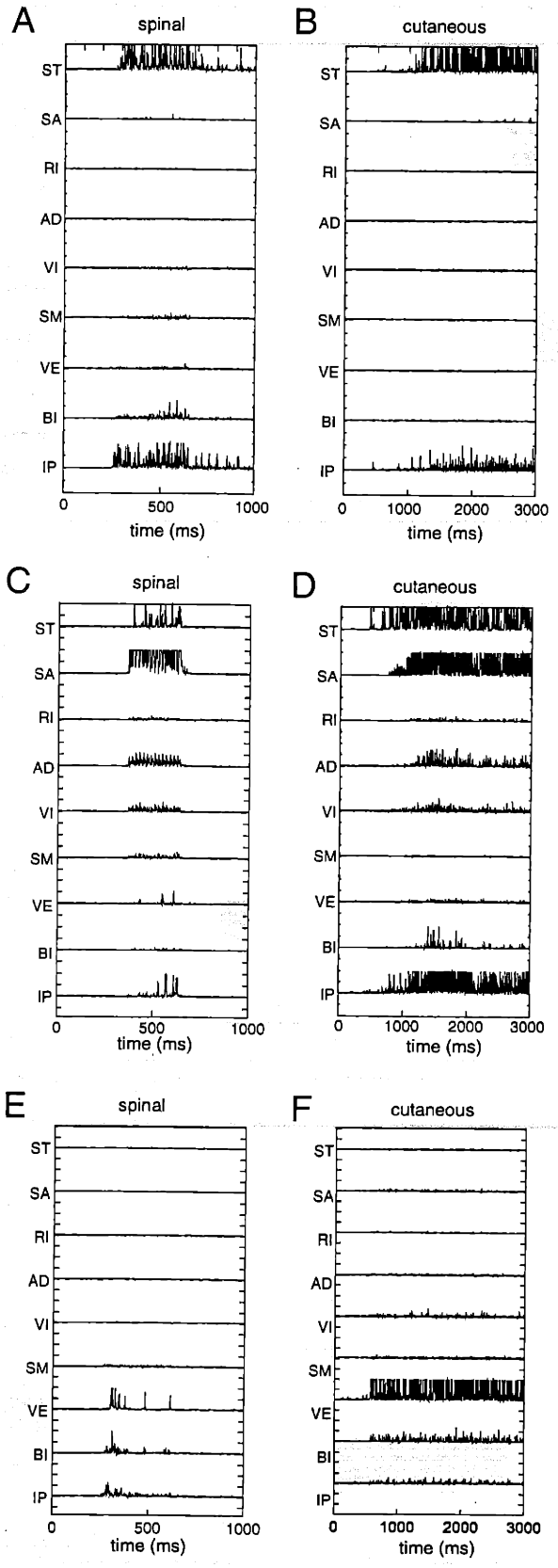

Figure 1. Examples of good correspondence between spinal and cutaneous responses. (A) shows a response evoked from microstimulation of the spinal cord at a site with a receptive field on the back of the metatarsus. (B) shows a response evoked from cutaneous stimulation of the same region of the hindlimb. (C) shows a response evoked from stimulation of a spinal site with a receptive field on the front of the calf near the knee and (D) shows the corresponding response from cutaneous stimulation. (E) shows a response from stimulation of a spinal site with a receptive field on the back of the calf and (F) shows the corresponding response from cutaneous stimulation.

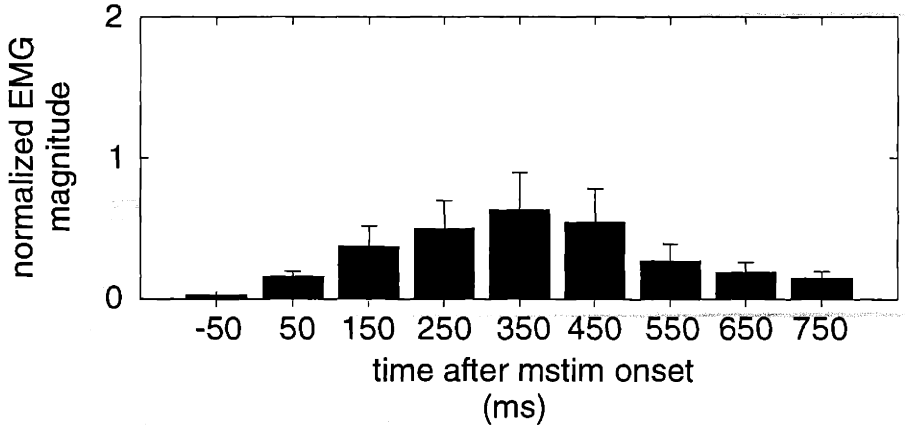

Figure 2. The magnitude of EMG response as a function of time following the onset of spinal stimulation. Each bar represents the mean magnitude of all responses observed within one animal. Magnitudes were taken as the length of the vector of muscle activations, with each muscle normalized to its maximum value observed. Error bars represent one standard deviation away from the mean.

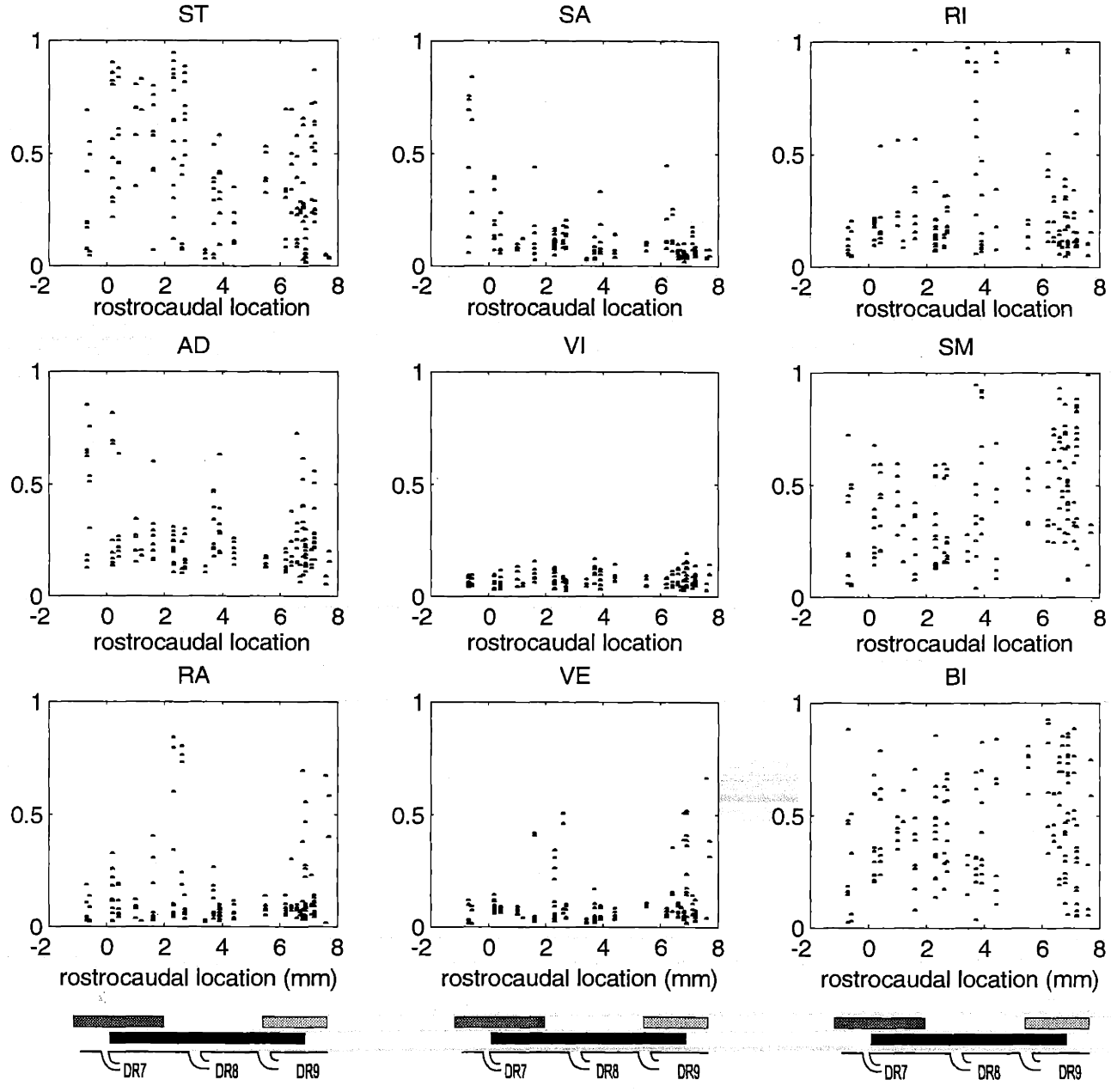

Figure 3. Muscle activations from different rostrocaudal regions of the spinal cord. The normalized activation level of each muscle is plotted against the rostrocaudal location in the spinal cord from which each response was evoked. Each muscle was normalized to its maximal value and then each response was then normalized to be of unit magnitude. Each data point shown here therefore reflects the relative contribution of each muscle to one response. On the bottom of the figure is shown a schematic of the spinal cord of this animal, showing the locations of the dorsal roots. The shaded bars at the bottom of the figure represent the boundaries of the sensory representation of the front of the leg (dark gray), the foot (black), and the back of the leg (light gray) obtained from recording the receptive field of spinal sites.

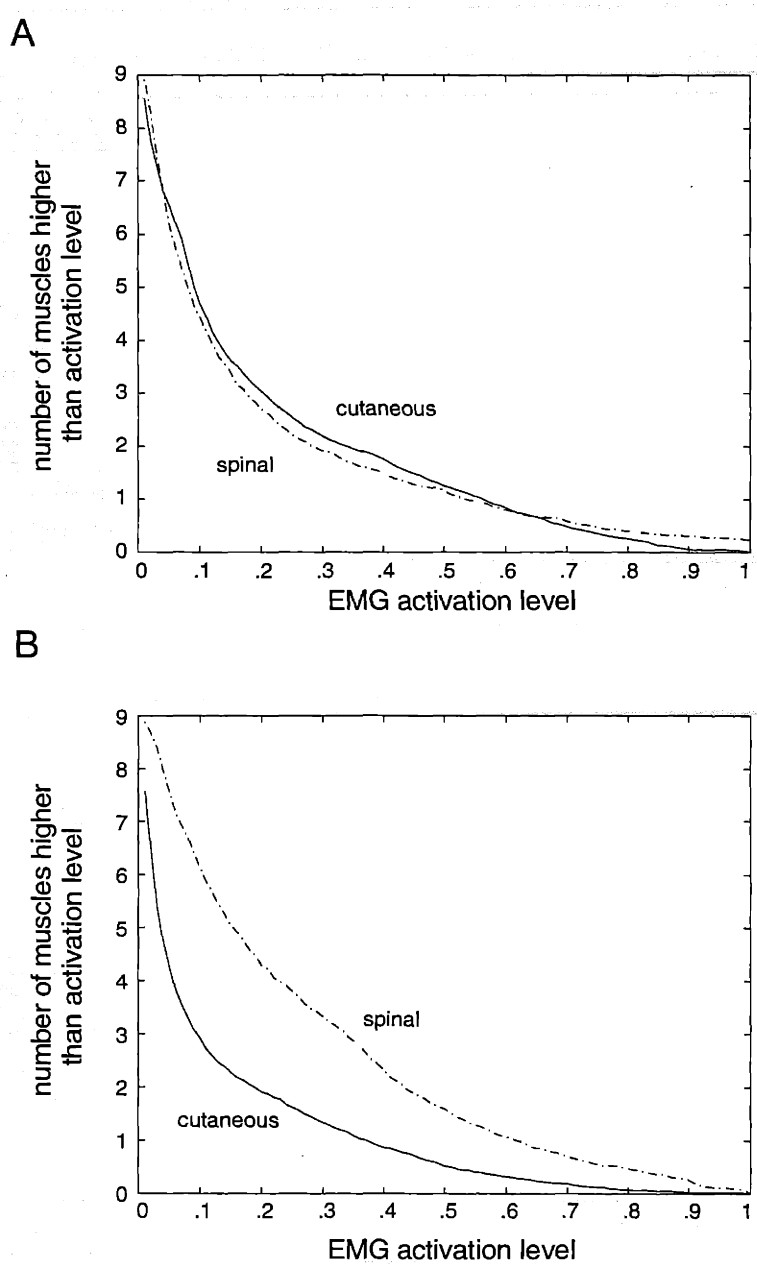

Figure 4. The number of muscles activated in spinal and cutaneous responses. Two animals are shown here, in (A) and (B). The curved lines indicate the average number of muscles activated above a certain level in a response from either cutaneous or spinal stimulation. The further a curve is to the right, the more muscles which are activated in the responses.

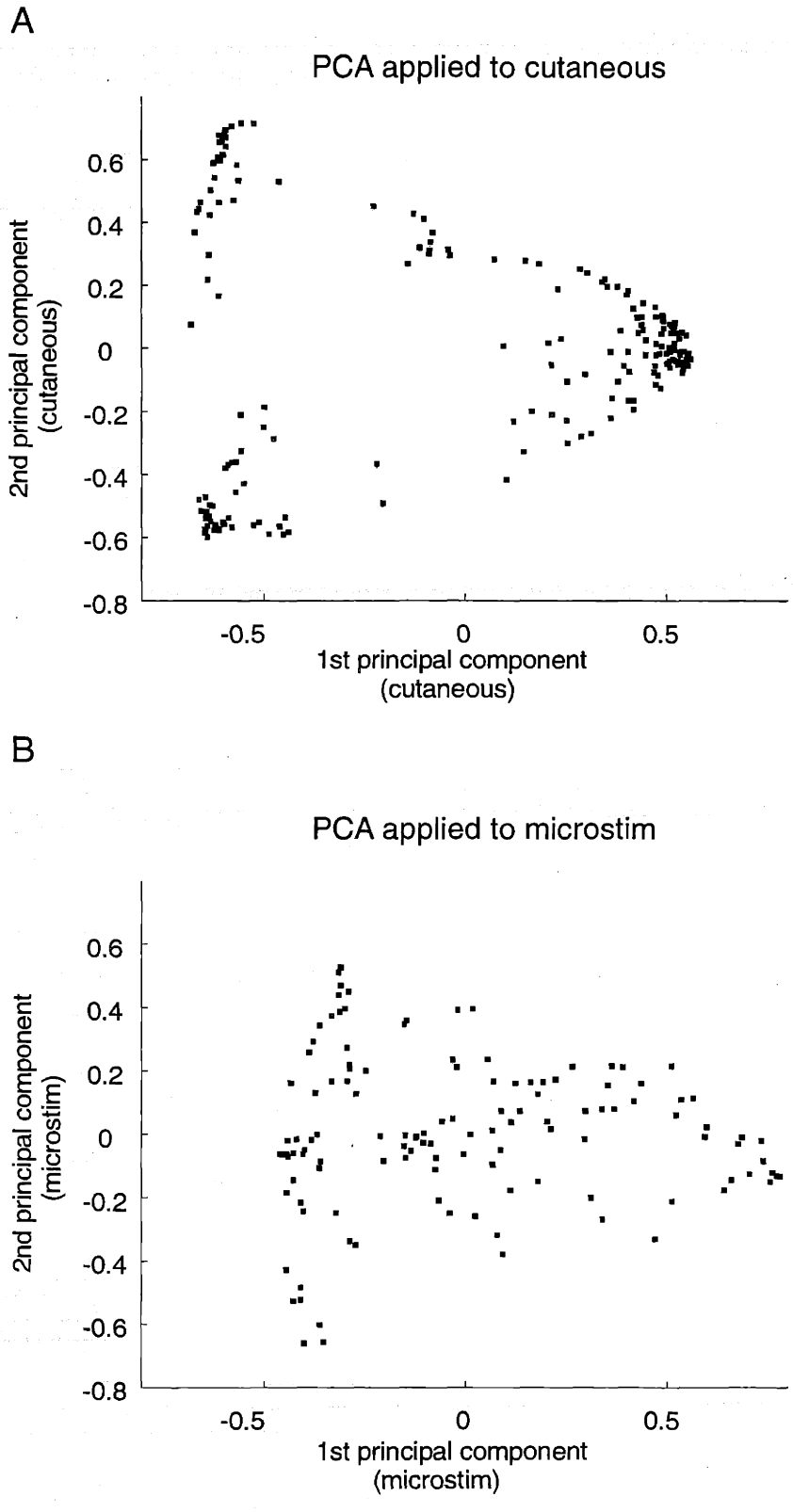

Figure 5. Principal component analysis of responses from cutaneous stimulation (A) and spinal stimulation (B). Responses were projected onto the first two principal components found separately for each data set.

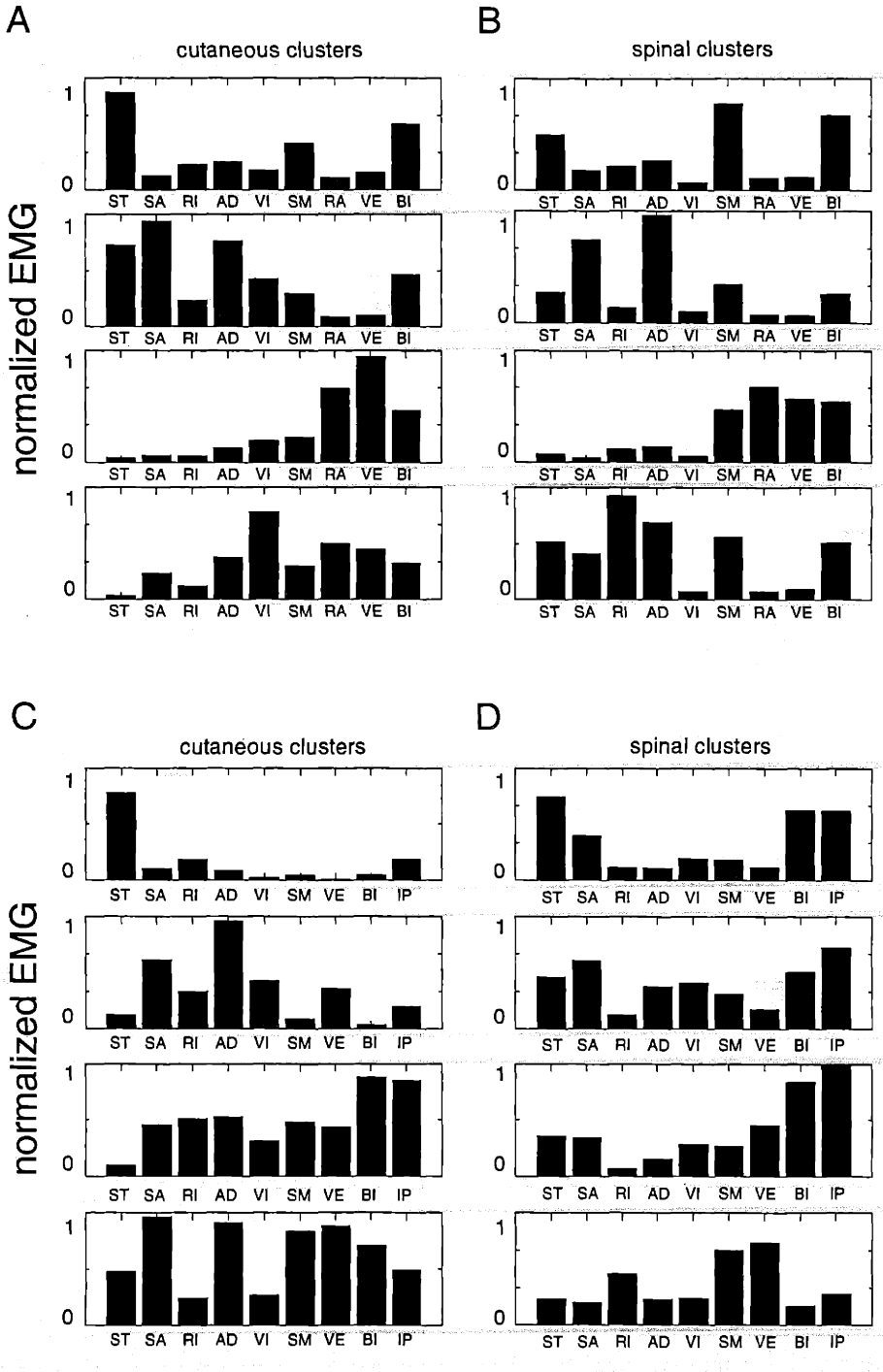

Figure 6. K-means clustering for spinal and cutaneous responses. The k-means algorithm was applied to the responses from cutaneous and spinal stimulation separately. The clusters found for one animal are shown in (A) and (B) and for another animal in (C) and (D). Each bar chart represents the centers of each of the clusters found using k-means. The height of each bar indicates the mean value of each muscle within that cluster.

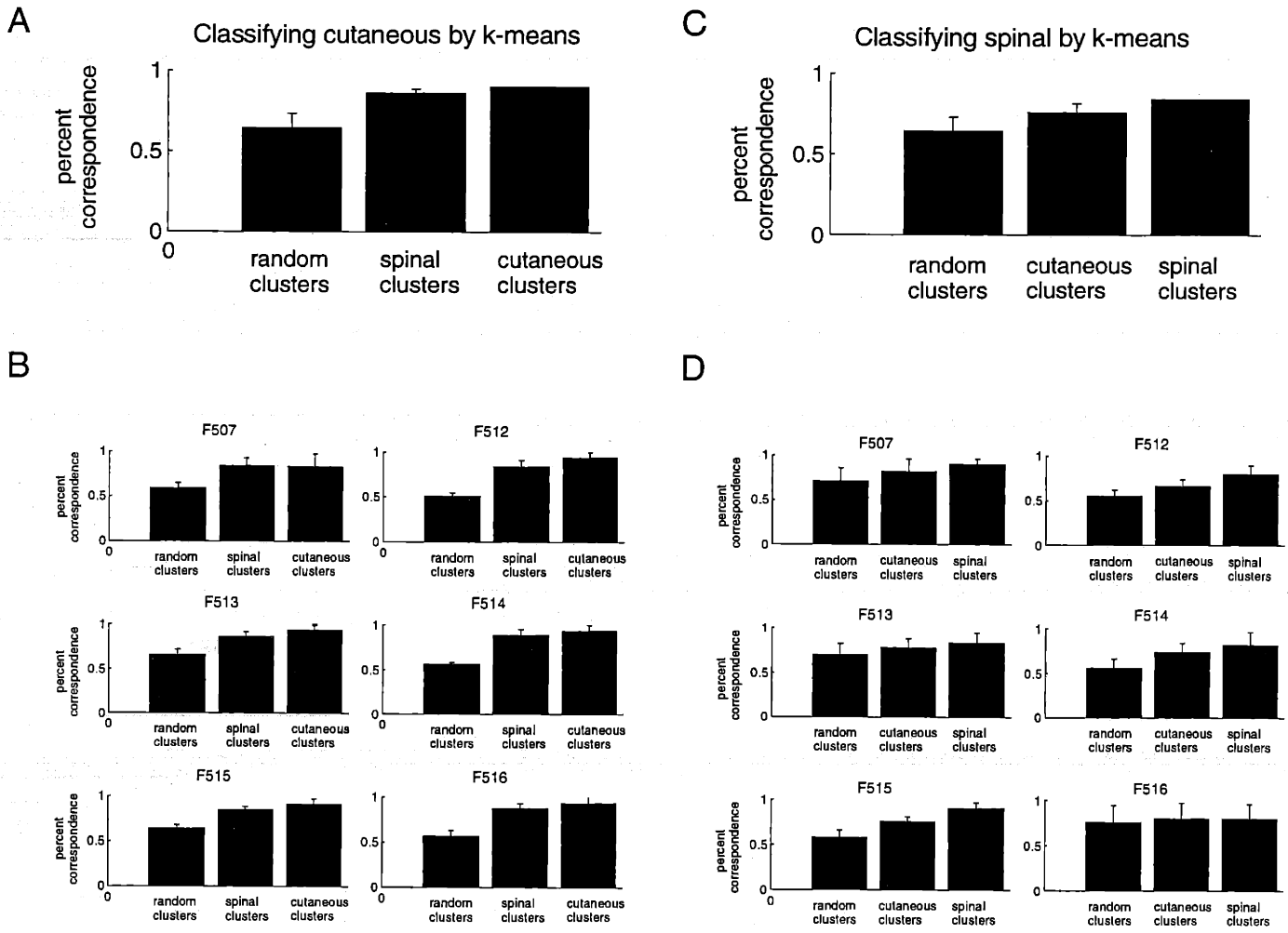

Figure 7. The correspondence between classification of one set of responses by the other. (A) and (C) show the results of the classification of cutaneous and spinal responses, respectively. In (A), the bar labeled 'cutaneous clusters' indicates the amount of correspondence between the classification of cutaneous responses obtained from two different sets of k-means clusters derived from the cutaneous responses themselves. These k-means clusters could vary since they were derived from different subsets of the data (see Methods). The bar labeled 'spinal clusters' indicates the amount of correspondence between the classification of cutaneous responses based on a set of k-means clusters derived from the cutaneous responses to the classification based on a set of k-means clusters derived from the spinal responses. The bar labeled 'random clusters' indicates the amount of correspondence expected by chance. (A) shows these values averaged across all animals. (B) shows these values for each individual animal. (C) and (D) show the analogous analyses for the responses from spinal stimulation.

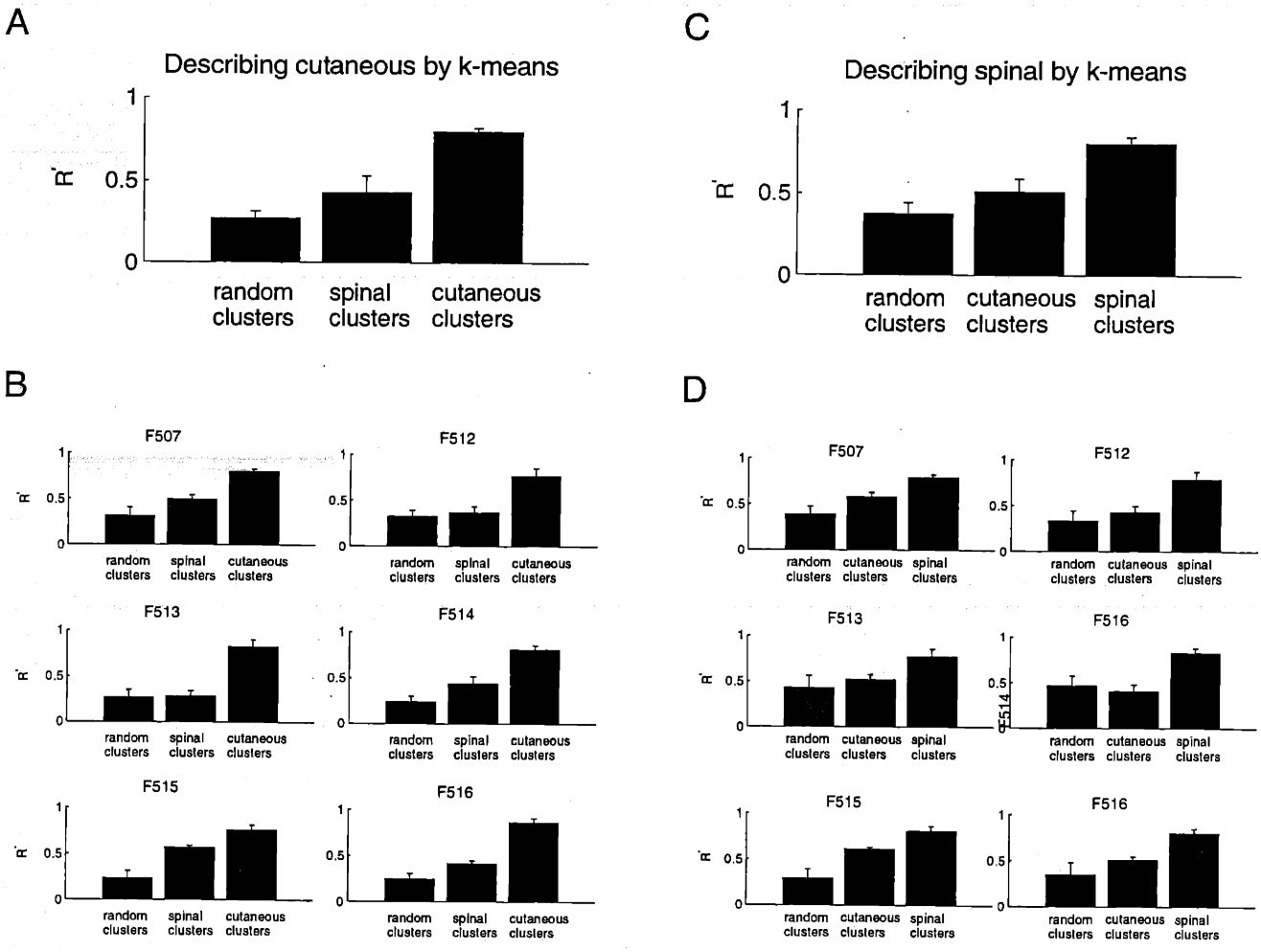

Figure 8. The amount of variance explained by k-means clustering from one set of responses applied to the other. (A) and (C) show the analyses for cutaneous and spinal responses, respectively. In (A), the bar labeled 'spinal clusters' indicates the amount of variance explained by applying the k-means clusters obtained from spinal responses to the cutaneous responses. The bar labeled 'cutaneous clusters' indicates the amount of variance explained by the applying the k-means clusters obtained from a subset (90%) of cutaneous responses to the responses not used (10%) to actually find the k-means clusters. The bar labeled 'random clusters' indicates the amount of variance explained by clusters chosen randomly. (A) shows these values averaged across all animals. (B) shows these values for each individual animal. (C) and (D) show the analogous analyses for spinal responses.

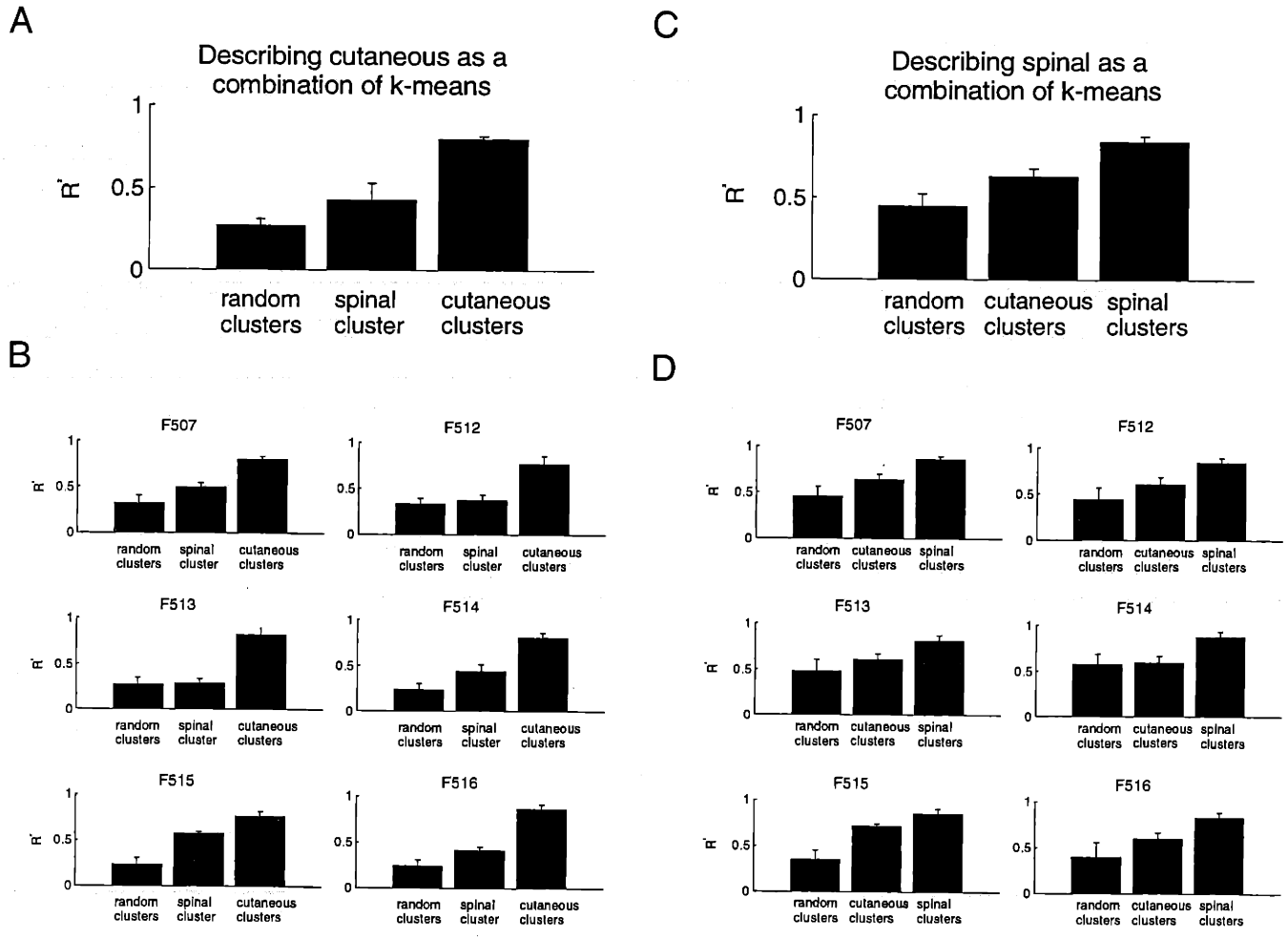

Figure 9. The amount of variance explained by fitting one set of responses to a linear combination of k-means clusters from the other. (A) and (C) show the analyses for cutaneous and spinal responses, respectively. In (A), the bar labeled 'spinal clusters' indicates the amount of variance explained by fitting the cutaneous responses as a non-negative linear combination of the k-means clusters found from the spinal responses. The bar labeled 'cutaneous clusters' indicates the amount variance explained by fitting a subset (10%) of cutaneous responses as a linear combination of the k-means clusters found from the remaining subset (90%) of cutaneous responses. The bar labeled 'random clusters' indicates the amount of variance explained by a linear combination of clusters chosen at random. (A) shows these values averaged across all animals. (B) shows these values for each individual animal. (C) and (D) show the analogous analyses for spinal responses.

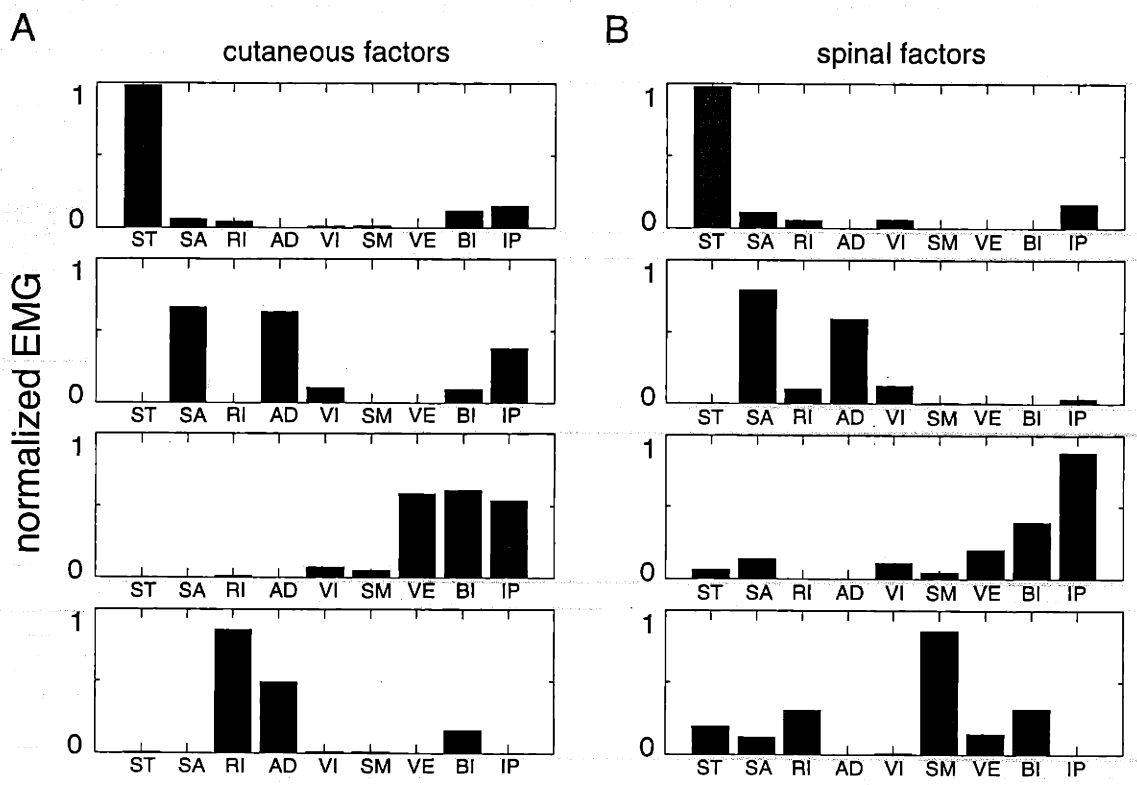

Figure 10. An example of the sets of EMG weighting coefficients, or 'factors', found by the gradient descent algorithm described in the text applied to the cutaneous responses (A) and the spinal responses (B) in one animal.

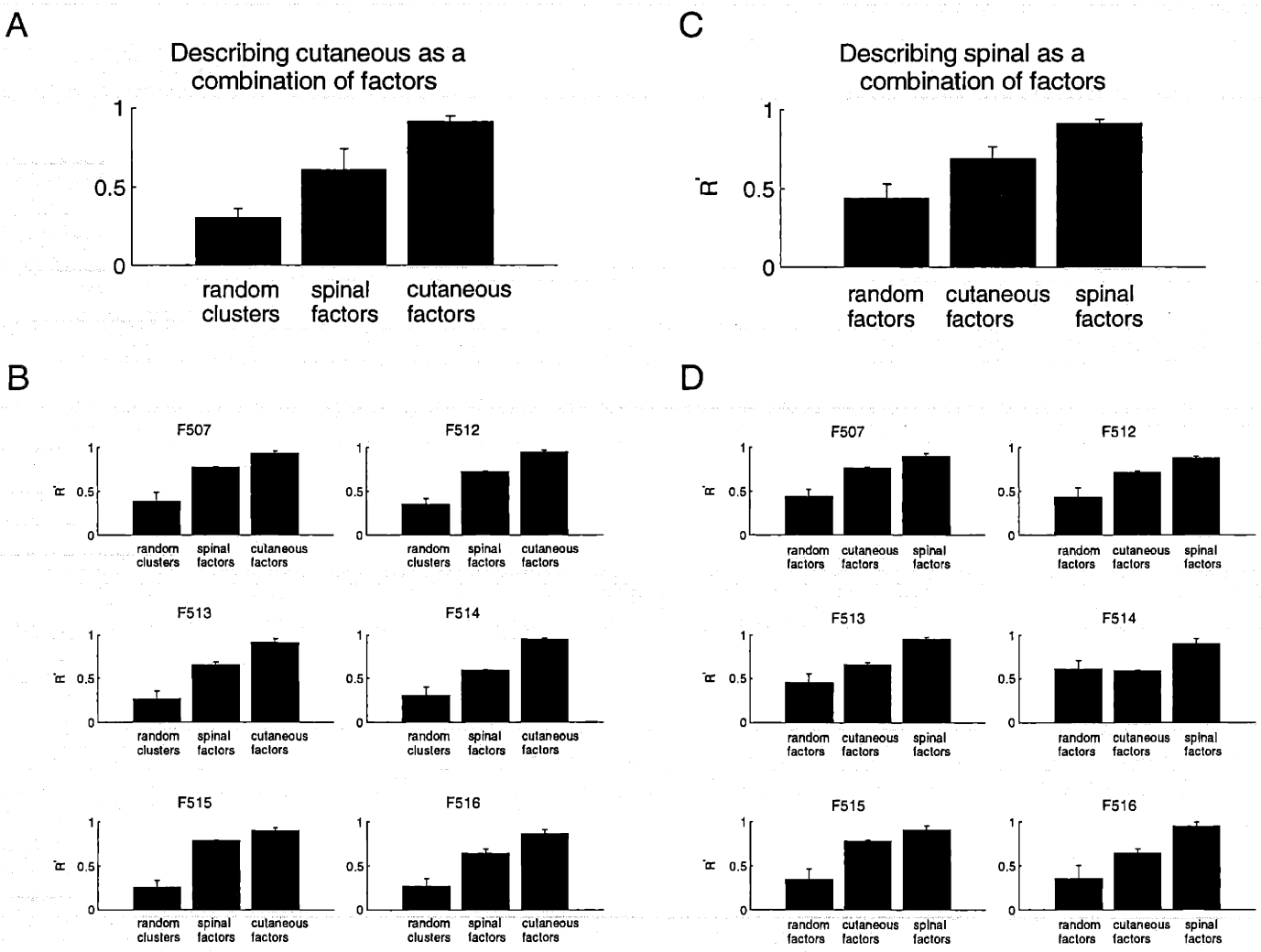

Figure 11. The amount of variance explained by fitting one set of responses to a linear combination of 'factors' found from applying the gradient descent algorithm (see Methods) to the other set of data. (A) and (C) show the analyses for cutaneous and spinal responses, respectively. In (A), the bar labeled 'spinal factors' indicates the amount of variance explained by fitting the cutaneous responses to a non-negative linear combination of the factors found from spinal responses. The bar labeled 'cutaneous factors' indicates the amount of variance explained by fitting a subset (10%) of cutaneous responses to a linear combination of factors found from the remaining subset of the responses (90%). The bar labeled 'random factors' indicates the amount of variance explained by a linear combination of factors chosen randomly. (A) shows these values averaged across all animals. (B) shows these values for each individual animal. (C) and (D) show the analogous analyses for spinal responses.

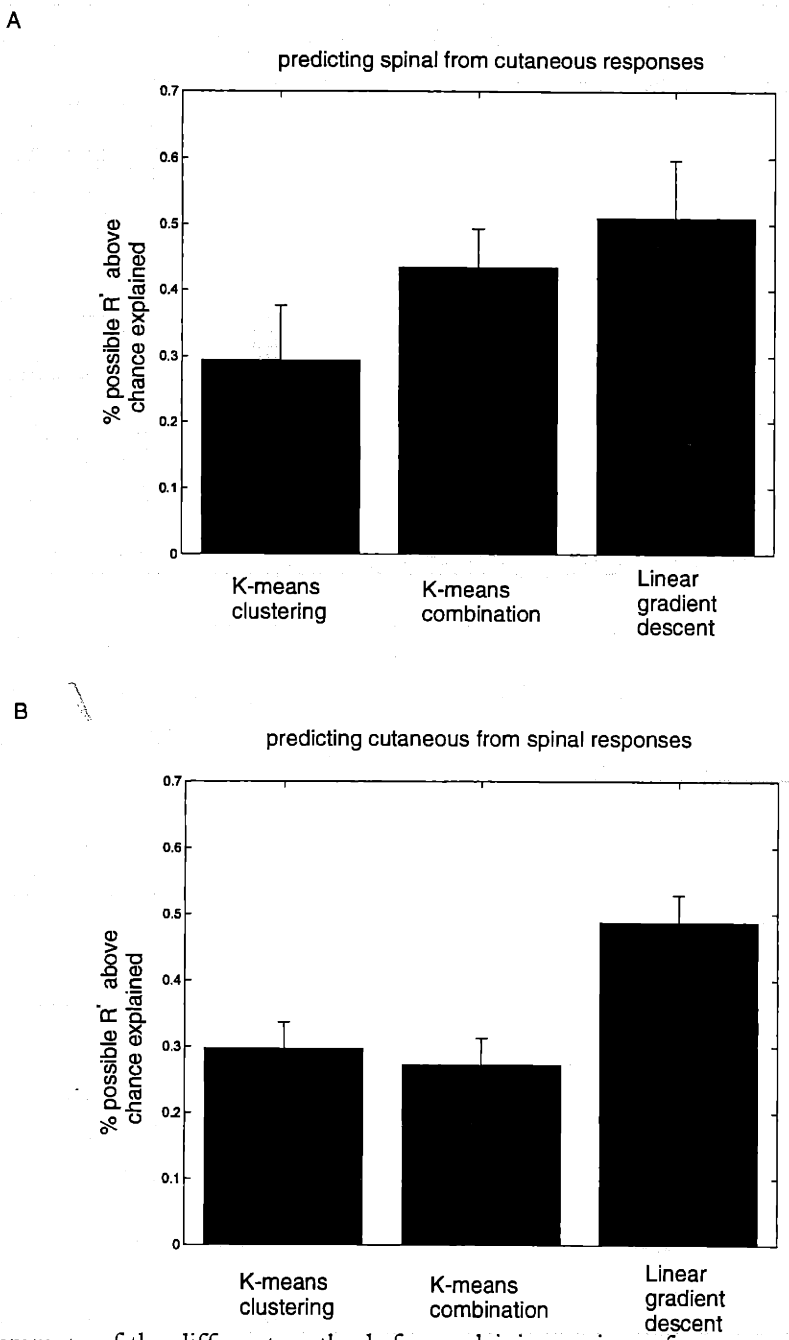

Figure 12. Summary of the different methods for explaining variance from one set of responses to another. Each bar indicates the fraction of explainable variance above chance actually explained by the model. In (A), we examine this fraction for the responses from spinal stimulation. For the bar labeled 'k-means clustering' we subtracted the amount of explained variance expected by chance from the amount of variance explained by the spinal clusters and from the amount of variance explained by the cutaneous clusters themselves. We then found the ratio of the variance expected above chance for the spinal clusters to the variance expected above chance for the cutaneous clusters. This ratio expresses how close the clusters from spinal responses are to explaining the same amount of variance in the cutaneous responses as the cutaneous clusters. A value of 1 for this ratio indicates that the two clusters explain the same amount of variance, a value of 0 indicates that the spinal clusters explain no more variance than expected by chance. The other two bars in (A) indicate the results of the same summary for the linear combination of k-means clusters and for the linear combination of factors. (B) shows the analogous results for the responses from spinal stimulation.

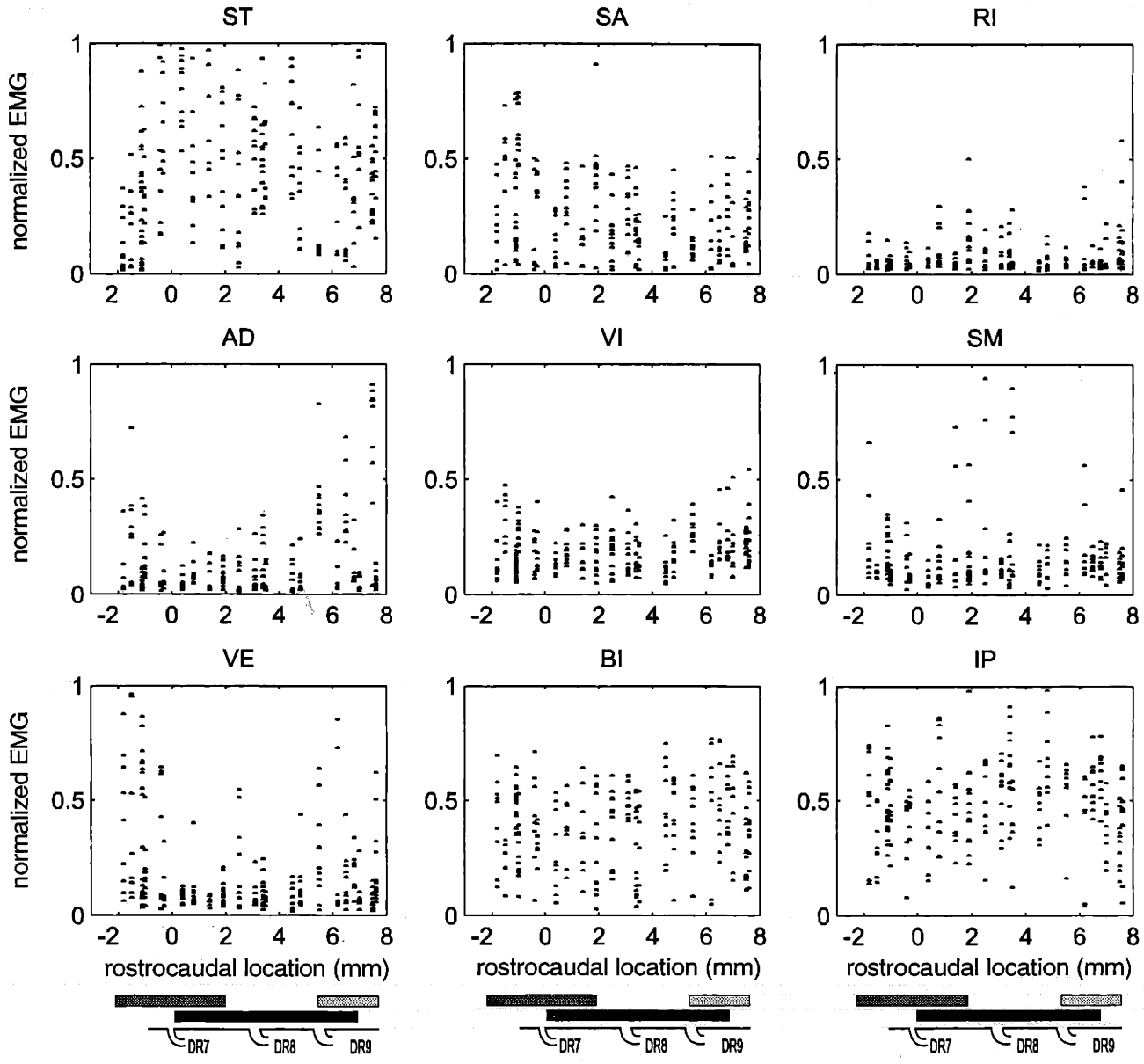

Figure 13. The rostrocaudal distribution of responses from spinal stimulation. Conventions same as Figure 2.

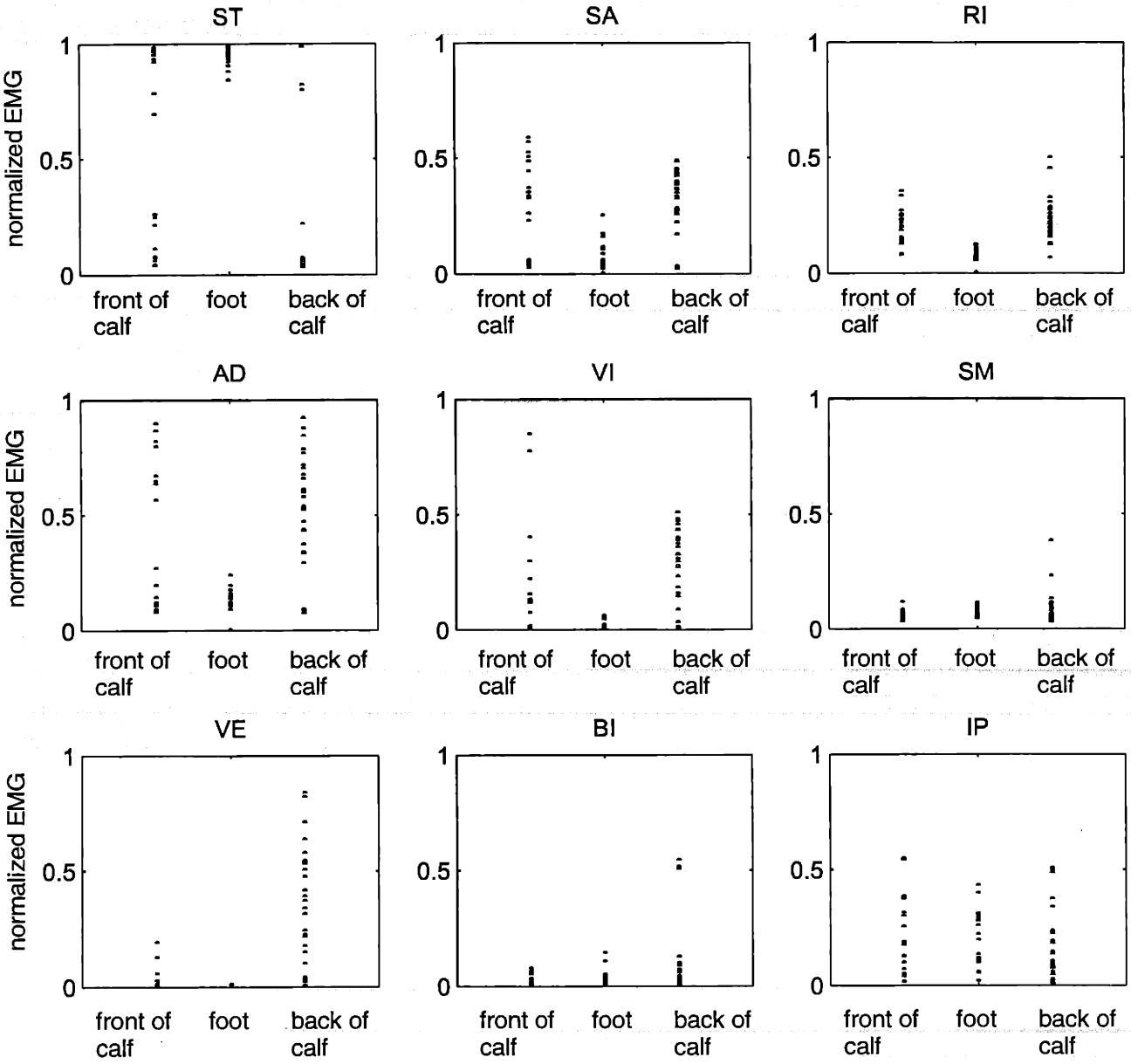

Figure 14. The responses from cutaneous stimulation in the same animal shown in Figure 13. In this animal, not every site of the hindlimb was stimulated so sites were divided into three main categories as suggested by the results of Chapter 3: the back of the calf, the foot, and the front of the calf.

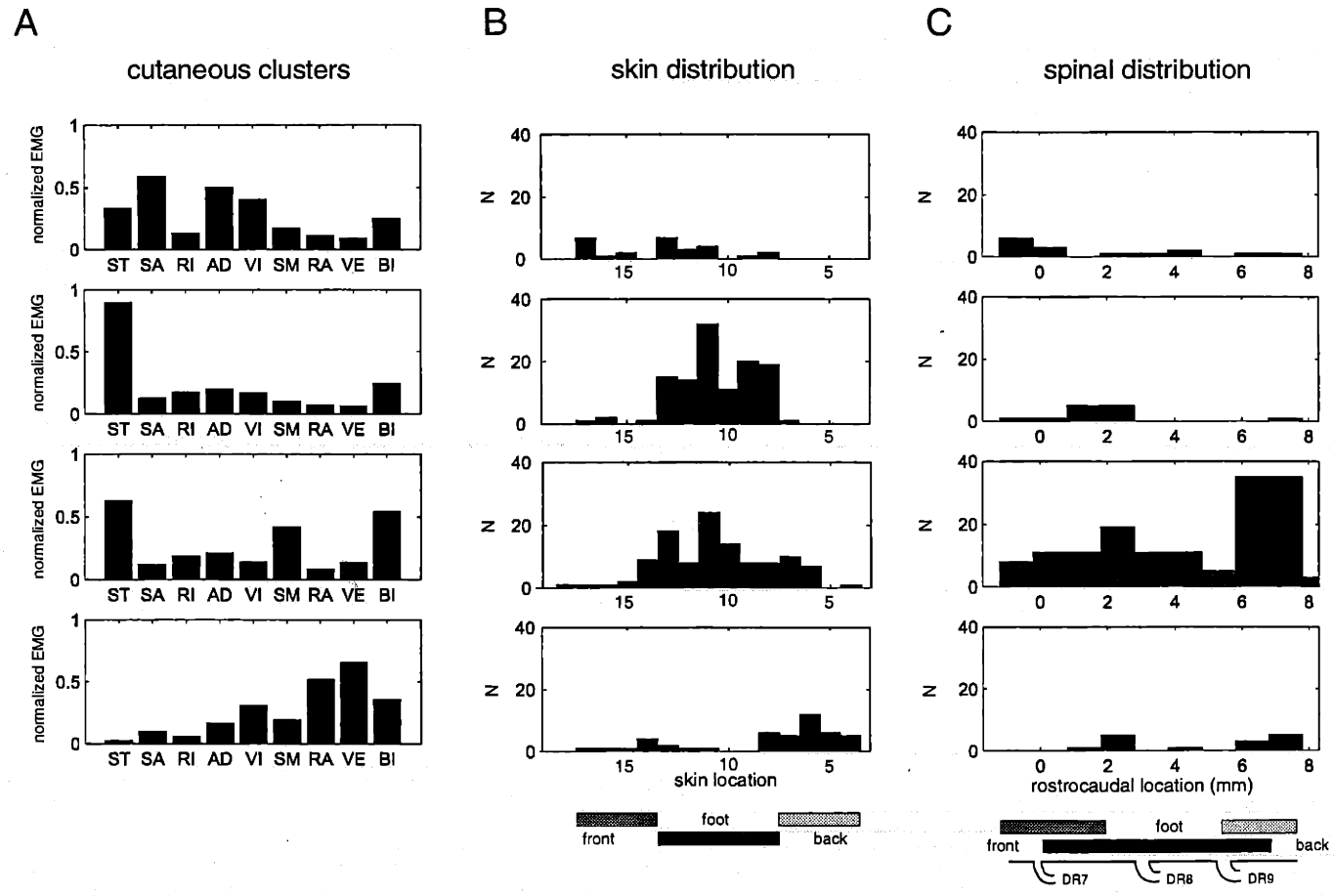

Figure 15. Comparison of the distribution of responses from different regions of the leg to the distribution of different rostrocaudal regions of the spinal cord. (A) shows the clusters found by applying k-means to the cutaneous responses. Conventions are the same as for Figure 6. (B) shows the frequency of these clusters evoked from stimulation of the skin in the same animal. Note that the x-axis is shown in decreasing order so that the skin locations correspond to the somatotopy of the spinal cord. The schematic at the bottom of this figure indicates the different regions of the skin surface. (C) shows the frequency of responses classified as each of these clusters from different regions of the spinal cord. The schematic at the bottom indicates the somatotopical organization of the spinal cord found from unit recording at the site of stimulation.

	Number of responses	
Animal	cutaneous	spinal
F507	192	326
F512	69	251
F513	128	118
F514	202	112
F515	323	155
F516	372	225

Table 1. The number of responses for each animal evoked from the two types of stimulation. These are responses with magnitude above one standard deviation of the distribution of all response magnitudes.

Chapter 5: Summation of muscle synergies underlying withdrawal reflexes in the spinalized frog

Introduction

The results of the previous chapters demonstrated a number of similarities between the responses from cutaneous and spinal stimulation. For both means of stimulation, only a few types of responses were observed. The types of responses observed in spinal and cutaneous stimulation were similar to one another, as assessed both qualitatively and quantitatively. The two sets of responses could also be related to one another by the somatotopy of the spinal cord: spinal sites with a receptive field on a region on of the skin tended to produce responses which were similar to those produced from stimulating that region of the skin. All of these results suggest that the features described for the organization of responses from spinal microstimulation could also be observed in the responses from cutaneous stimulation.

One feature of the responses from spinal microstimulation which has not been addressed, however, is the summation of different responses. Simultaneous stimulation of multiple sites within the spinal cord results in a response which is a simple combination of the response evoked from individual stimulation of each site (Mussa-Ivaldi et al., 1994). This simple combination is observed for both the forces and for the muscle activations produced from costimulation (Galagan et al., 1997). This ability of responses from spinal stimulation to combine flexibly has been suggested as a mechanism by which the nervous system can produce a wide range of movements through the specification of a small number of different types of responses (Bizzi et al., 1991). This mechanism of summation, however, has not been observed in behaviors produced by the nervous system in a physiological context.

In this regard, it is interesting that of the methods for describing responses examined in the previous chapter, the best method allowed the responses from either cutaneous or spinal stimulation to combine together in a means similar to that described for the costimulation of sites within the spinal cord. This method provided the best explanation of the set of responses evoked from one means of stimulation by the other. The observation that cutaneous responses were better described with this method suggested that there might be evidence of flexible combination of basic muscle activation patterns in these responses as well. The observation in Chapter 3 that there was not a sharp distinction between responses from the foot and from the front of the calf also suggested that there might be a combination of responses across this region of the skin surface. In the present chapter, we examine these possibilities more directly.

Methods

All procedures used here were as described previously. Cutaneous responses in muscles from different regions of the ipsilateral hindlimb (shown in Figure 1) were averaged for each response and each muscle was normalized to its maximal value. The gradient descent algorithm described in the previous chapter was then applied to these responses as explained previously. The patterns of muscle activations, or factors, found by the algorithm were then examined with regard to how they were utilized to produce the observed responses.

Results

We show again the variation of muscle activation patterns across the skin surface for two different animals in Figure 2. In both animals, there was a sharp transition between responses evoked from the back of the calf and the foot, as was described in Chapter 3. The transition between responses evoked from the foot and from the front of the calf, however, was different for

the two animals. In the animal shown in Figure 2A, this transition was sharp, as can be seen by comparing the activation of ST and AD across this transition. In the animal shown in Figure 2B, this transition appeared to be more gradual, with the activation of AD gradually increasing from the foot to the front of the knee. Figure 3 shows the average correlation of each muscle's activation to the stimulation site (Fig. 3A) and the average slope of the regression of each muscle's activation to stimulation site (Fig. 3B). These analyses were both performed on only the responses evoked from stimulation of the foot and the front of the calf (sites 8 through 18) since muscle activations appeared to change gradually across these regions. In both analyses, it can be seen that the activation of SA, AD, VI, VE, and IP each increased as the stimulation site approached the front of the calf while the activation of ST and BI decreased. These results were reported in Chapter 3.

We then examined whether the activation of these muscles covaried within responses irrespective of which stimulation site produced the response. To this end, we found a set of muscle activation weightings which could be combined together in order to produce the responses observed from cutaneous stimulation. We found these weightings, or factors, using the gradient descent algorithm described in the previous section. This algorithm assumes only that the responses are a non-negative combination of a smaller number of muscle activation patterns and that the coefficients of this combination were uniformly distributed. These factors represent a set of muscles which tend to vary with one another between different responses.

In Figure 4 we show an example of how this algorithm described responses from cutaneous stimulation. Figure 4A shows a series of five responses evoked from stimulation of a site in the middle of the front of the calf (site 15). Figure 4B shows these same five responses averaged from the onset to the offset of the responses and with each muscle normalized to the maximal value observed for that muscle. These five responses, although generally similar, were not identical.

Figure 4C shows the set of factors found by the gradient descent algorithm. Figure 4D shows the how these factors were combined in order to describe the responses shown in Figures 4A and 4B. In general, the best fit predicted responses captured much of the variation within the responses actually observed. There were deviations, such as the underestimation of the activation of SA, but overall the basic features of the muscle activations were well described.

The good quality of these fits was confirmed by the large amount of variability in the set of responses that these fits explained. Table 1 shows the R^2 values obtained for the 90% of the data used to actually find the set of factors and the R^2 values obtained for the 10% of data not used to find these factors. The lack of difference between these R^2 values in each animal suggests that these factors were capturing general features of the responses. This ability to generalize to new data, along with the high R^2 values, together suggest that this model described the responses from cutaneous stimulation observed here well.

In Figure 5 we show the sets of factors found for three other animals. In each of these animals, we found three very similar types of factors (see also Figure 4C). These three factors were observed in the responses of every animal we studied. The first factor usually consisted of a large weighting of ST which was usually activated alone or with either BI or IP. The second factor consisted of activation of SA and AD with either VI or IP or of all four muscles. The third factor consisted of activation mainly of VE, sometimes along with VI or along with BI and IP. There could be variations between animals in the exact composition of each of the factors, but overall each factor was consistent between different animals. The fourth factor differed between animals although certain patterns were also observed more than once. In particular, two animals showed a factor with coactivation of RI and SM, and two animals showed a factor with coactivation of BI and IP.

The consistency between different animals was also suggested by examining the contribution of each of these factors from different parts of the skin. The average contribution of each factor to

the responses evoked from different stimulation sites is shown for three animals in Figure 6. The order of these distributions corresponds to the order of the factors shown in Figure 5. In each animal, the factor with activation of ST was activated the highest for sites on the foot, but fell off for sites on the front or back of the calf. The factor with activation of SA and AD was activated highest for sites on the front of the calf, but fell off for sites on the foot. The factor with activation of VE was activated highest for sites on the back of the calf and very little from sites on the foot while it was activated weakly from the front of the calf. As would be expected from the data shown in Figure 2, the transition from activation of this VE factor to the ST factor was very sharp in all animals examined. The transition from activation of the ST factor to activation of the SA and AD factor was more gradual in the animals shown in Figure 6A and 6B. This latter transition was much sharper in the animal shown in Figure 6C. The animals shown in Figure 6B and 6C are the same as those shown in Figures 2A and 2B, respectively. The factors found by the algorithm for these two animals are shown in Figure 5B and 4C, respectively. The similarity between these factors is striking given that one animal recruited each factor primarily in isolation while the other animal recruited them primarily in combination.

Discussion

These results suggest that withdrawal reflexes in the frog are created from the combination of a small number of muscle activation patterns. These muscle activation patterns were similar between different animals and were recruited in a similar manner from different regions of the skin surface. Further, these muscle activation patterns could be combined together simultaneously in order to produce different types of responses. The capability of these patterns to combine flexibly appeared to be exploited in different ways by different animals, with some animals producing responses which were usually a combination of patterns while other animals produced responses which were usually one of the patterns in isolation. These results therefore support the hypotheses reviewed in the first chapter claiming that complex behaviors can be created from the combination of a small number of muscle activation patterns (Grillner, 1981; Sherrington, 1910).

These results were based on the results obtained from a particular statistical model. Interpreting the results of such a statistical analysis in physiological terms is always difficult. We believe that such an interpretation is warranted in the present study by of the ability of this model to generalize to new data, by the large amount of variance that this model explained, and by the overall consistency of the results it produced between different animals. The differences which were observed could be due to differences between individual animals or between differences in pickup of muscle activity by the implanted electrodes. These similarities were found even in animals which did not usually produce the patterns in isolation of one another, such as the animal shown in Figure 2B. Each of these results suggests that the algorithm was capturing consistent patterns underlying the production of withdrawal reflexes in the spinalized frog. This algorithm, or ones like it, might therefore be of general use in the study of the composition of behaviors by a small number of muscle activation patterns.

We believe that the results of the present study support the notion of distinct patterns of muscle activations within the spinal cord. In principle, the patterns of covariation extracted by the algorithm we used here could result from the independent and parallel specification of synergistic muscles to the stimulation site: a group of muscles with similar mechanical actions will tend to be recruited in a similar manner for a given task. Such a pattern of covariation of muscle activations has in fact been shown for postural stabilization in the cat (Jacobs and Macpherson, 1996). This covariation of synergists could result from the independent control of muscles just as easily as from the control of distinct muscle groups.

There were several observations in the present study which argue that these patterns of muscle activations reflect the control of distinct muscle groups. First, the set of muscles included within a pattern did not strictly consist of the activation of functional synergists. For instance, the factor with activation of SA and AD often also had activation of VI and/or IP. These muscles, although not strict antagonists, are clearly not agonists either: in particular, SA and AD both produce knee flexion while VI produces knee extension. Second, muscle activations within a pattern covaried even for responses evoked from stimulation of the same site. In the responses illustrated in Figure 4A, for instance, the responses from the same region of the skin could be described as a combination of the same set of factors, but with different weightings of combination for each response. If the patterns were created from the specification of synergistic muscles independent of one another, then stimulation of the same location of the skin should produce independent variation of the different muscles. The observation that muscle activation patterns covaried with one another even at the same stimulation site strongly suggests that this set of muscles was controlled as a distinct entity.

It should be noted, however, that two of the factors consisted mainly of the strong activation of a single muscle, one with strong ST, and the other with strong VE. There could be other associated muscles, but generally these muscles dominated the activation pattern. The results from Chapter 3 suggest that these two patterns are activated in exclusion of one another and are therefore distinct. We therefore consider them as distinct 'patterns' of muscle activations, but the 'pattern' is mainly of a single muscle. Such a pattern is clearly not what one would immediately expect from the notion of a muscle synergy.

Although the factors underlying the withdrawal reflexes in different animals were generally similar to one another, they appeared to be utilized in slightly different ways. All animals tended progressively to recruit the factor with SA and AD as the stimulation site approached the front of the knee. The progression of this recruitment, however, could differ dramatically between different animals. For example, the animal shown in Figure 6C recruited this factor almost entirely in opposition to the factor with ST so that only a few sites produced a coactivation of the two factors. The animals shown in Figure 6A and 6B, however, recruited the factor with SA and AD systematically as the stimulation was moved from the foot toward the knee so that almost all responses consisted of the simultaneous combination of multiple factors. This differential control of a similar set of underlying factors between different animals is consistent with the notion that basic patterns of muscle activations organized within the spinal cord can be used flexibly in order to create a range of different movements. These results therefore provide evidence that the mechanism of summation shown for responses from spinal microstimulation can be used in a physiological manner during the production of behaviors.

Finally, it is interesting that these basic patterns were observed in withdrawal reflexes. In many ways, withdrawal reflexes can be considered as one of the fundamental behaviors produced by all vertebrates, fundamental both in the phylogenetic and behaviorally relevant sense. Because withdrawal reflexes are critical for survival, it likely that they were one of the behaviors first developed by the nervous system, along with other basic behaviors. Withdrawal reflexes are also behaviorally demanding in that they require the nervous system to respond quickly and to be optimally adaptive to the environment. Such pressures are not as immediately obvious for other basic behaviors for survival. This fundamental position of withdrawal reflexes might suggest that much of the organization of spinal systems is based around withdrawal reflexes. Other behaviors developed subsequently might then take advantage of these preexisting systems in order to simplify their creation. In this context, it will be interesting to examine whether more complex behaviors, such as scratch reflexes or those produced by descending systems can utilize the same patterns of muscle activations observed here within the withdrawal reflexes.

Summary and conclusions

The experiments described in this thesis have found that in both the frog and the rat, movements produced from microstimulation of the spinal cord are closely related to the organization of spinal cutaneous systems. It also appeared that the withdrawal reflexes produced by the isolated spinal cord were organized into a small number of distinct patterns of muscle activations. These patterns were organized across a group of muscles; i.e. each response could be described as a muscle synergy. Within each pattern, however, there were significant modulations of individual muscles, suggesting that these patterns were not fixed balances of muscles but could be altered. The responses from spinal stimulation were related to these patterns of muscle activations underlying withdrawal reflexes. Finally, it appeared that the summation of responses observed for spinal microstimulation could also be observed in the responses from cutaneous stimulation.

These results imply that the stimulation of the spinal cord activated systems involved in the production of withdrawal reflexes. This connection to a particular behavioral system helps place the responses from spinal stimulation in a physiological context, in which their contribution to more complex behaviors can be assessed. This connection, however, also makes the interpretation of results from spinal stimulation more problematic and determining the extent to which the responses from spinal stimulation and from cutaneous stimulation are activating the spinal cord in the same way will be important in order to establish in future work.

References

Bizzi, E., Mussa-Ivaldi, F. A., and Giszter, S. F. (1991). Computations underlying the execution of movement: a biological perspective. Science *253*, 287-291.

Galagan, J. E., Lemay, M., Loeb, E. P., and Bizzi, E. (1997). EMG activity during vector summation of spinal force field primitives. Soc. Neurosci. Abstr. *23*.

Grillner, S. (1981). Control of locomotion in bipeds, tetrapods, and fish. In Handbook of Physiology: Sect I. The Nervous System, V. B. Brooks, ed. (Bethesda, MD: American Physiological Society), pp. 1179-1236.

Jacobs, R., and Macpherson, J. M. (1996). Two functional muscle groupings during postural equilibrium tasks in standing cats. J. Neurophys. *76*, 2402-2411.

Mussa-Ivaldi, F. A., Giszter, S. F., and Bizzi, E. (1994). Linear combinations of primitives in vertebrate motor control. Proc. Nat. Acad. Sci. *91*, 7534-7538.

Sherrington, C. S. (1910). Flexion-reflex of the limb, crossed extension reflex and reflex stepping and standing. J. Physiol. *40*, 28-121.

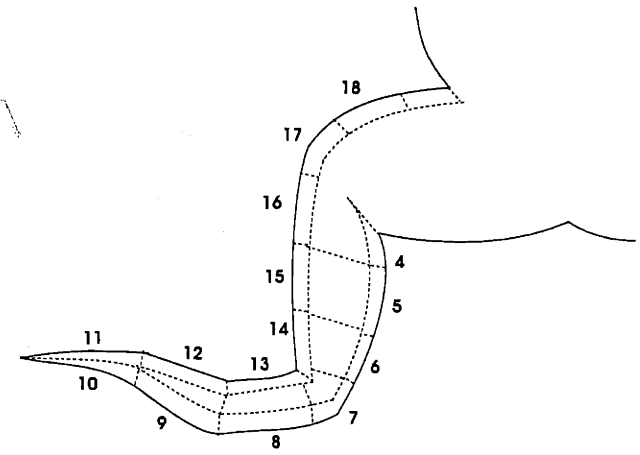

Figure 1. The stimulation sites from which responses were evoked. The left leg is shown from a dorsal aspect.

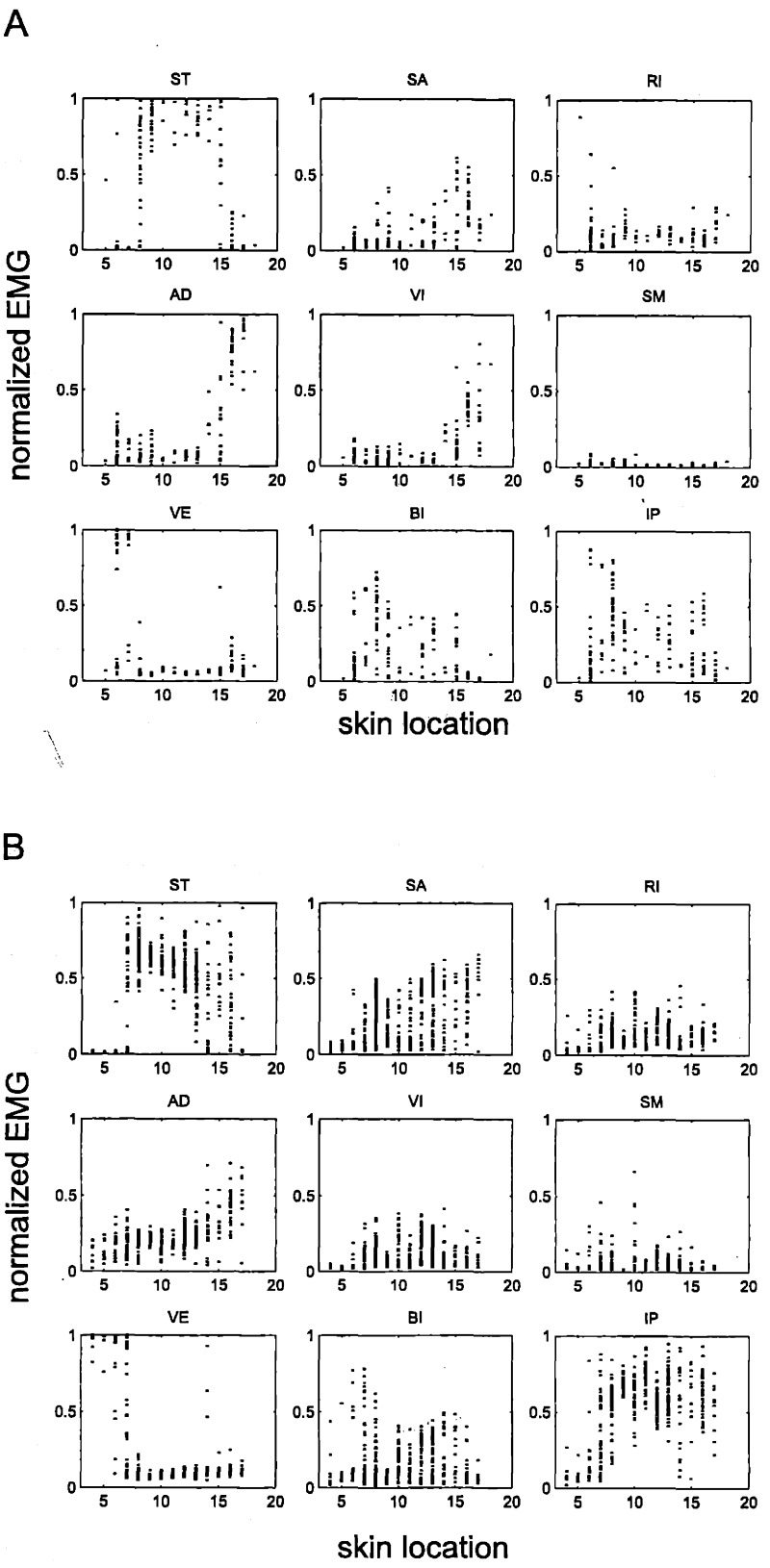

Figure 2. Variation of muscle activations with stimulus location for two animals. The EMG activity in nine muscles was recorded and averaged for each evoked response. Each muscle was normalized to the peak value observed for that muscle in any trial of stimulation, including stimulation of the contralateral ankle, back, or forelimb. The values shown here were then normalized so that each response was of unit magnitude. These values therefore represent the fraction that each muscle contributed to the observed response. Skin locations refer to the numbering shown in Figure 1.

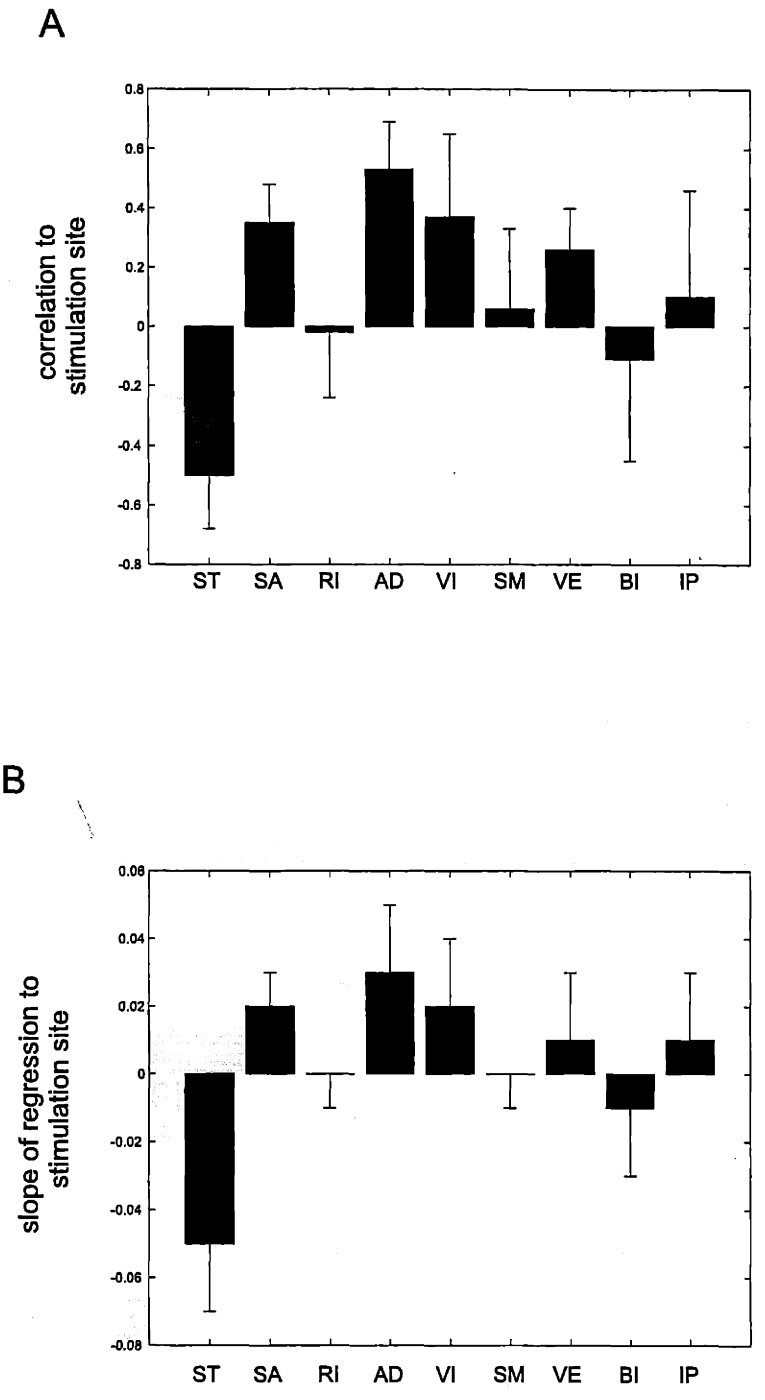

Figure 3. The normalized activation of each muscle was regressed and correlated to the skin location. Only responses evoked from regions of the foot or front of calf (sites 8 through 18) were used for this analysis. (A) shows the mean slopes for the linear regression. Note that the small slopes were a result of differences between the magnitudes of the range of the muscle activation and skin location, respectively. These values are the average slope observed across all animals. Error bars represent one standard deviation from the mean. (B) shows the mean correlation coefficient of each muscle to stimulus location.

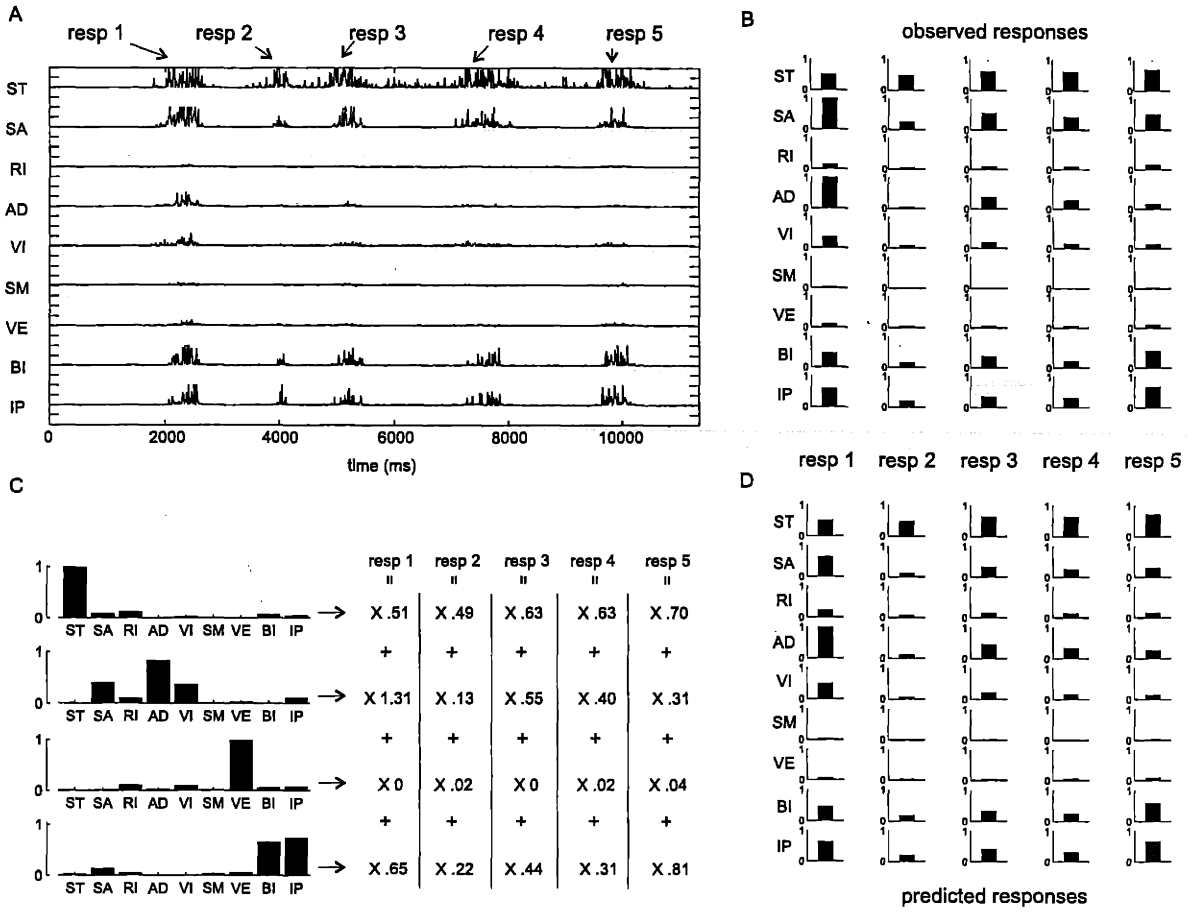

Figure 4. An example of the linear combination of factors. (A) shows an example of responses evoked from stimulation of the same site of the hindlimb in the middle of the front of the calf (site 15). Five responses were evoked, each with a different balance of muscle activations. (B) shows the activation of each muscle, averaged from the onset to the offset of the response and with each muscle normalized to its maximal value. Note that although AD and VI are activated at a similar level for the first response in the raw data shown in (A), this level is near the maximal value observed for AD while this is less than half of the maximal value observed for VI. This difference is reflected in the difference between the level of these two muscles in the normalized activity shown in (B). In (C) we show the results obtained from the gradient descent algorithm described in the text. The factors shown to the left were found by the algorithm applied to the entire set of responses evoked from stimulation of the foot and calf. To the right of these factors are shown the weighting coefficient of each of these factors which will give the best fit to the responses observed in (A) and (B). For example, the first response is composed of .51 of the first factor, 1.31 of the second factor, 0 of the third factor, and .65 of the fourth factor. The patterns of muscle activations predicted from these combinations are shown in (D) for each response.

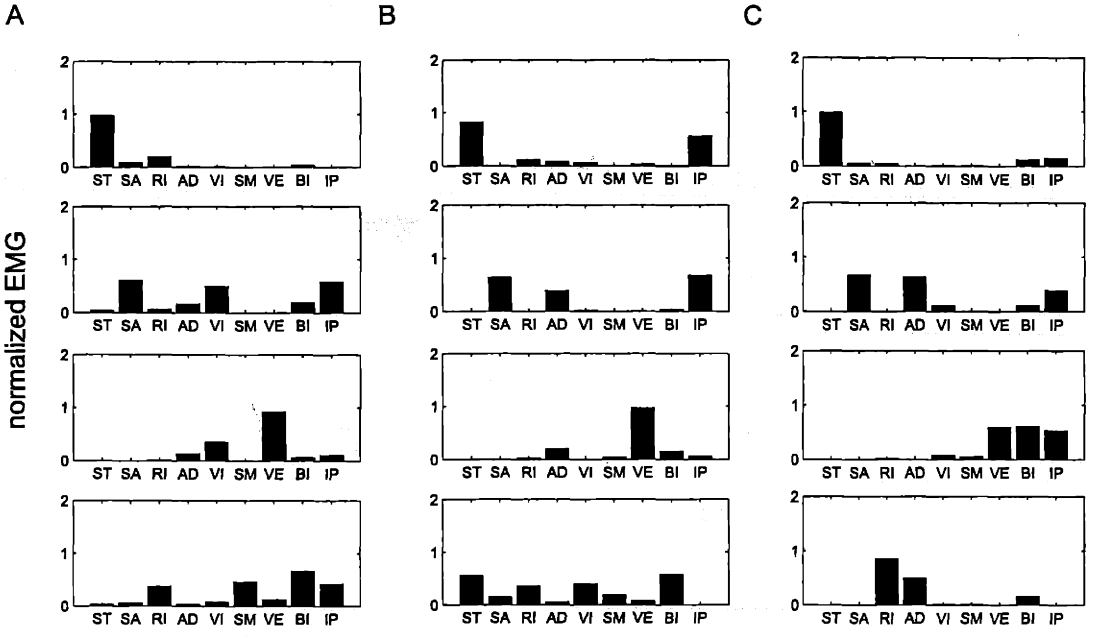

Figure 5. Three examples of the factors found by the algorithm for different animals. The factors were arranged so that similar factors are shown next to one another. Compare also these factors to those obtained for the animal shown in Figure 4C.

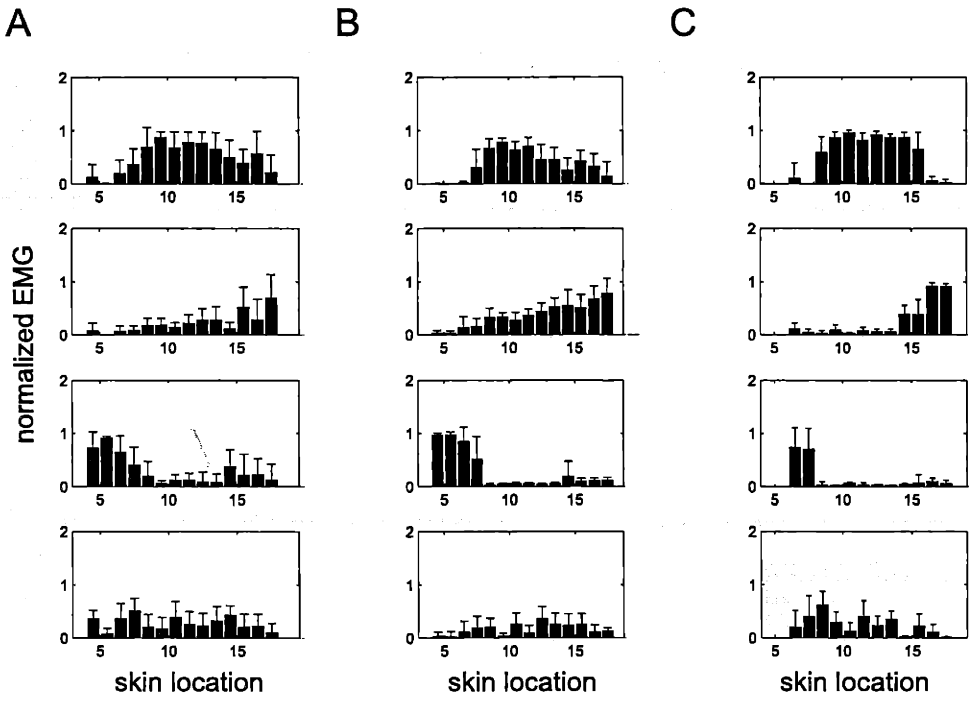

Figure 6. The strength of contribution of each factor in the responses evoked from different parts of the skin surface. The order of factors shown here corresponds to the order shown in Figure 5. Each observed response was fit to a combination of factors as shown in Figure 4. At each location, the weighting coefficients for each factor were averaged. These coefficients were found for the responses normalized to be unit magnitude. The mean of these coefficients is indicated by the height of each of the bars shown here. Error bars represent one standard deviation of the means of these coefficients. Skin locations represent the numbering scheme illustrated in Figure 1.

	Rsq		
Animal	To fit data	To test data	
F507	0.94	0.93	n.s.
F508	0.93	0.94	n.s.
F509	0.96	0.96	n.s.
F511	0.91	0.91	n.s.
F514	0.94	0.95	n.s.
F515	0.9	0.9	n.s.
F516	0.86	0.86	n.s.
Avg	0.92	0.92	

Table 1. The R squared values describing the amount of variance explained for each set of responses by the model found by the gradient descent algorithm. Each R2 value represents the mean R2 value for 10 repetitions of the algorithm for 10 different subsets of the set of responses. Each iteration was fit to 90% of the data, chosen randomly. The mean R2 value to this fit data is shown in left hand column. The mean R2 value of this model describing how well it described the 10% of the data which was not used to fit the model is shown in the right hand column. This ability of a given model to describe the 10% test data describes how well the model generalizes to new data. The significance of the difference between these R2 values was assessed using a bootstrap statistic (500 steps) for each animal.

ACKNOWLEDGEMENTS

There are many people who have been more than patient with my idiocy and I name but a few of them here. First, I must of course thank my advisor, Emilio Bizzi. As I shifted between research topics and interests from one meeting to the next, he was always supportive and gave me every opportunity that I could ask for. The range of topics that I was exposed to while in his lab was unique and has given me an appreciation for many different aspects of the field. His grace and ease will always serve as models to which I will aspire but never acheive. The group of fellow first years, and I'll always think of us as such, have been a huge part of my graduate career and I hate to imagine what my time here would have been like without them. Chris Moore is an incredibly nice guy and his ability to find positive sides of any situation always astounds me, especially when I'm acting in my typically cynical and negative manner. Brad Postle is similarly an incredibly nice guy and this is all the more noteworthy since he manages to combine it with a healthy dose of cynicism and skepticism. His ability to combine hard work with a rich personal and intellectual life has always been something to strive for. Stephen Gilbert is also incredibly nice – so nice in fact that I really don't believe it. Of all the people having to put with my idiocy, perhaps Raj Sheel had to put up with the most and I can only thank him for the interactions we did have during our first years here. I-han Chou similarly had to put up with an incredible litany of foolishness from me and I look back in gratitude at her patience and understanding. Turning to the lab, I would like to thank Judy Schotland who served as outside member on my thesis committee and as a constant reminder that one doesn't need to sacrifice integrity and sanity in order to survive in the field. I thank Simon Giszter for his support during my first months (years) spent getting used to graduate school and for his continued support subsequently – and for showing that it's possible to shift between being grownup and being completely absurd at the mere mention of frogs. I also thank Sandro Mussa-Ivaldi for showing me that one can be generous and selfless and humble while still being successful and entirely sincere – a combination that I still can't understand how to acheive. Eric Loeb should be thanked for his insanity and fundamental good nature and for telling me enough wild theories of politics and neuroscience that I began being able to deal rationally with ideas which make no absoltuely sense to me, an ability which is very helpful in this field – I now appreciate him for the Zen master that he is. I thank Philippe Saltiel for many great conversations of both a personal and intellectual nature – I enjoyed the time we spent and regret my lack of patience which prevented me from spending more. Thanks to Andrea D'Avella for being intense enough about the research to convince me that it might actually be worth while and to James Galagan for being lax enough to convince me that it's not and to both for being easy targets in Doom. I promised myself I'd only spend one page on this, so in the interests of space: to all else in lab and in the department and in outside life who can't be enumerated here and with whom I've interacted and annoyed, thanks. Finally, I of course must thank my parents, sister, and brother for their support and blind faith in me, irrespective of the stupidest things that I've done at various stages in my life.