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RESEARCH ARTICLE

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Responses to spinal microstimulation in the chronically spinalized rat and their relationship to spinal systems activated by low threshold cutaneous stimulation

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Abstract We describe the responses evoked by microstimulation of interneuronal regions of the spinal cord in unanesthetized rats chronically spinalized at T10–T12. One to three weeks after spinalization, sites in the lumbar spinal cord were stimulated using trains of low current microstimulation. The isometric force produced by stimulation of a spinal site was measured at the ankle. Responses were reliably observed from stimulation of a region within the first 1250 μm from the dorsal surface of the spinal cord. These responses were clearly not due to direct motoneuronal activation and were maintained after chronic deafferentation. The force evoked by microstimulation and measured at the ankle varied smoothly across the workspace. Simultaneous stimulation of two sites in the spinal cord produced a response that was a simple linear summation of the responses evoked from each of the sites alone. Microstimulation generally produced a highly non-uniform distribution of response directions, biased toward responses which pulled the limb toward the body. Within these distributions there appeared to be two main types of responses. These different types of responses were preferentially evoked by microstimulation of different rostrocaudal regions of the spinal cord. This anatomical organization paralleled the spinal cutaneous somatotopy, as assessed by recording cutaneous receptive fields of neurons at sites to which the microstimulation was applied. This relationship was maintained after chronic deafferentation. The findings described here in the rat spinal cord in large part replicate those previously described in the frog.

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Introduction

A recent set of experiments has described the organization of motor responses evoked by microstimulation of the spinal cord of the frog (Bizzi et al. 1991; Giszter et al. 1993; Mussa-Ivaldi et al. 1994). These experiments showed that stimulation of the interneuronal regions of the frog lumbar spinal cord evoked only one of a few different types of responses. Responses other than these few types, however, could be produced when stimulation was applied to two sites simultaneously, and the resulting response was a simple combination of the response evoked from each site separately. Further, it appeared that these different types of motor responses were preferentially evoked by stimulation of different anatomical regions of the spinal cord. These results suggested that the movements organized within the spinal cord could be exploited in a simple manner in order to create a wide range of movement.

For several reasons, however, these results raised a number of questions. First, because these results were described for the frog, their generalization to other animals, especially mammals, is difficult: although there are many similarities between the organization of the frog spinal cord and the spinal cord of other vertebrates, there are also significant differences (Simpson 1976). Second, the movements evoked by microstimulation are difficult to interpret since their physiological relevance has not been established. Microstimulation is a good method for characterizing the movements evoked after activation of different regions of the nervous system in a controlled, systematic, and reproducible manner. However, in order to be able to interpret the results obtained with microstimulation of the nervous system, it is clearly helpful to find a physiological correlate to the organization of these movements. For instance, the movements evoked by microstimulation of sites within the cat motor cortex

(Asanuma et al. 1968) and of the primate superior colliculus (Schiller and Stryker 1972) could be systematically related to the sensory receptive field of neurons at the same site. These relationships suggested that the responses from microstimulation reflected aspects of the physiological organization of these systems.

Because of these concerns, the present study examines the organization of movements within the spinal cord of a mammal: the rat. The first issue we examine here is whether the results described previously for the frog spinal cord can be generalized to the spinal cord of a mammal. The second issue we examine is whether the organization of responses evoked by spinal microstimulation can be related to known aspects of the physiological organization of the spinal cord. In particular, we examine whether there is a relationship between the response evoked by microstimulation of a site in the spinal cord and the sensory receptive field of neurons at that site.

Preliminary results from these experiments have been reported in abstract form (Tresch and Bizzi 1995, 1996).

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 MIT.

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.) and methylprednisolone (35 mg/kg i.m.) (Behrmann et al. 1994) and prepared for aseptic surgery. The spinal cord was exposed by dorsal laminectomy of T8, the dura was opened, the cord was infiltrated with lidocaine, the dorsal spinal vessel was cauterized, and the spinal cord was then transected at T10–T12 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. After the animals recovered from anesthesia, they 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 to 3 days after surgery. Antibiotics (ampicillin 25 mg/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 66 ml/kg daily. Bladders were expressed manually at least twice daily. Male rats were initially used in this study but we later switched to female rats since their maintenance after spinalization was generally much easier. There were no apparent differences between the results from male and female rats, so data from both were combined.

In some animals (*n*=6), a complete unilateral hindlimb deafferentation was also performed in the same surgical session as the spinal transection. Dorsal roots from L1 to S1 were exposed by partial dorsal laminectomy and cut intradurally after lidocaine had been applied topically in order to eliminate injury discharge. Completeness of the deafferentation was verified post-surgically 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 after the experiments by dissection of the spinal cord. Only those animals with complete L1 to S1 deafferentations are reported in this study.

Rats were maintained for one to three weeks after surgery. These periods are sufficient to eliminate the possibility of severed axons being able to conduct action potentials (Tsao et al. 1994; Boyes and Veronesi 1988). This loss of conduction was confirmed in the present experiments by stimulating the proximal end of cut roots and observing no motor response after these survival periods.

At the end of this survival period, the rats were prepared for the acute experiment.They were anesthetized with a single dose of ketamine/xylazine (87/13 mg/kg i.p.) given with atropine (0.02 mg/kg s.c.) and dexamethasone (18 mg/kg i.p.). The carotid arteries were tied off. The animals were transferred to a stereotaxic frame, supported by a platform which allowed the limbs to hang free. They were then decerebrated precollicularly and anesthesia was discontinued.

In two animals bipolar EMG electrodes were next placed in hindlimb muscles. A pair of electrodes (braided stainless steel Teflon insulated, ~1 mm exposed tip) were placed in the following muscles: semitendinosus (ST), iliopsoas (IP), vastus lateralis (VL), and biceps femoris (BF). Methods for recording EMG activity were as described elsewhere (Giszter et al. 1993).

The lumbar spinal cord was then exposed by opening T13, L1, and/or L2 vertebrae by dorsal laminectomy. In some animals only one or two vertebrae were opened, 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. Vertebrae immediately rostral and caudal to the exposure were clamped to secure the spinal cord and a coccygeal vertebra was also clamped to secure the hip.

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

Data collection

Microelectrodes (stainless steel, tip diameter 0.5–1 μm, 10 MΩ) were inserted into the spinal cord under visual inspection through a microscope. We applied trains of negative current stimulation (cathodal stimulation of 1–15 μA, 200–600 ms train, 70 Hz, 0.3 ms pulse) through this electrode (BAK stimulator, 180 V compliance). The current levels typically used in these experiments, 2, 8 and 15 μA, would be expected to directly activate volumes of tissue of approximately 80, 170, and 260 μm radius, respectively (Gustafsson and Jankowska 1976; Ranck 1975; see Discussion). Sites between 300 and 800 μm lateral to the cord midline were examined.

The left hindlimb was attached to a six-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.

One method used to describe the responses to spinal microstimulation was to examine how the response evoked by 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 (Bizzi et al. 1991; Giszter et al. 1993). Due to the mechanical fixation of the spinal column such movement of the limb did not induce any significant movement of the spinal cord.

The interaction between the force fields evoked from different sites in the spinal cord was also examined. Electrodes were placed

in two different spinal sites and the force field evoked by stimulating each site separately was measured. Microstimulation was then applied to both electrodes simultaneously and the force field resulting from this co-stimulation was measured.

Force fields were collected to describe how the evoked forces changed depending on the position of the limb in the workspace. Because of the time required to collect the forces at 10–20 ankle positions within a force field, it is difficult to collect enough force fields to perform a systematic analysis across many spinal sites, especially within a particular animal. In order to assess the distribution of responses evoked from 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 rats. We tested different ankle positions between rats in order to distinguish between the different types of microstimulation responses (see Results). Because forces in different animals were not collected at the same ankle position, data from different animals were not combined. For these experiments, in which many sites in the spinal cord were examined, the electrode was advanced in steps of 250 µm from the dorsal surface of the spinal cord and currents of either 2, 8, or 15 µA 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 strong cutaneous stimulation often evoked motor responses in these unanesthetized animals, sensory receptive fields were mapped using light brushing of the hair and occasional light pressure on the skin. 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.

At the end of the experiment we placed electrolytic lesions (10 µA, 20 s) at sites of interest, dissected the spinal column with cord within the vertebrae, and placed the cords in fixative (10% formalin). These fixed cords were then cut into 80 µm transverse sections and stained with Prussian blue or cresyl violet.

Data analysis

We generally only report the results based on an analysis of the force evoked in the sagittal plane containing the hip. 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. This background force results from the passive properties of the skin, tendon, and muscle of the hindlimb, as well as the effects of gravity. Only evoked force responses of a magnitude of greater than 0.022 N were analyzed. Responses less than this magnitude could have large variations in their direction due to limitations in the sensitivity of the force sensor. Analyses of directional data were done using standard circular statistics (Fisher 1993; Mardia 1972).

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. For each electrode penetration, we took the mean direction of the most superficial site examined, usually 250 µm from the top of the spinal cord. This mean direction was then subtracted from each direction along that electrode penetration, the absolute value of these deviations was taken, and the deviations at different depths were combined for each animal. A statistical test was then performed to examine the effect of depth on the deviation of force direction from the top of the spinal cord for each animal. The effect of depth on the deviation of force direction was assessed by a bootstrap test (500 bootstrap steps) using the non-parametric Y statistic for the comparison of multiple samples of circular data (Fischer 1993), essentially a one-way ANOVA. If there was a significant effect of depth, pair-wise 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 examined whether the force fields evoked from different dorsoventral regions of the spinal cord differed in how smoothly the evoked force changed with ankle position across the workspace of the hindlimb. To assess this possibility, 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 forces measured across the workspace as a function of ankle position:

$$\begin{bmatrix} F_x^1 & F_x^2 & \cdots & F_x^N \\ F_y^1 & F_y^2 & \cdots & F_y^N \end{bmatrix} = \mathbf{B} \begin{bmatrix} x_1 & x_1 & (x_1)^2 & (y_1)^2 \\ x_2 & y_2 & (y_2)^2 & (y_2)^2 \\ \vdots & \vdots & \vdots & \vdots \\ x_N & y_N & (x_N)^2 & (y_N)^2 \end{bmatrix}^T \quad (1)$$

where F_x and F_y are the x and y components of the evoked force in the sagittal plane and the superscript indexes over the 1 to N limb positions within the force field. The 4 by N matrix on the right contains columns with the x and y positions of the hindlimb at each of the N positions and their squares. The **B** matrix is a 2 by 4 matrix giving the dependence of the x and y force components on the x and y positions of the hindlimb. The **B** matrix was calculated through regression. The quality of the fit for a particular force field was assessed using the coefficient of determination, R^2, calculated from the residual sum of squares of this fit. 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 different dorsoventral regions of the spinal cord.

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 estimated using a statistical test described by 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. The distributions of evoked force directions were fit as a mixture of von Mises distributions, using the EM algorithm (Dempster et al. 1977; Saltiel et al. 1998). 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 (Hsu et al. 1986; Saltiel et al. 1998).

Rostrocaudal and mediolateral variation of force direction

To determine whether the direction of evoked force was related to the rostrocaudal or mediolateral location 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:

$$\theta = ar + br^2 + cm + dm^2 + e \quad (2)$$

where θ is the force direction, r and m are the rostrocaudal and mediolateral location of the stimulation site and a–e are the re-

gression coefficients. This analysis was applied to data obtained from each animal separately since location measurements were not standardized for differences between the sizes of spinal cords in different animals. The distribution of force directions for each animal was rotated so that the mean of the distribution was at zero degrees in order to reduce discontinuities in the distributions of direction. 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.

EMG analysis

The EMG activity evoked by spinal microstimulation was sampled at 1000 Hz, rectified, and the activity within a muscle was averaged over a period of 100 ms at various times after the onset of the stimulation train. The activity in each muscle was normalized so that the maximum level recorded across all stimulation trials for that muscle was 1. To compare responses of different magnitudes we normalized each response so that the total magnitude (taken as the vector norm) of the EMG activity from all recorded muscles was 1. This second normalization allowed us to compare the relative contributions of each muscle to different responses. The results from this normalization to total EMG activity were very similar to the results obtained from the normalization of each response to the recorded force magnitude (as would be expected from the close relationship between total EMG activity and force magnitude, see Fig. 11A).

Results

Responses to microstimulation of the rat spinal cord

Microstimulation of the rat spinal cord produced forces measured at the ankle both during the stimulation train and for a period of time after the offset of stimulation. Figure 1A shows an example of the responses evoked along an electrode penetration through the L4 spinal segment, along the track indicated in Fig. 2B. The directions of the forces evoked by spinal microstimulation were typically consistent throughout the stimulation train and often outlasted the stimulation train. In some cases, the force recorded after the offset of the stimulation train changed in either magnitude or direction (see Fig. 11 for clearer examples). Stimulation of the same site was also similar between repeated stimulation trials (data not shown). Figure 1B shows, for one animal, the changes in direction between the forces recorded at two times separated by 200 ms within the stimulation train. Figure 1C shows the same changes in direction for two forces also separated by 200 ms, but one before and one after the offset of the stimulation train. As can be seen in the figure, while the responses within the stimulation train were very similar to one another, there was often an alteration in the responses evoked after the offset of stimulation. Across all animals, the average angular change for forces within the stimulation train was significantly lower than that for forces before and after the stimulation offset (7.85±1.79 vs 18.16±8.97°, $P<0.05$). Because of the relative variability in the responses evoked after the offset of the stimulation train, only responses evoked within the train of stimulation were analyzed further. In particular, the force response measured at a latency of 230 ms was used for subsequent analyses. As would be expected from the consistency of responses throughout the train of stimulation, the results reported here were essentially unchanged when responses were examined at other latencies.

Figure 1A also shows that responses were evoked from a region of the spinal cord between the dorsal surface of the cord and around 1250 μm more ventral. Figure 2A shows for one animal the fraction of stimulation trials that evoked a threshold response as a function of depth of the stimulation site and the level of stimulation current. Responses were obtained most reliably within the first 1250 μm from the dorsal surface of the spinal cord. Responses from intermediate regions of the cord were comparatively infrequent, even with the highest stimulation levels used in this study. At deeper sites, there was a slight increase in the frequency of evoked responses. Figure 2B shows the locations of electrolytic lesions placed at varying depths in different lumbar segments, illustrating the relationship between the spinal anatomy at different rostrocaudal levels and the depths measured from the dorsal surface of the cord. The histology shows that the dorsal region from which responses were observed corresponded to lamina I to V.

Figure 1A also shows that the directions of the forces evoked within this dorsal region were similar at different depths. To evaluate this observation, the change in the direction of force from the dorsal surface of the spinal cord was assessed. The majority of animals examined (7/11) showed the first significant change ($P<0.05$) in force direction at a depth of 1500 μm, three showed the first change at 1250 μm, and one showed a change at 750 μm.

The force fields evoked from dorsal and deep sites also appeared to have different characteristics. Figure 2C shows a force field evoked from the dorsal region of the spinal cord, at a depth of 500 μm. In this response, the force measured at the ankle varied smoothly as the ankle was moved between positions. Figure 2D shows a force field measured from a site 1500 μm deeper along the same electrode penetration as the site in Fig. 2C. In contrast to the more superficial force field, the force measured at the ankle for this response showed much more marked changes between nearby ankle positions. We quantified this difference between the force fields from dorsal and deep sites by examining how well the change in evoked force across the workspace could be described as a smooth function of ankle position (equation 1). Force fields evoked from dorsal sites were significantly better fit as a second order polynomial function of ankle position than the force fields from deeper sites (dorsal R^2=0.83±0.01, deep R^2=0.70±0.16, $P<0.05$). Figure 3 shows that the force fields evoked from this dorsal region were also well structured when their action in three dimensions was examined.

These basic features of the responses from microstimulation in chronically transected animals were preserved after a unilaterally complete chronic deafferentation of

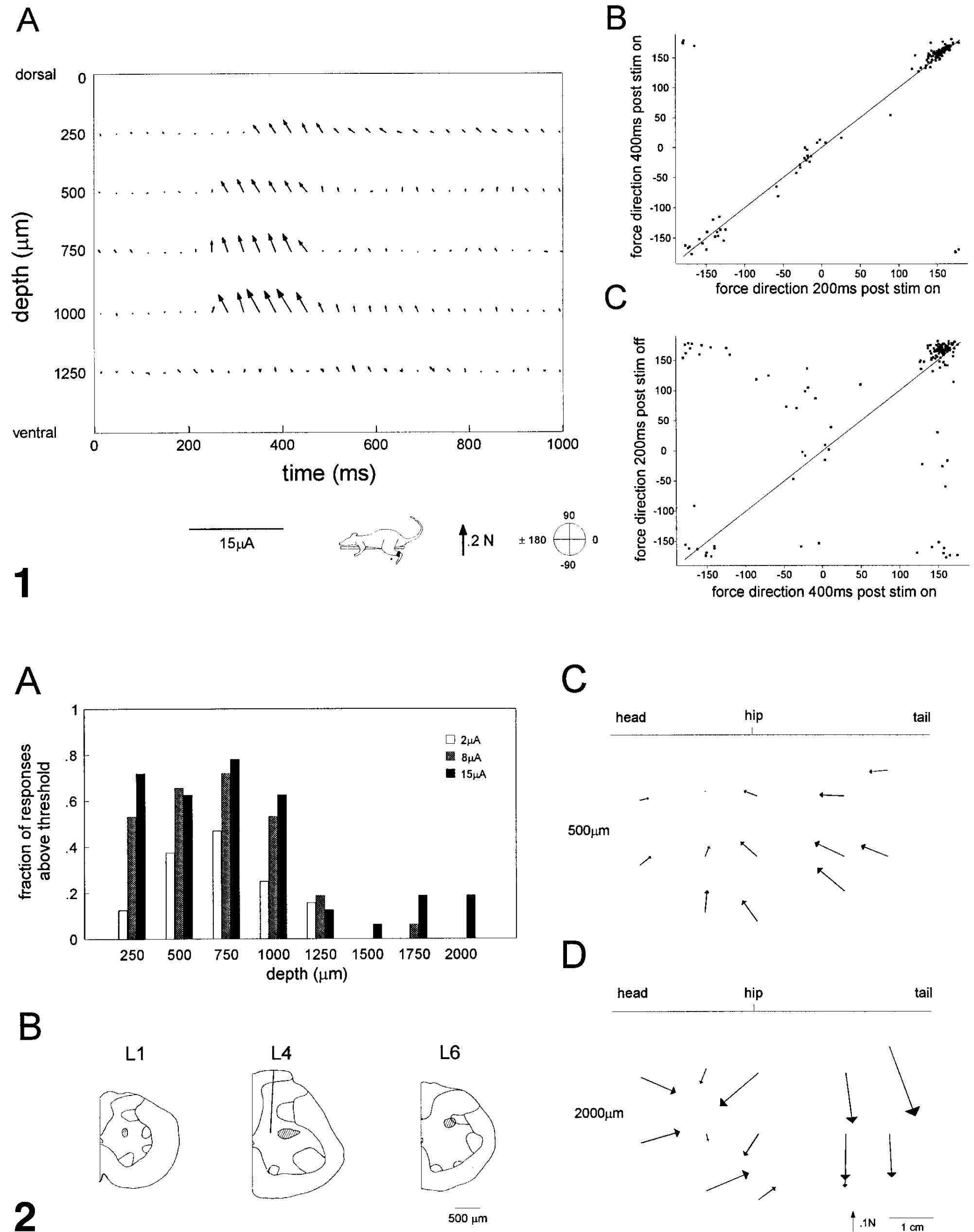


Fig. 1 **A** An example of the responses evoked by microstimulation along a single electrode penetration in L4, 400 µm lateral from the midline. The temporal evolution of the evoked force is shown for different depths along this penetration. The force at each time is indicated by the *arrows* in the 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 at the lower right of **A**, 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 (15 µA, 70 Hz, 0.3 ms pulses) was applied for 200 ms, indicated by the solid line below the figure. **B** shows the relationship between the direction of force evoked for two responses separated by 200 ms within the stimulation train. **C** shows the relationship between the direction of force evoked for two responses also separated by 200 ms, but with one response within the stimulation train and the other after the stimulation train. Only trains of 400 ms or longer and only responses which were above threshold at all three times were included in this analysis. The direction of force relative to the rat's body is indicated in the lower part of **A**

Fig. 2 **A** The variation in the fraction of stimulation trials which evoked a response above threshold as a function of depth from the surface of the spinal cord and of current level. **B** The location of elecotrolytic lesions placed in different regions of the spinal cord to show the relationship between depth from the dorsal surface of the cord and spinal anatomical structures, taken from camera lucida drawings. The shaded area in each region shows the locations of lesions placed in different animals. The lesion in *L1* was placed at a depth of 1000 µm and 500 µm lateral to the midline, the lesion in *L4* was placed at a depth of 1250 µm and 600 µm lateral to the midline, and the lesion in *L6* was placed at a depth of 750 µm and 600 µm lateral to the midline. The outlined region in the ventral cord shows the location of presumed motoneuron pools while the outlined region in the dorsal cord shows the location of the lateral reticulated region characteristic of lamina V. The *vertical line* shows the electrode track of the penetration illustrated in Fig. 1A. This track was seen in the histology in a section 400 µm rostral from the section in which the lesion was placed. There is a good correspondence between the depth as assessed from the histology and the depth as assessed at the time of the experiment. **C, D** Force fields evoked from two sites along the same electrode penetration in L6. The force field shown in **C** was taken at 500 µm from the dorsal surface of the cord with a current of 8 µA. Stimulating at a site 1500 µm deeper along the same penetration at the same current strength produced the force field shown in **D**

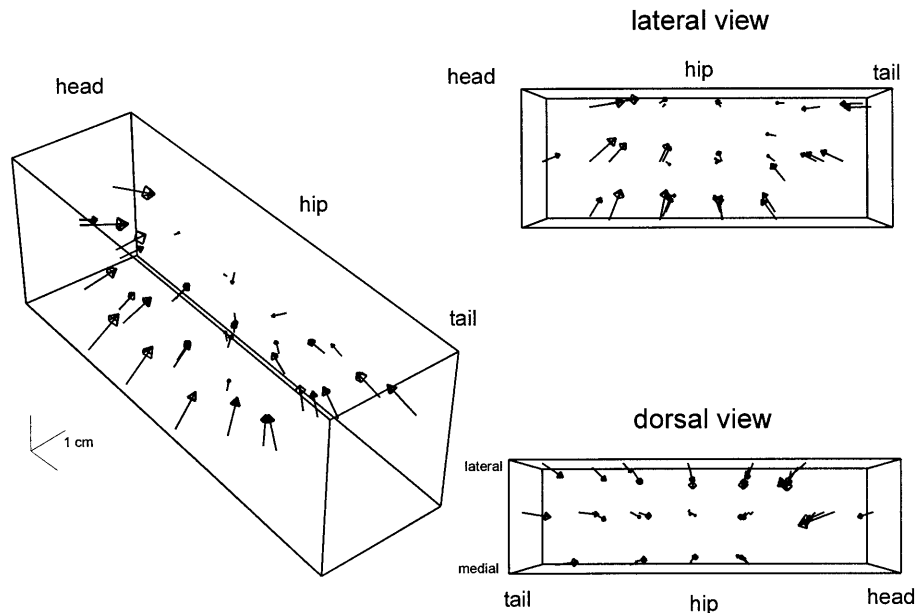
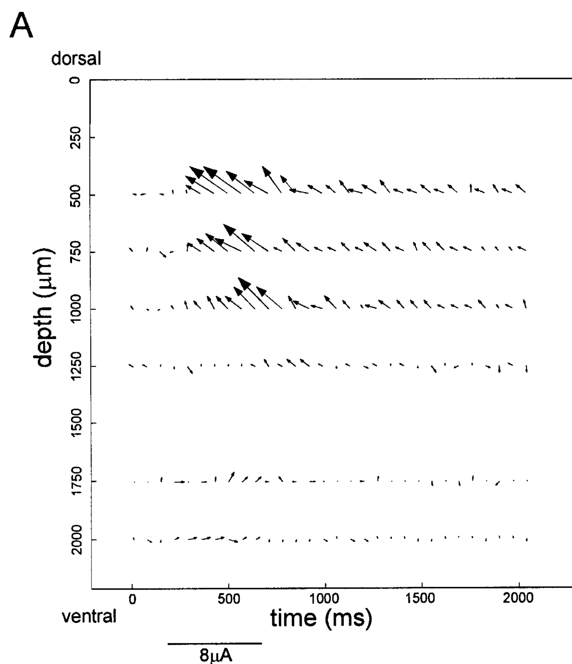


Fig. 3 An example of a force field measured in the three-dimensional workspace of the rat hindlimb. Forces evoked from microstimulation in the spinal cord were measured at ankle positions in three sagittal planes, one directly below the hip, one 1 cm lateral to the hip, and one 1 cm medial to the hip. Three views of the force field are shown here. In addition to the perspective view shown on the left, a *lateral view* (top right), and a *dorsal view* (bottom right) are shown. This response was obtained from microstimulation of a site at a depth of 750 μm with a current of 8 μA in L5



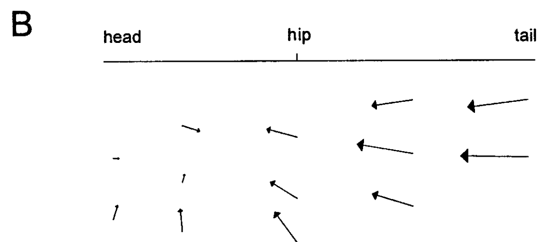


Fig. 4A,B Responses evoked by microstimulation of a chronically deafferented rat. **A** Responses evoked by microstimulation of different depths along an electrode penetration (conventions the same as in Fig. 1). Note that at 250 μm and at 1500 μm no response was observed to microstimulation at this level of current. **B** A force field obtained at a depth of 750 μm with 8 μA current in L5

one to three weeks (n=6). Figure 4A shows that microstimulation could still evoke responses from the dorsal region at similar stimulation strengths as those used for animals with chronic spinal transection but with afferents intact. Responses from these regions were consistently observed in every deafferented animal (see also Fig. 8B). We have not measured a large number of force fields in chronically deafferented animals. In the force fields we have measured, however, there did not appear to be major qualitative differences in their organization after chronic deafferentation. An example of a force field from a chronically deafferented animal is shown in Fig. 4B.

The responses evoked from this dorsal region are clearly not due to direct stimulation of motoneurons and, since they are maintained after chronic deafferentation, are not dependent on the direct stimulation of sensory afferents. On the other hand, it is difficult to rule out a potential contribution of direct motoneuronal stimulation to the responses evoked from the deeper regions of the spinal cord. In order to focus on the organization of spinal interneuronal systems, only responses obtained between 250 μm and 1250 μm from the dorsal surface were therefore included in subsequent analyses.

Co-stimulation

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 Fig. 5. Stimulation of site A produced a force field directed caudally and dorsally (Fig. 5A), while stimulation of site B evoked a force field directed ventrally (Fig. 5B). The force field

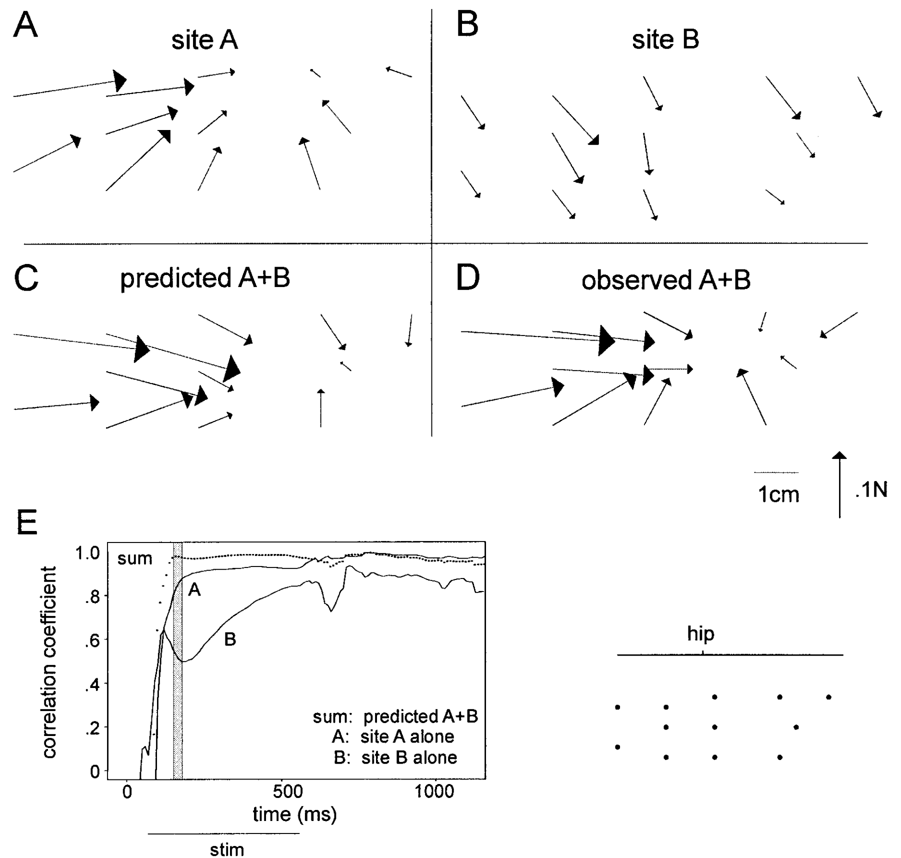


Fig. 5A–E An example of the linear combination of force fields evoked by co-stimulation. The force fields evoked by separate microstimulation of*site A* and of *site B* are shown in **A** and **B**. *Site A* was obtained by stimulation of a site 1000 μm deep with a current of 6 μA in L2. *Site B* was obtained by stimulation of a site 1000 μm deep with a current of 5 μA in L5. **C** Force field predicted if the two force fields combined linearly. **D** Force field produced by simultaneous stimulation of the two sites. **E** Correlation of the observed co-stimulation force field to the separate force fields from *sites A* and *B*, 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. The *shaded area* indicates the period where the criteria for summation were met. The *inset* at the bottom right shows the ankle positions relative to the hip at which the force fields in **A–D** were taken

predicted from a linear summation of each separate response is illustrated in Fig. 5C. The force field observed from co-stimulation of the two sites is illustrated in Fig. 5D. Qualitatively, the observed co-stimulation force field is similar to that predicted by linear summation, but is different from the force fields evoked by separate stimulation of the spinal sites.

This qualitative similarity was assessed using the force field correlation measure described by Mussa-Ivaldi et al. (1994). As a correlation, this measure varies from –1 to 1, with values near 1 indicating that the responses were similar. The response evoked by co-stimulation was considered to be a good case of summation if two criteria were met: (1) the similarity measure between the observed co-stimulation force field and the force field predicted from a simple linear vector addition of each separate force field was greater than 0.8, and (2) this similarity measure was at least 0.1 greater than the similarity measure between the force field produced from co-stimulation and from stimulation of each separate spinal site. The first criterion ensures that the co-stimulation force field is similar to the predicted summation force field, while the second criterion ensures that the co-stimulation 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.

Figure 5E shows this force field correlation measure for each of these comparisons during the period of microstimulation. It can be seen that the correlation of the co-stimulation response to the predicted summation was very high throughout the train of stimulation and was higher than the correlations with either of the separate force fields. This example met both criteria described above for a good case of summation only in the initial phases of the response, indicated by the shaded region in the figure. In all, we examined seven cases of co-stimulation 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 six of these cases the co-stimulation response met both criterion at some point during the stimulation train. In the other case, it turned out that the responses were too similar 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 up-

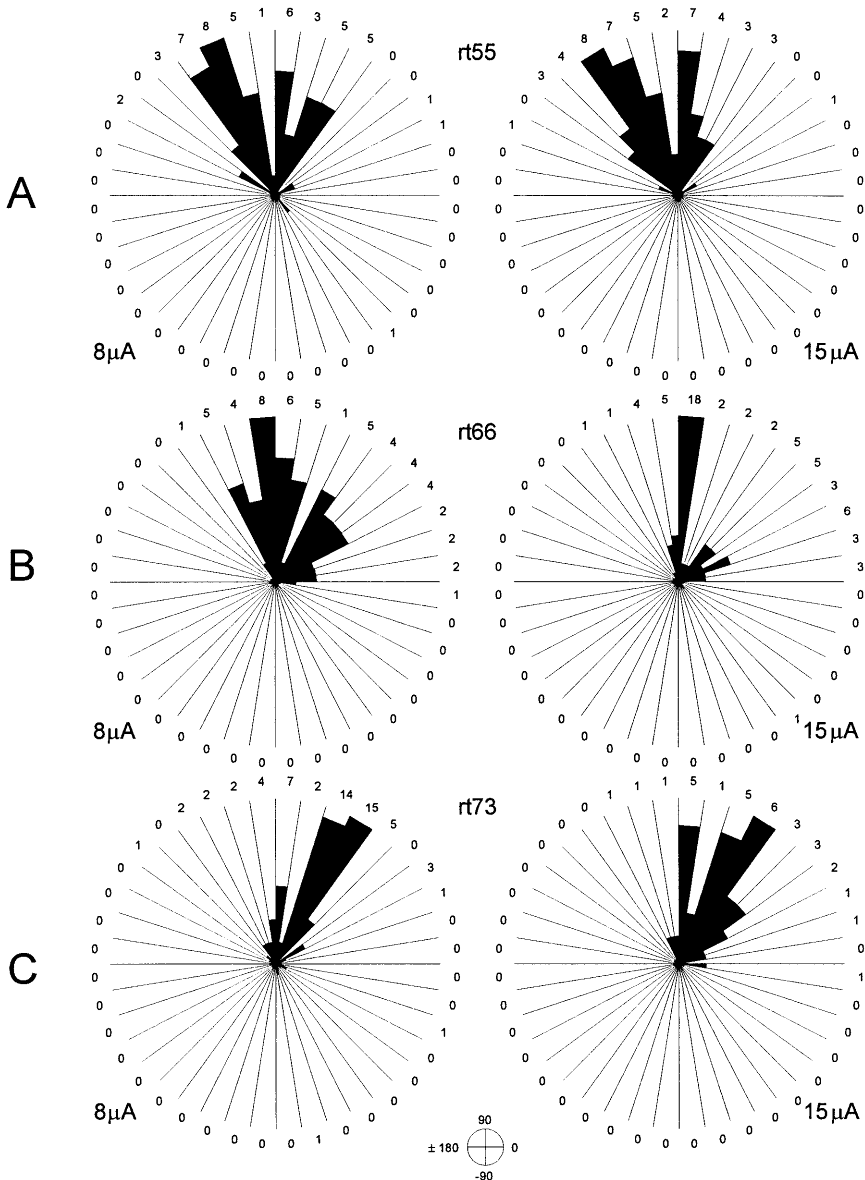


Fig. 6A–C Angular histograms of the force directions evoked by spinal microstimulation in three different rats, shown for two different levels of current. Angular histograms of 9° bins are shown. Force data are measured for a single position of the ankle in the workspace. In the animals illustrated here, this position was directly below the hip, with the knee extended. Only responses from sites between 250 μm and 1250 μm from the dorsal surface of the spinal cord and with a magnitude greater than 0.022 N are included

wards to the body. Closer examination of the distributions shown in Fig. 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 show that it was not due to different stimulation strengths. Similar distributions were observed from spinal microstimulation in chronically deafferented rats (see Fig. 8B). 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; Saltiel et al. 1998). Applying this analysis to each individual rat showed that there was evidence (P<0.05) for at least two modes in the majority of animals (8/11), for at least three modes in two animals, and only one mode in one animal.

The force fields corresponding to the two classes indicated in the distributions are illustrated in Fig. 7A, B. 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 Fig. 7C, a third type of force field is illustrated, which drives the limb away from the body. This third type of force field was observed in 14% (6/42) of the force fields we measured and we did not observe it in each animal, as evidenced by the analysis described above. When we did observe it, it was distinct, and we therefore include it here as a third type of response.

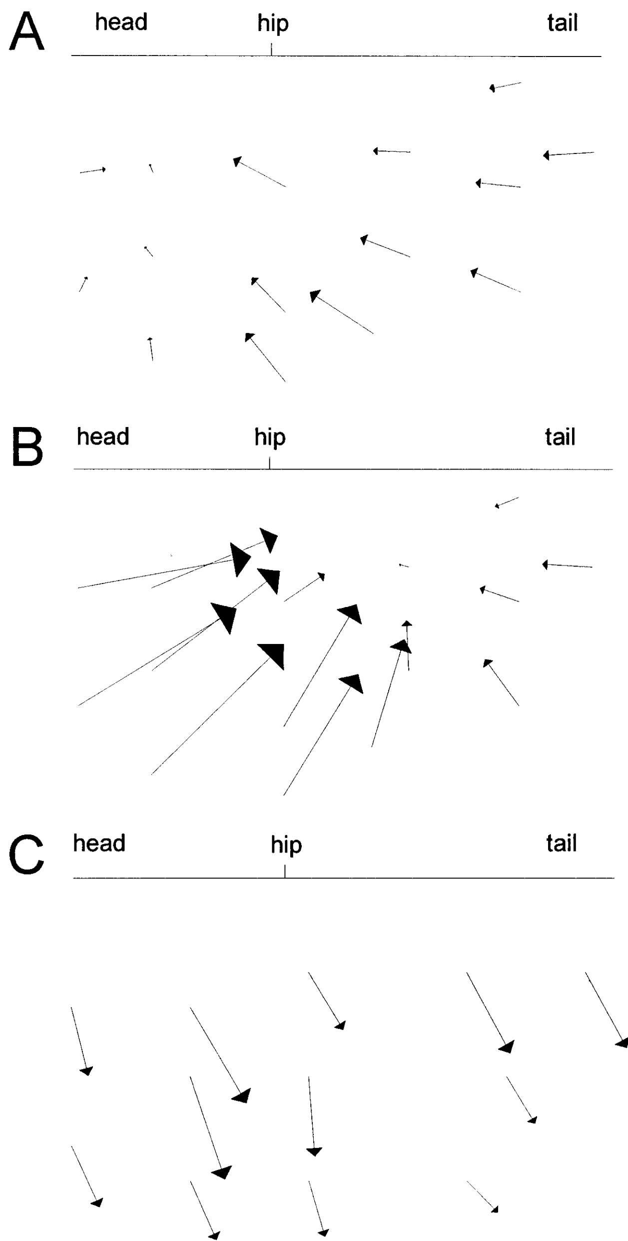


Fig. 7A–C Three types of force fields observed from spinal microstimulation. **A** Force field drawing the limb dorsally and rostrally. **B** Force field drawing the limb dorsally and caudally. **C** Force field not commonly observed, which drove the limb away from the body

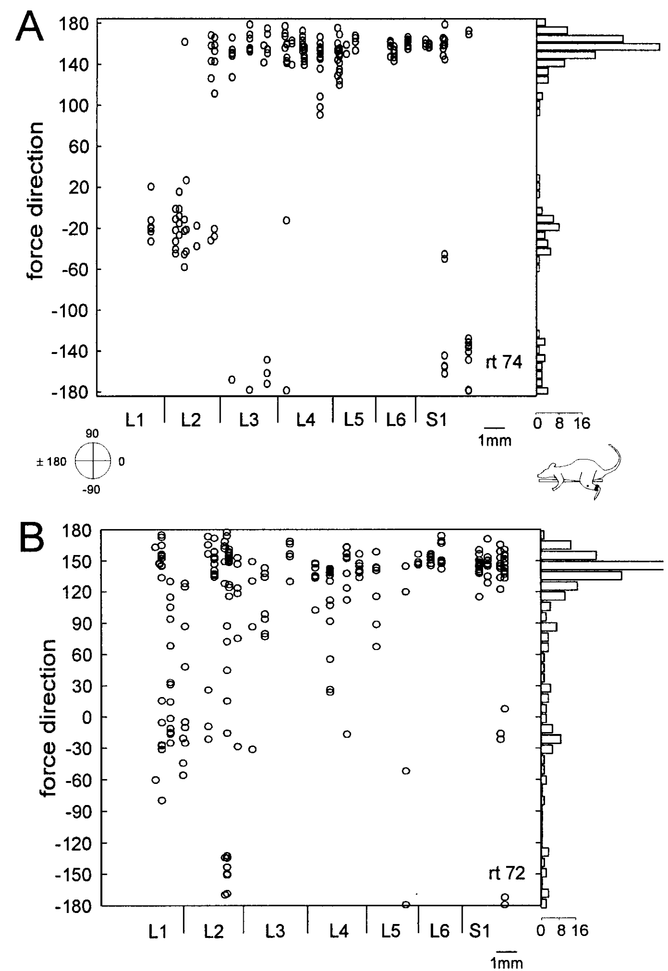


Fig. 8A,B The variation of force responses with rostrocaudal location. **A** Responses measured at a single ankle position after stimulation of sites between 250 and 1250 μm from the dorsal surface of the cord with currents of 2, 8, or 15 μA, in a chronically spinalized animal with afferents intact. The histogram on the right shows the frequencies of the different force directions. The ankle position at which the measurements were taken is indicated by the dot in the schematic of the rat shown at the right. Each column of measurement points reflects the responses evoked at different sites and different currents along a site of electrode penetration. **B** The variation of force responses with rostrocaudal location in a chronically spinalized and deafferented animal

Anatomical organization of microstimulation-evoked responses

In several animals (*n*=8: five with afferents intact, three deafferented), we explored many different sites throughout the rostrocaudal and mediolateral extent of the spinal cord. In the animal shown in Fig. 8A the direction of force evoked by 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 (equation 2). Four of the five animals showed a significant relationship to the rostrocaudal coordinate of the stimulation site, and one of these animals showed a significant relationship to the mediolateral location of the stimulation site. Figure 8B 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

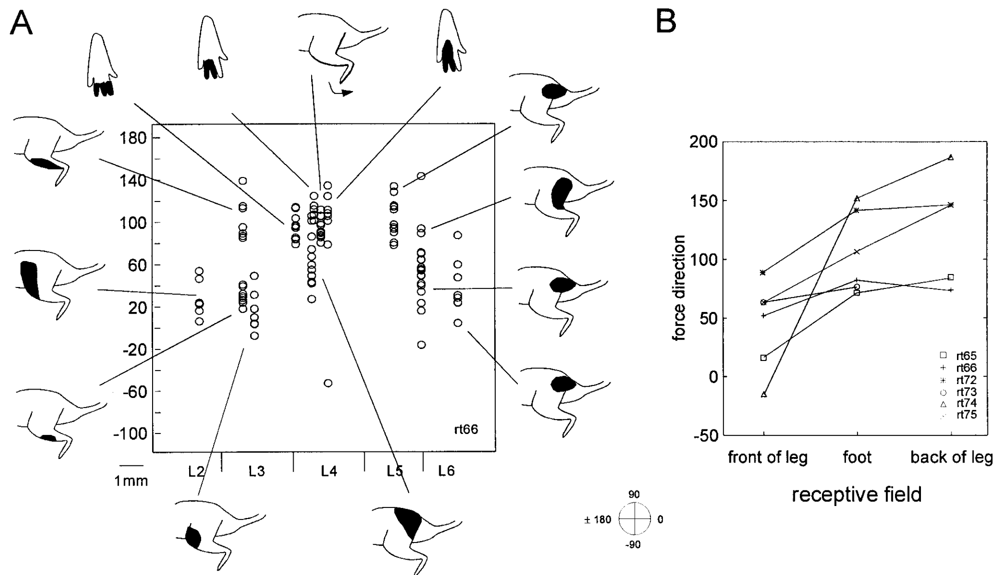


Fig. 9A,B The relationship between force response, rostrocaudal location, and receptive field of sites in the spinal cord. **A** Composite receptive fields obtained from each penetration. Receptive fields of multiple units and occasional single units were mapped using light brushing of the hair or gentle pressure on the skin which did not produce a measurable reflex. Other conventions as in Fig. 8. **B** 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 that 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 side. The findings in **A** are indicated by the findings with the plus sign (rt66) in **B**

a dependence on the mediolateral coordinate. In the animals that had a significant mediolateral dependence, more medial responses tended to produce responses directed dorsally and rostrally, while lateral responses tended to produce responses directed dorsally and caudally (data not shown).

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 correspond roughly to the somatotopic organization of the spinal cord shown in previous studies (Brown 1981). By combining 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 Fig. 9A, illustrating the well-described variation of sensory receptive field with rostrocaudal location. This relationship between receptive field and rostrocaudal location paralleled the variation of the direction of the response evoked by microstimulation. In the animal shown in Fig. 9A, microstimulation at sites with receptive fields on the front of the leg produced caudally directed responses, sites with receptive fields on the foot produced responses directed dorsal and rostrally, while sites with receptive fields on the back of the leg again produced caudally directed responses. These caudally directed responses from the caudal cord were not observed in most other animals (see Figs. 8A, B, and Fig. 9B; but see also Fig. 1D): stimulation of caudal regions of the cord usually evoked more rostrally directed responses. This pattern of caudal responses evoked from caudal spinal regions appeared to result from a difference in the relationship between microstimulation-evoked responses and the receptive field of a site, since the cutaneous somatotopy of all animals was similar. A few of the penetrations in the animal shown in Fig. 9A were inconsistent with this general relationship between receptive field and evoked force, such as the sites at the rostral and caudal boundaries of the middle region, which produced a force direction atypical for their receptive field.

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 to light cutaneous stimulation 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 Fig. 9A, 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 four rats examined are shown in Fig. 9B (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 9B also illustrates that responses from sites with receptive fields on the back of the leg tended to drive the limb more rostrally than sites with receptive fields on the

front of the leg. The effect of receptive field location on the response produced by microstimulation was found to be significant in each of these rats (bootstrap *Y* statistic, $P<0.05$).

This correspondence between receptive fields to light cutaneous stimulation and the responses evoked by microstimulation was also examined in animals with chronic unilateral deafferentation ($n=2$). We examined the relationship between the response to 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. Findings from these two rats are illustrated in Fig. 9B (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, $P<0.05$) but did not reach significance for rt72 ($P>0.05$). This correspondence was supported in a third deafferented rat in which responses from stimulation sites roughly paralleled the spinal cutaneous somatotopy in the contralateral spinal cord, but in which a paired microstimulation and receptive field mapping was not performed.

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. Nineteen days after 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 (e.g. Pubols and Goldberger 1980; Koerber and Mirncs 1995). When these sites with aberrant receptive fields were stimulated, however, they produced responses significantly different (*Y* statistic bootstrap, $P<0.05$) from those evoked by stimulation of sites with receptive fields on the back of the leg. This result, although from a single animal, further demonstrated that the response to microstimulation is not due to the activation of sensory afferents.

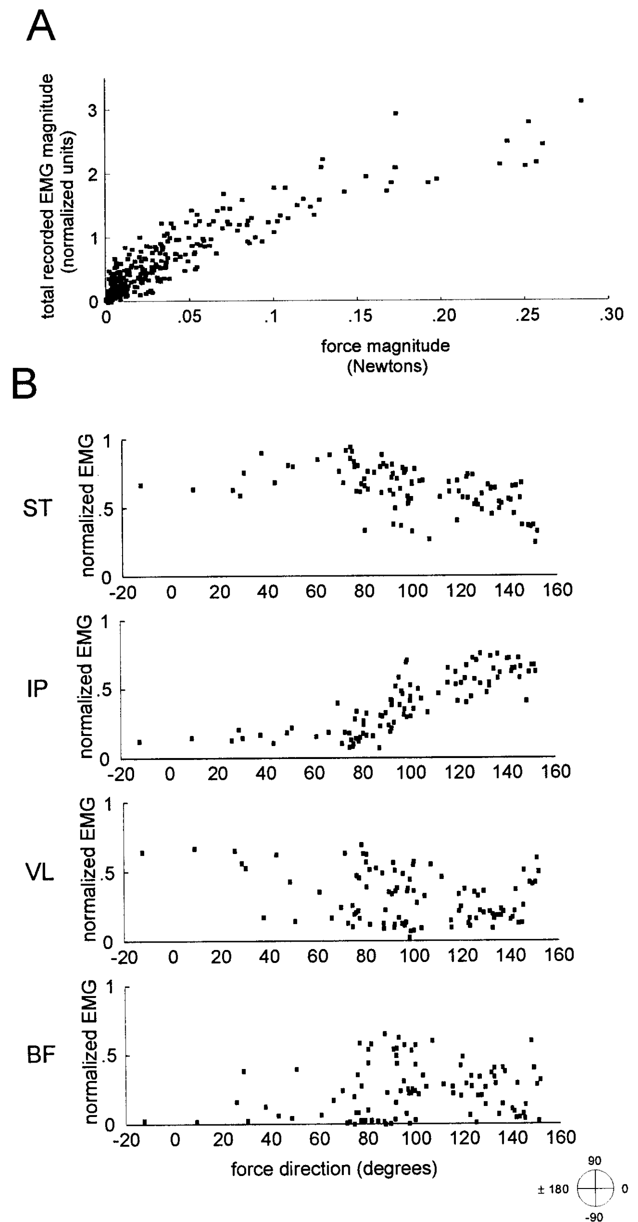


Fig. 10 **A** The relationship between total EMG activity and the force magnitude of responses evoked by spinal microstimulation. For this plot the EMG activity of each muscle was normalized to its maximum value across all stimulation trials. All stimulation trials, regardless of the evoked force magnitude, are included in this plot. **B** The relationship between force direction and EMG activity of each muscle. For these plots, the activity of each muscle was normalized to its maximum as for **A**, then each response was normalized so that the magnitude (taken as the vector norm) of all recorded EMG activity was 1. This second normalization was performed to examine the relative contributions of each muscle to the observed response

EMG activity evoked from spinal microstimulation

Finally, the activity evoked by spinal microstimulation in some proximal hindlimb muscles was recorded ($n=2$, chronically spinalized, afferents intact). The EMG activity in each muscle was averaged over a period of 100 ms preceding the force reported here (from 130 ms to 230 ms after stimulation onset). As can be seen in Fig. 11A–C, the EMG responses were generally similar throughout the stimulation train, and there was often some locking of the EMG activity to the 70 Hz stimulation train (see Fig. 11B). Figure 10A shows the relationship between the total recorded magnitude of EMG activity and the magnitude of the evoked force for all stim-

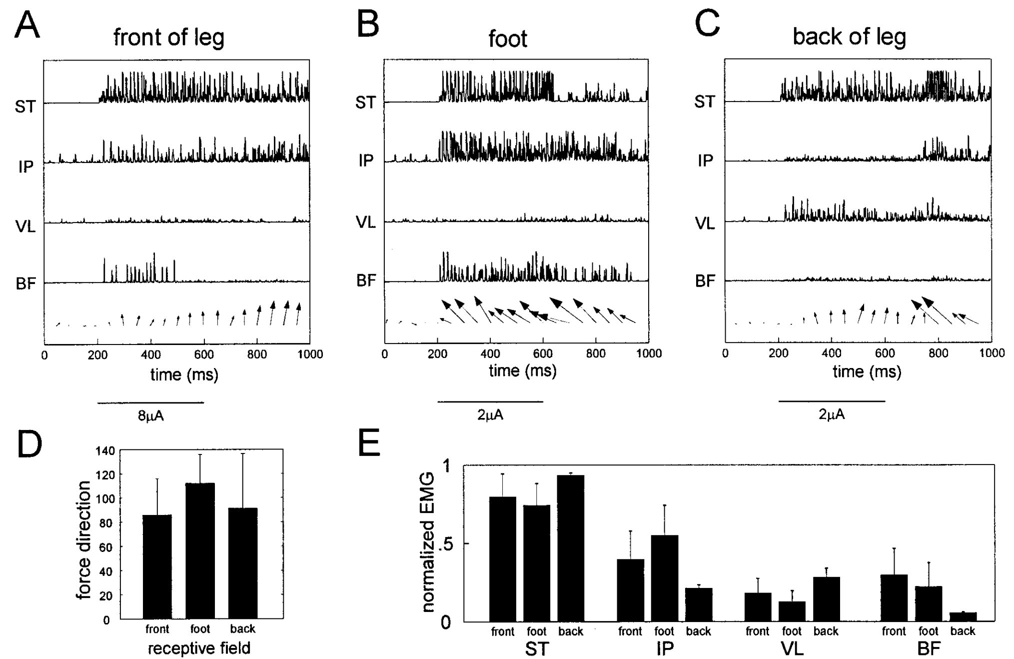


Fig. 11A–E Muscle activation patterns evoked by spinal microstimulation. **A–C** Three examples of single-stimulation trials applied to sites in the spinal cord with a receptive field on the front of the leg, on the foot, and on the back of the leg. Conventions as in Fig. 1A. **D** Average force angle evoked from sites with these three receptive fields. **E** Average contribution of each muscle to the responses evoked from spinal sites with different receptive fields. *ST* semitendinosus, *IP* iliopsoas *VL* vastus lateralis *BF* biceps femoris

ulation trials in one animal. As seen in the figure, there was a systematic relationship between the magnitude of the evoked force and the magnitude of the recorded EMG activity (Pearson r=0.89, 0.91 for the two animals, P<0.05 both). The figure shows that few responses with a small force magnitude also had a large EMG signal, suggesting that responses with small forces were not produced through co-contraction of antagonist muscles. Figure 10B shows the relationship between the recorded muscle activity and the direction of evoked force. Both ST (r= –0.35, –0.50, P<0.05 both) and IP (r=0.73, 0.81, P<0.05 both) were significantly correlated with the direction of evoked force, in the direction expected from their mechanical actions: ST was more active when the force was directed caudally in hip extension and knee flexion while IP was more active when the force was directed rostrally in hip flexion. The activation of VL (r= –0.22, –0.33, P<0.05 both) had a similar though weaker relationship to the force direction as that of ST, while the activation of BF was not significantly correlated to the force direction (r=0.07, –0.16, P>0.05 both). The activations of VL and BF in these responses are therefore difficult to explain in terms of the mechanical actions of these muscles in a simple way. The relationship between the force responses described here and the particular patterns of muscle activation underlying them is therefore not necessarily straightforward and must be interpreted cautiously.

In one of these animals, we examined the relationship between the pattern of evoked EMG activity and the receptive field of spinal sites. Figure 11A–C shows examples of the EMG activity evoked from three different sites in this animal, with receptive fields on the front of the leg, on the foot, and on the back of the leg. Consistent with the analyses described above, the force response evoked by stimulation of sites with a receptive field on the foot (Fig. 11B) was more rostrally directed than stimulation of sites with a receptive field on the front of the leg (Fig. 11A). In this animal, sites with receptive fields on the back of the leg evoked responses similar to those evoked from sites with receptive fields on the front of the leg (Fig. 11C), similar to findings in the animal shown in Fig. 9A. The average force direction for these different receptive fields is shown in Fig. 11D. This difference in evoked force was paralleled by a difference in the evoked EMG activity levels. The average levels of EMG activity evoked from spinal sites with different receptive fields are shown in Fig. 11E for each muscle. The activity level of IP, VL, and BF was significantly dependent on the receptive field of the spinal site (ANOVA, P<0.05), while that of ST was not.

Discussion

There were two main results of this study. First, we were in large part able to replicate in the rat the results previously described for the frog (Bizzi et al. 1991; Giszter et al. 1993; Mussa-Ivaldi et al. 1994). In particular, the findings of smooth force fields, the non-uniform distribution of force directions, and the summation of simultaneously evoked responses described here in the rat, have each been described previously in the frog. It should be noted that there are some differences in the particular distributions of force responses between the two species (see Saltiel et al. 1998). This general replication suggests that the previous results described in the frog were not unique to the frog or to amphibians but can also be observed in a mammal. Second, we demonstrated a relationship between the organization of the responses from

spinal stimulation and the organization of spinal systems activated by low threshold cutaneous afferents. Demonstration of this relationship suggests that the responses to spinal microstimulation are reflecting aspects of the physiological organization of the spinal cord.

As mentioned in the Introduction, the results from microstimulation in the nervous system can be difficult to interpret, though microstimulation has previously been used to describe aspects of the organization of motor systems (e.g Asanuma et al. 1968; Schiller and Stryker 1972; Yamaguchi 1986; Drew and Rossignol 1990). The levels of current used in the present study would be expected to directly activate a sphere of approximately 80–260 µm radius (Gustafsson and Jankowska 1976; Ranck 1975).These values were obtained after correcting for the slightly longer pulse duration used here than in those studies (see Ranck 1975). The direct effects of the microstimulation train will preferentially excite axons near the stimulating electrode, both axons of passage and axons of neurons with cell bodies near the stimulating electrode. In addition to the region directly activated by microstimulation, the spread of activation away from this directly activated site would be expected to recruit elements beyond this region (Jankowska et al. 1975). Because of the nonspecific nature of microstimulation and its widespread activation, the responses evoked by microstimulation must be interpreted cautiously. However, both the replication between species and the relationship to spinal cutaneous systems described here support the physiological relevance of the results from spinal microstimulation.

Other observations also lend support to the physiological relevance of the results of spinal microstimulation. First, not every region of the spinal cord was equally capable of producing responses to microstimulation. Given the widespread connectivity of the spinal cord, one would expect stimulation of nearly every region to readily evoke a response. The observation that some regions of the spinal cord were not capable of producing a response suggests that microstimulation was primarily reflecting the activation of the site at which the stimulation was applied. Second, a recent study in the frog (Saltiel et al. 1998) has indicated that focal application of *N*-methyl-D-aspartic acid (NMDA) to sites within the spinal cord produced a similar pattern of results to that described with electrical microstimulation. NMDA iontophoresis has the advantage of activating only somata and dendrites at the site of stimulation, and thus can be better interpreted as reflecting the activation of elements near the electrode. Third, the results in the present study from the animal with partial deafferentation suggest that the activation of sensory afferents by microstimulation does not significantly contribute to the evoked response, again suggesting that the responses to spinal microstimulation reflect the actions of particular regions near the electrode. This evidence supporting the local effects of spinal microstimulation suggests that even if such stimulation is primarily exciting axons, the observed response is dominated by the activation of the axons of neurons near the stimulating electrode.

We emphasize, however, that the results obtained from spinal microstimulation will reflect only relatively gross principles of organization within the spinal cord. Microstimulation would not be expected to be able to isolate the different interneuronal systems intermingled in the same region of the spinal cord (Jankowska 1992). Instead, microstimulation should reflect organizational features common to these different interneuronal systems. The relationship between microstimulation responses and spinal cutaneous systems observed here is consistent with this expectation: since neurons within a given region of the spinal cord are involved in processing information from the same general region of the body (Brown 1981; Light and Durkovic 1984; Bullit 1991; Rivero-Melian 1996; Riddel and Hadian 1998), their aggregate activation by microstimulation might be expected to reflect this organization.

Microstimulation was also observed in many cases to evoke a response after the offset of the stimulation train. When tested, this late response was clearly coupled to the offset of the stimulation train, as could be seen by varying the duration of the stimulation train. These offset responses are similar to the "after contractions" evoked by stimulation of peripheral cutaneous nerves in semichronically spinalized animals (see for example Fig. 3 in Sherrington 1910). There are many potential explanations for these offset responses. One potential explanation is that they are due to a rebound from a latent inhibitory effect produced by the stimulation train. Another possible explanation is that they might be due to electrode polarization effects, especially prominent with the monopolar stimulation used in these experiments. However, in one animal in which bipolar stimulation was used, thereby reducing electrode polarization, offset responses were still observed (data not shown). Although we have not examined the organization of these responses in detail, it appeared that several features of these offset responses were not the same as for the responses evoked during the train of stimulation. In particular, the multimodal distributions and the topographical organization of offset responses appeared to be less clear than for responses taken during the stimulation train. Because of these differences and because of the difficulty in the interpretation of offset responses, we focused here on the responses evoked during the train of stimulation. Further experiments would be required to understand the nature of these offset responses in more detail.

Regional localization of responses

Spinal microstimulation consistently evoked responses in a region of the spinal cord ranging from the dorsal surface to depths of approximately 1250 µm. Neurons in the superficial part of this region receive direct projections from the periphery and have no outputs to the motoneurons, and they have been implicated in the processing of sensory information (Brown 1981). In the deeper parts of this region, however, there are neurons

with direct projections to motoneurons which have been implicated in the production of behaviors such as withdrawal reflexes (Moschavakis et al. 1992; Kitazawa et al. 1993; Schouenborg et al. 1995). These deeper parts of this region also receive substantial inputs from descending systems such as the corticospinal and rubrospinal systems (Antal et al. 1992; Kuang and Kalil 1990). It is therefore possible that there are qualitative differences between the responses evoked by stimulation of these different depths, ranging from the activation of a sensory representation to the evocation of a motor output. Establishing these differences might require more subtle analyses than the basic description presented here.

It is somewhat surprising that we were unable to evoke responses easily from the deep intermediate regions of the spinal cord. These regions contain last-order interneuronal systems (Jankowska 1992) as well as many passing fiber systems on their way to make connections with motoneurons (Brown 1981). Activation of these elements should produce a response readily. It might be that the effect of activating these regions is predominantly inhibitory or that anatomical aspects of these regions make them more difficult to excite (e.g. Scheibel and Scheibel 1969), possibilities that we have not explored in the present study.

Smooth variation with ankle position, summation, and non-uniform distribution of evoked forces

As in the frog, we found that the force fields from the superficial regions of the spinal cord consistently evoked responses that varied smoothly with the position of the ankle in the workspace (Bizzi et al. 1991; Giszter et al. 1993). Force fields evoked from deep regions of the spinal cord generally did not have such a smooth variation. It is important to realize that some type of variation in the magnitude and direction of isometric force with limb configuration is expected, due to the mechanical actions of muscles: the moment arm and length of a muscle, along with changes in the Jacobian translating joint torques into endpoint forces, can all vary with limb configuration, resulting in a position-dependent force field. The smooth variation in force with limb configuration could also reflect modulation of the evoked response by position-dependent afferent feedback. The observation that similar force fields were observed after deafferentation suggests that the smooth variation is not due to afferent modulation, as has also been shown for the responses in the frog (Loeb et al. 1993). Other aspects of the responses from spinal stimulation, however, might be modulated by afferent feedback. This type of smoothly varying force field has previously been characterized as convergent (Bizzi et al. 1991; Giszter et al. 1993), driving the ankle to a particular position within the workspace. In the present experiments, however, we have not attempted to make this characterization. In the majority of sites examined here, the evoked responses drove the limb toward the extremes of the workspace, making it difficult to identify a limb configuration to which the ankle was always driven. We have therefore refrained from describing these responses as convergent or as specifying a stable limb configuration within the workspace.

The summation of force fields observed here and previously in the frog (Mussa-Ivaldi et al. 1994) evoked from co-stimulation of spinal sites is somewhat surprising. This result suggests that responses evoked from different sites are produced independently of one another and combine only at the level of the motor output. It is possible that our stimulation electrodes were placed far enough apart so that they activated non-overlapping interneuronal populations. Given the wide connectivity of the spinal cord and the expected spread of microstimulation away from the electrode location (Jankowska et al. 1975), however, one might have expected to observe interactions even between distant stimulation sites. The summation of responses observed here and in the frog might be exploited in order to produce a range of motor responses in a fairly simple manner, either to produce behavior or to produce limb movements after spinal cord injury. In the frog, it has been shown that it is possible to use the measured isometric force field to predict the trajectory of the limb when it is free to move (Giszter et al. 1993). Other work has demonstrated the ability of the summation of spinally evoked responses to produce a range of motor outputs in a simple and controllable manner (Mussa-Ivaldi and Giszter 1992; Lemay et al. 1997).

We consistently observed that the distributions of force directions evoked by spinal microstimulation were highly non-uniform. Examination of these distributions also suggested the presence of a few different response directions that were preferentially evoked by spinal microstimulation. Given the redundancy in the actions of the hindlimb musculature, however, it is possible that a direction of force preferentially observed here was produced from the activation of different sets of muscles. Such a possibility is suggested in the results shown in Fig. 11, where similar forces are produced by activation of sites in the rostral and caudal regions of the spinal cord, even though the muscle activation patterns underlying them are different. Note that this redundancy is not simply the result of co-contraction of antagonist muscles, which might have been expected if spinal microstimulation were activating spinal systems non-specifically. Further work will be necessary to explore this issue of the relationship between force production and muscle activation. However, such redundancy would, if anything, tend to obscure the evidence for a small number of distinct responses, making the multimodal and non-uniform distributions of force direction observed here unexpected. These features of the distributions of force directions are therefore likely to reflect the organization of the interneuronal systems activated by spinal microstimulation.

Relationship of microstimulation responses to spinal cutaneous systems

This study describes a relationship between the anatomical organization of responses evoked by spinal microstimulation and the somatotopical organization of spinal systems activated by low-threshold mechanical cutaneous stimulation. Although we have only examined the relationship between low threshold afferents and microstimulation responses, there are close relationships between the somatotopical organization of these afferents and other afferent systems (Light and Durkovic 1984; Schouenborg 1984). It is therefore likely that we would have observed a similar relationship if we had characterized the organization of different afferent systems, such as nociceptive afferents. This relationship to the organization of spinal cutaneous systems suggests that the motor responses described here reflect the actions of interneuronal systems involved in processing cutaneous information in the spinal cord. This relationship might help to explain the non-uniform distribution of responses from spinal microstimulation observed here. Given that hindlimb cutaneous stimulation in the spinalized animal generally evokes responses which flex the limb toward the body (e.g. Sherrington 1910; Hagbarth 1952; Schouenborg and Kalliomaki 1990), it might have been expected that the responses evoked by activation of spinal cutaneous interneuronal systems would also tend to drive the limb toward the body. Further, both the spinalized turtle (Stein et al. 1982) and the spinalized frog (Berkinblitt et al. 1986) have been described as producing a small set of different behaviors in response to cutaneous stimulation, observations possibly related to the multimodal distributions described here and in the frog (Giszter et al. 1993). In the rat, it has been observed that after acute spinalization the precisely adapted responses observed in the intact animal (Schouenborg and Kalliomaki 1990; Schouenborg and Weng 1994) become much coarser, losing much of their precision (Schouenborg et al. 1992). This loss of precision after spinalization might be reflected in the few distinct types of responses to spinal microstimulation observed here.

It should be noted that the organization of responses described here is broadly in agreement with the organizational principles described by Schouenborg et al. (Schouenborg and Kalliomaki 1990; Schouenborg and Weng 1994) for withdrawal reflexes in the rat. The general observation that responses from sites with receptive fields on the front of the leg produced more caudally directed responses than sites from the foot and the back of the leg is similar to the principle of local variations in withdrawal reflexes described by Schouenborg and Weng (1994). Similarly, the greater contribution of IP compared to ST and BF for responses from sites with foot receptive fields parallels the patterns of muscle activation described in those experiments, although the activation of VL from sites with back of the leg receptive fields (Fig. 11E) is unexpected, based on the results of Schouenborg et al. (Schouenborg and Kalliomaki 1990; Schouenborg et al. 1992). However, other studies have shown that under certain conditions such knee extensor activation can be observed (Hagbarth 1952). It is therefore not clear if this co-contraction is due to the microstimulation techniques used in this study or to changes in the organization of spinal systems after spinalization. Although the broad similarities between microstimulation responses and spinal cutaneous systems suggest that spinal microstimulation may be activating neural pathways involved in the production of withdrawal reflexes, further work will be required to establish this connection more definitively.

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