Broadly Tuned Spinal Neurons for Each Form of Fictive Scratching in Spinal Turtles

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Berkowitz, Ari. Broadly tuned spinal neurons for each form of fictive scratching in spinal turtles. *J Neurophysiol* 86: 1017–1025, 2001. Behavioral choice can be mediated either by a small number of sharply tuned neurons or by large populations of broadly tuned neurons. This issue can be conveniently examined in the turtle spinal cord, which generates each of three forms of scratching—rostral, pocket, and caudal—in response to mechanical stimulation in each of three adjacent regions of the body surface. Previous research showed that many propriospinal neurons are broadly tuned to either the rostral scratch region or the pocket scratch region, but responses to caudal scratch stimulation could not be examined in that reduced preparation. In the current study, individual spinal neurons were recorded extracellularly from the gray matter of the turtle spinal cord hindlimb enlargement, while sites in the rostral, pocket, and caudal scratch regions were mechanically stimulated. Many neurons were broadly tuned to the caudal scratch region; other neurons were broadly tuned to either the pocket scratch or rostral scratch region. All three types were typically found within a single animal. These data are consistent with the hypothesis that the turtle spinal cord relies on large populations of broadly tuned neurons to select each of the three forms of scratching. In addition, neurons that were broadly tuned to each of the scratch regions were typically found in each spinal cord segment and within the same range of mediolateral and dorsoventral locations. Providing that these neurons are related to the selection and generation of the three forms of scratching, this would indicate that cells of this type are not segregated into distinct regions of the spinal cord gray matter.

INTRODUCTION

How do nervous systems integrate sensory information to generate an appropriate behavioral choice for each circumstance? In some cases, sensory signals are funneled into a single neuron or a small number of neurons that is dedicated to producing a single type of behavior (Croll et al. 1985; Edwards et al. 1999; Frost and Katz 1996; Huang and Satterlie 1990; Kovac and Davis 1980; Newsome et al. 1989; Salzman and Newsome 1994; Zottoli et al. 1999). Each such neuron is sharply tuned; that is, its receptive field is sufficiently precise to select reliably a single, appropriate behavior on its own. More commonly, however, a distributed population of broadly tuned neurons integrates sensory information and selects an appropriate behavior; the receptive field of each such neuron is insufficiently precise to select reliably an appropriate behavior on its own, but the neurons act as a population to encode a behavioral choice with the required precision (Berkowitz and Stein 1994a,b; Bosco and Poppele 1993; Bosco et al. 2000; Georgopoulos et al. 1986; Heiligenberg 1987; Kristan and Shaw 1997; Lee et al. 1988; Levi and Camhi 2000; Lewis and Kristan 1998; Liebenthal et al. 1994; Lockery and Kristan 1990; Miller et al. 1991; Ritzmann and Pollack 1988; Sparks et al. 1976, 1997; Westin et al. 1988). Most studies of population coding have focused on behaviors that can vary continuously, just as the activity in an array of sensory neurons can vary continuously. But can a population of broadly tuned neurons also select among a set of discrete behavioral choices?

This issue can be conveniently addressed by examining the spinal cord control of the three forms of scratching in turtles. Turtles respond to mechanical stimulation of their shell or skin by rhythmically rubbing their ipsilateral hindlimb against the stimulated site (Mortin et al. 1985). Due to biomechanical constraints, the turtle selects one of three forms of scratching—rostral, pocket, or caudal—to rub against each of three adjacent (and slightly overlapping) regions of the body surface (Fig. 1). The three forms of scratching differ in which part of the hindlimb contacts the body and in the timing of knee extension within the hip movement cycle (Mortin et al. 1985). Spinalized and immobilized turtles produce an appropriate form of fictive scratching for each site stimulated; knee extensor activity occurs in a distinct phase of the hip flexor activity cycle in each form of fictive scratching (Fig. 1) (Robertson et al. 1985); thus the spinal cord can perform the selection and generation of an appropriate form of scratching even in the absence of input from the brain and movement-related sensory feedback (Robertson et al. 1985).

Previous research demonstrated that there are populations of spinal interneurons that are broadly tuned to either the rostral scratch region or the pocket scratch region; sharply tuned neurons for either of these regions are rare or absent (Berkowitz and Stein 1994a). This suggested that the turtle spinal cord may rely on a distributed population of broadly tuned neurons to select each form of scratching. These experiments, however, utilized a reduced spinal cord preparation that did not include the spinal cord segments that innervate the caudal scratch region, so it could not be determined whether spinal neurons that respond to stimulation of the caudal scratch region are broadly tuned or sharply tuned.

Interneurons involved in caudal scratching might be sharply tuned, even though interneurons involved in rostral and pocket scratching are generally broadly tuned, for the following reason. Sensory innervation of the rostral scratch region is pro-

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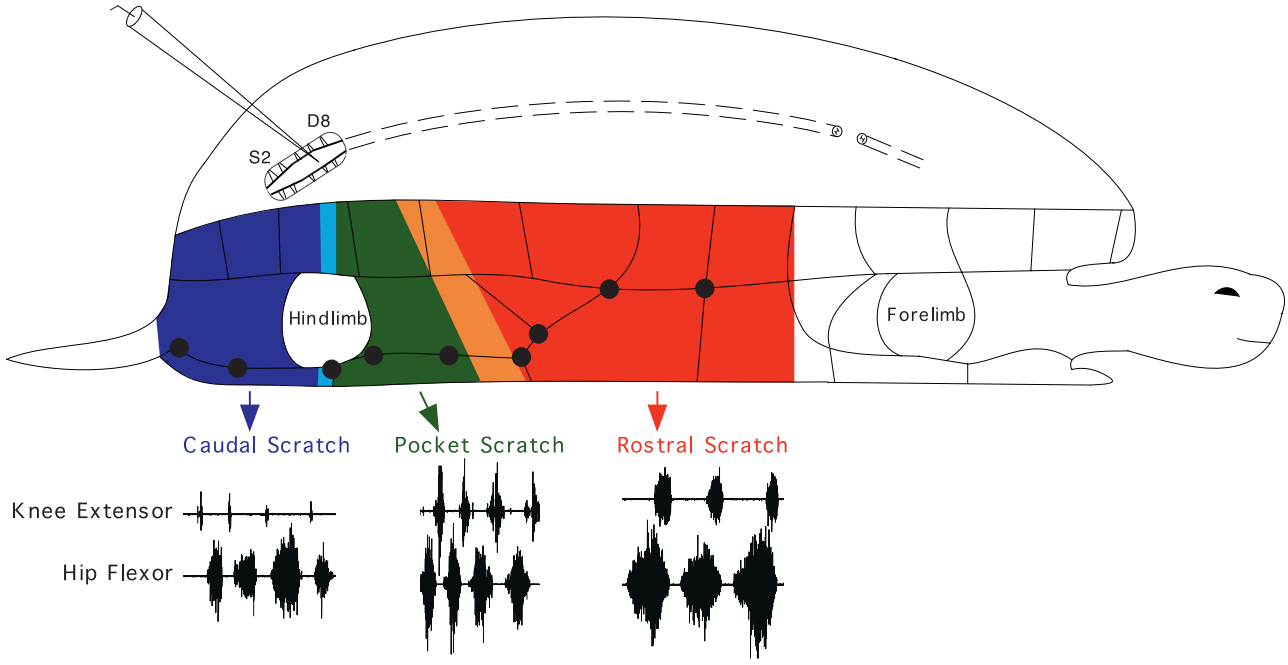


FIG. 1. Diagram of the preparation, showing the receptive fields for caudal scratching (blue), pocket scratching (green), and rostral scratching (red), along with the transition zones (teal and orange). Examples of the scratch motor patterns produced in an immobilized, spinal turtle are also shown; in all figures, "knee extensor" refers to FT-KE and "hip flexor" refers to VP-HP (see METHODS). Single units were recorded from the hindlimb enlargement (D_8–S_2) spinal cord. Usual sites of mechanical stimulation are indicated by black circles.

vided by the 3rd dorsal (postcervical) through 6th dorsal (D_3–D_6) spinal cord segments; sensory innervation of the pocket scratch region is provided by the D_6–D_8 segments (Mortin and Stein 1990). These spinal segments are adjacent and partly overlapping. In contrast, sensory innervation of the caudal scratch region is provided by the 2nd sacral (S_2) and more posterior segments (Mortin and Stein 1990); these segments are separated from the D_3–D_8 segments by three intervening segments that provide sensory innervation to the hindlimb instead. Thus spinal neurons that are activated by stimulation in the caudal scratch region might not be activated during other forms of scratching (i.e., might be sharply tuned), in contrast to neurons activated by stimulation in the rostral scratch and pocket scratch regions. Therefore one goal of the current study was to determine whether or not there is a population of spinal neurons that are broadly tuned to the caudal scratch region.

Previous work also examined the anatomical organization of spinal interneurons that are activated by stimulation of the rostral scratch or pocket scratch regions (Berkowitz and Stein 1994a). Recordings were made from the spinal white matter; the recorded axons did not appear to be arranged according to the region of the body surface to which they responded maximally. Nonetheless, the cell bodies of such neurons might be segregated within the gray matter. Thus a second goal of the current study was to determine whether the cell bodies of spinal neurons are arranged in the gray matter according to their sensory tuning.

These experiments have been reported previously in an abstract (Berkowitz 1999).

METHODS

Surgical procedures

Adult red-eared turtles (*Trachemys scripta elegans; n* = 16) of both sexes, weighing 500–1,500 g, were immersed in crushed ice for at least 2 h prior to surgery to induce hypothermic analgesia (Melby and Altman 1974). Each animal was kept partially submerged in crushed ice throughout surgery. The first surgical procedure on each animal was a dorsal laminectomy to expose the spinal cord just posterior to the forelimb enlargement; the spinal cord was then completely transected midway between the dorsal roots of the D_2 and D_3 spinal segments (Fig. 1). The exposed spinal cord was then covered with Gelfoam surgical sponge (Upjohn, Kalamazoo, MI) moistened with turtle saline (Stein and Schild 1989) and the opening sealed with warm dental wax glued to the shell with cyanoacrylate adhesive.

All remaining surgical procedures were carried out on regions of the body posterior to this complete spinal transection. A second dorsal laminectomy was performed to expose the spinal cord from the D_6 through the D_{10}, S_1, or S_2 spinal cord segments (Fig. 1). The meninges were torn over the dorsal surface of the spinal cord in just the region from which single spinal neurons were to be recorded in that animal. The spinal cord was then covered with saline-moistened Gelfoam. Finally, several hindlimb muscle nerves were dissected free from surrounding tissue through one or two additional exposures; each nerve was then tied with surgical suture near its entrance to the muscle and cut just distal to the tie, to prepare it for electroneurographic recordings (ENGs). The nerves prepared for ENGs were as follows: the hip flexor muscle nerve, VP-HP (innervating puboischiofemoralis internus, pars anteroventralis), on both sides of the body (or, in early experiments, on the right side alone), and three knee extensor muscle nerves, IT-KE, AM-KE, and FT-KE (innervating triceps femoris, pars iliotibialis, pars ambiens, and pars femorotibialis, respectively) on the right side (Robertson et al. 1985). FT-KE is the monoarticular knee extensor and provides a clear indication of the timing of knee extension (Fig. 1) (Robertson et al. 1985).

Recordings

The turtle was allowed to warm up to room temperature and was injected intramuscularly with 8–10 mg/kg of the neuromuscular blocker gallamine triethiodide (Flaxedil, Rhone-Poulenc Rorer Canada, Montreal, Canada); in some experiments, a combination of gal-

lamine triethiodide (Sigma, St. Louis, MO) and D-tubocurarine (Sigma, St. Louis, MO) was used instead. Warm dental wax was formed into water-tight wells surrounding each of the hindlimb nerve exposures and a third surrounding the posterior spinal cord exposure; these wax wells were glued to the shell with cyanoacrylate adhesive. The nerve wells were filled with mineral oil and the spinal cord well with turtle saline. The animal was intubated and maintained on an artificial respirator (Harvard Apparatus, Holliston, MA) for the remainder of the experiment. Dental wax was used to secure the immobilized animal's shell to a Plexiglas dish; the dish was bolted to a vibration isolation table (Newport Corporation, Irvine, CA).

ENGs were obtained via pairs of 100-μm silver wire, insulated except for 2–4 mm at the tip, immersed in the pool of mineral oil (Robertson et al. 1985). Single-unit recordings from spinal neurons were obtained via Woods metal-filled glass microelectrodes whose tips were plated with gold and then platinum (Frank and Becker 1964). The microelectrode was lowered into the right side of the hindlimb enlargement spinal cord using a piezoelectric microdrive (Burleigh Instruments, Fishers, NY). The segmental location (D_8, D_9, D_{10}, S_1, or S_2) and the mediolateral location (visually estimated as a percentage of the distance from the midline to Lissauer's tract, which are both visible on the dorsal surface of the spinal cord) of each microelectrode penetration were noted; the microelectrode depth was obtained from the digital readout of the microdrive, which was set to zero when the microelectrode first touched the surface of the spinal cord. Recordings were typically made in a single segment in each animal, at the rostrocaudal level of the dorsal roots; D_8 recordings were made in more animals than other segments for surgical reasons. Recordings were always made at depths of $<$1,200 μm and were made at depths of $<$900 μm for electrode penetrations of $>$60% laterality to avoid recording from motoneuron cell bodies. All isolated single units encountered were studied if they were activated during fictive scratching; stimulation in the caudal scratch region was usually used as a search stimulus. ENGs and single-unit recordings were amplified and filtered (100–1,000 Hz band-pass; A-M Systems, Carlsborg, WA). ENGs, single-unit recordings, and the force transducer output were digitized by pulse code modulation (DC-5 kHz band-pass) via an eight-channel Neurocorder video adapter (Cygnus Technology, Delaware Water Gap, PA) and stored on videotapes, along with a voice channel.

Stimulation

Mechanical stimulation (gentle rubbing at 2–4 Hz with approximately constant force for all sites stimulated, generally 0.8–1.2 N) was delivered via a glass probe with a fire-polished tip, attached to a force transducer (Grass-Telefactor, West Warwick, RI). Stimulation was delivered for 20–50 s to a single site to elicit several consecutive cycles of fictive scratching; 2-min breaks were provided between stimulations. Whenever possible, nine sites were stimulated on each side of the body, comprising an approximately posterior-anterior axis through the caudal scratch, pocket scratch, and rostral scratch regions (Fig. 1); consecutive stimulation sites were alternated among the three scratch regions.

Analysis

Each single-unit recording, along with the hip flexor ENG ipsilateral to the stimulation site, was redigitized (at 12.5 and 2.5 kHz, respectively) from videotape to a computer, using Datapac 2000 hardware and software (Run Technologies, Laguna Hills, CA); in some cases, additional ENGs were also redigitized (at 2.5 kHz/channel). For each single-unit recording, every available episode of mechanically elicited fictive scratching was redigitized and analyzed (a total of over 1,100 episodes of fictive scratching). Each hip flexor ENG was full-wave rectified and smoothed (with a 10-ms time constant); the onset and offset of each hip flexor burst was determined via user-defined positive and negative slope crossings, respectively, using Datapac 2000. When necessary, single-unit action potentials were isolated using a spike-sorting module of Datapac 2000, on the basis of clustering of waveform parameters. Each cycle of fictive scratching was defined to begin and end with the onset and offset, respectively, of a burst of the hip flexor nerve ipsilateral to the stimulation site. The single-unit firing frequency was calculated on a cycle-by-cycle basis as the total number of single-unit action potentials during the scratch cycle, divided by the cycle duration. For each stimulation episode, all cycles of fictive scratching conforming to a set of standard criteria were analyzed. These criteria were as follows: *1*) each cycle had to have a single clear hip flexor burst followed by a single clear hip flexor quiescent period, *2*) each cycle had to begin and end during the period of stimulation, *3*) the first cycle was always omitted, and *4*) any cycles that began more than 20 s after the beginning of the first acceptable cycle were omitted. The mean single-unit firing rate for each site was calculated as the average of the cyclical single-unit rates over all acceptable scratch cycles from one or more episodes of stimulation at that site. For some figures, instantaneous single-unit firing frequency is displayed; it was calculated as the reciprocal of the interspike interval.

Spinal units were classified as sharply tuned if they were activated by mechanical stimulation within the receptive field of only one form of fictive scratching. Units were classified as broadly tuned if they were activated by stimulation within the receptive fields of more than one form of fictive scratching and showed clear tuning; that is, they were most strongly activated by stimulation of one site and progressively less strongly activated by stimulation of progressively more distant sites. Broadly tuned units were further classified according to whether they were most strongly activated by stimulation within the ipsilateral caudal scratch, pocket scratch, or rostral scratch region; units that were activated approximately equally strongly by stimulation in two of the three ipsilateral regions or responded maximally to stimulation in the transition zone between two forms of ipsilateral fictive scratching (Mortin et al. 1985) were classified accordingly as caudal/pocket, pocket/rostral, or caudal/rostral. Units were classified as "contralateral only" if they were only activated consistently by stimulation in the contralateral scratch regions. The depth and laterality (see above) of the electrode tip during the recording of each tuned unit was plotted. Units were classified as untuned if they were activated by stimulation within all of the ipsilateral scratch receptive fields but showed no consistent change in firing rate as a function of stimulation location along the posterior-anterior axis of the body surface. Units were only included in this analysis if they were studied during stimulation of at least four ipsilateral sites including one site in each of the three scratch regions (before being lost).

Histology

In some experiments, electrolytic lesions were made by passing current from a Hyfrecator (Birtcher, El Monte, CA) through the recording electrode; sufficient current caused the gold/platinum electrode tip to detach and remain in place in the tissue. Animals were then given an overdose of pentobarbital sodium (1.0 ml of 390 mg/ml solution ip) and transcardially perfused 1–2 h later with 600 ml turtle saline (Stein and Schild 1989), including 0.1% sodium nitrite and 10,000 units/l heparin, followed by 200 ml fixative [4% paraformaldehyde, 1.25% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.4]. The spinal cord was postfixed overnight, transferred to 20% sucrose in PB for 4–8 h, and transferred to embedding medium (0.25% gelatin, 20% powdered egg albumin, 10% sucrose, in PB) overnight. The embedding medium was hardened by addition of glutaraldehyde to 2.5% and frozen-sectioned transversely at 60 μm on a sliding microtome. Sections were dipped in Albrecht's solution (0.75% gelatin in 40% ethanol) and mounted on gelatin-coated slides, then dried, fixed, lightly counterstained with cresyl violet, dehydrated, cleared, and coverslipped. Sections containing electrode tips were

traced using a drawing tube on a Nikon Optiphot-2 microscope and scanned for illustration.

These procedures meet guidelines of the American Physiological Society and were approved by the Institutional Animal Care and Use Committee.

RESULTS

The vast majority (48/56, or 86%) of the spinal units studied were broadly tuned. These neurons were activated by stimulation within the receptive fields for more than one form of scratching, but were maximally activated by stimulation within one region (or within 2 separate regions, in 2 cases) and were progressively less strongly activated by stimulation at sites progressively further away from this region, along an approximately posterior-anterior axis (Figs. 2 and 3). Of these broadly tuned neurons, 17 (35%) were tuned to an ipsilateral caudal scratch region (Figs. 2 and 3, *A* and *B*), 7 (15%) were tuned to a caudal/pocket region (i.e., maximally activated within both the caudal and the pocket region or in the transition zone between these regions; Fig. 3*C*, *4* and *5*), 8 (17%) were tuned to a pocket region (Fig. 3*C*, *6*), 3 (6%) were tuned to a pocket/rostral region (Fig. 3*D*, *7* and *8*), and 10 (21%) were tuned to a rostral region (Fig. 3*D*, *9*); in addition, 2 (4%) of the broadly tuned neurons were bimodally tuned to a caudal region and a rostral region (Fig. 4*A*), and 1 (2%) was activated only by stimulation of contralateral scratch regions.

Whenever several broadly tuned neurons were recorded from the same animal, they were tuned to quite different regions of the body surface. Figure 3 displays the tuning curves for nine broadly tuned neurons recorded from a single spinal segment of a single animal (the tuning curves are color-coded and arranged according to each neuron's tuning). Three of these neurons were caudal-tuned (Fig. 3, *A* and *B*, *curves 1–3*), two were caudal/pocket-tuned (Fig. 3*C*, *4* and *5*), one was pocket-tuned (Fig. 3*C*, *6*), two were pocket/rostral-tuned (Fig. 3*D*, *7* and *8*), and one was rostral-tuned (Fig. 3*D*, *9*).

Six (11%) of the spinal neurons studied were untuned (see METHODS). Only two neurons (4%) were sharply tuned; both of these neurons were tuned to a caudal scratch region (Fig. 4*B*).

The spinal segment, depth, and laterality (i.e., percentage of the distance from the midline to Lissauer's tract) of each recording location were noted (see METHODS). In each animal, units were typically recorded in the middle of one spinal segment, at the level of the dorsal roots, so there would be no

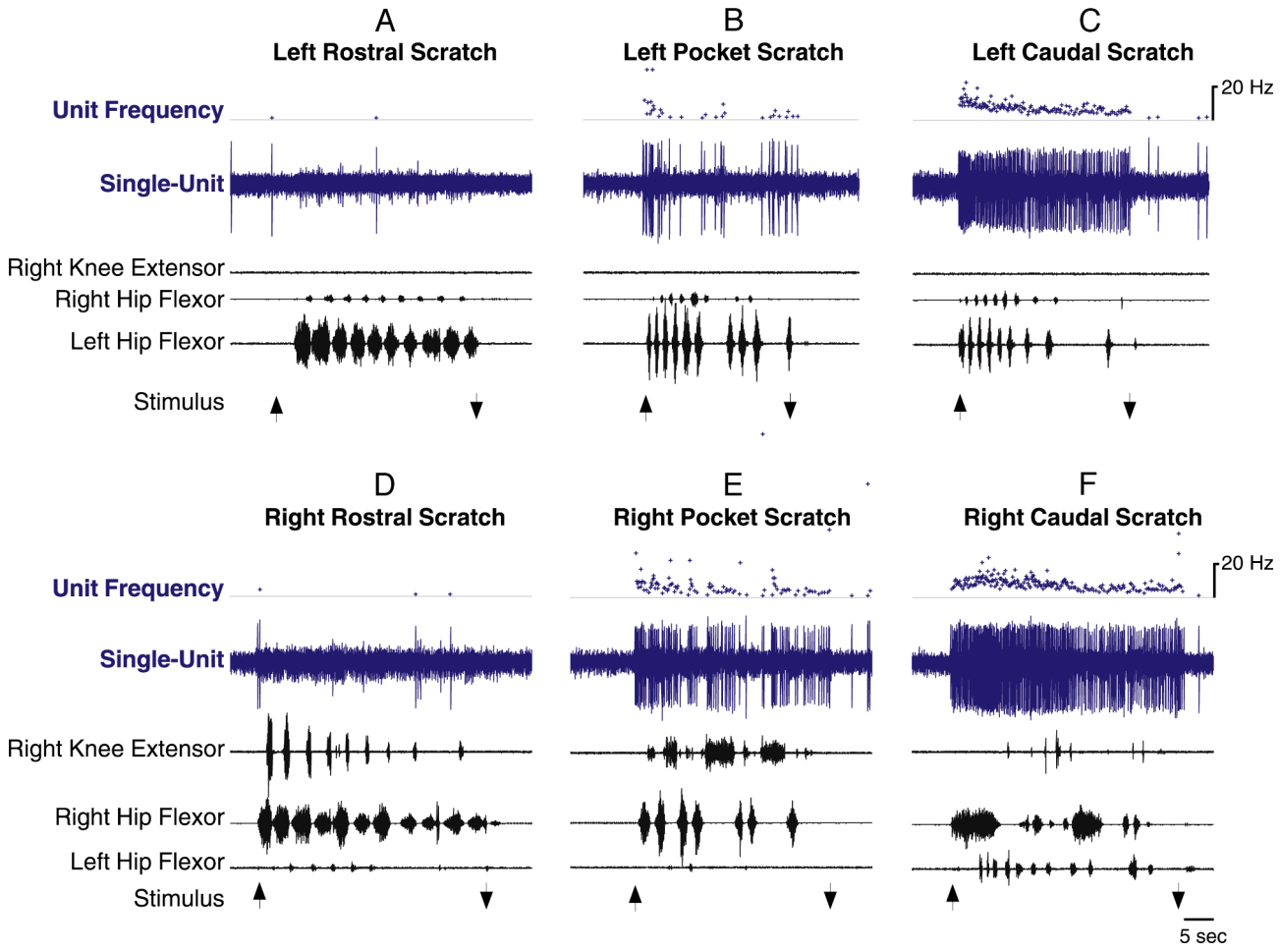


FIG. 2. Example of a spinal neuron that was broadly tuned to the caudal scratch regions. The raw single-unit recording and the instantaneous frequency of single-unit action potentials are shown in blue; the scratch motor patterns are shown in black. Note that this neuron was weakly activated by stimulation in the left rostral scratch (*A*) and right rostral scratch (*D*) regions, more strongly activated in the left pocket scratch (*B*) and right pocket scratch (*E*) regions, and still more strongly activated in the left caudal scratch (*C*) and right caudal scratch (*F*) regions.

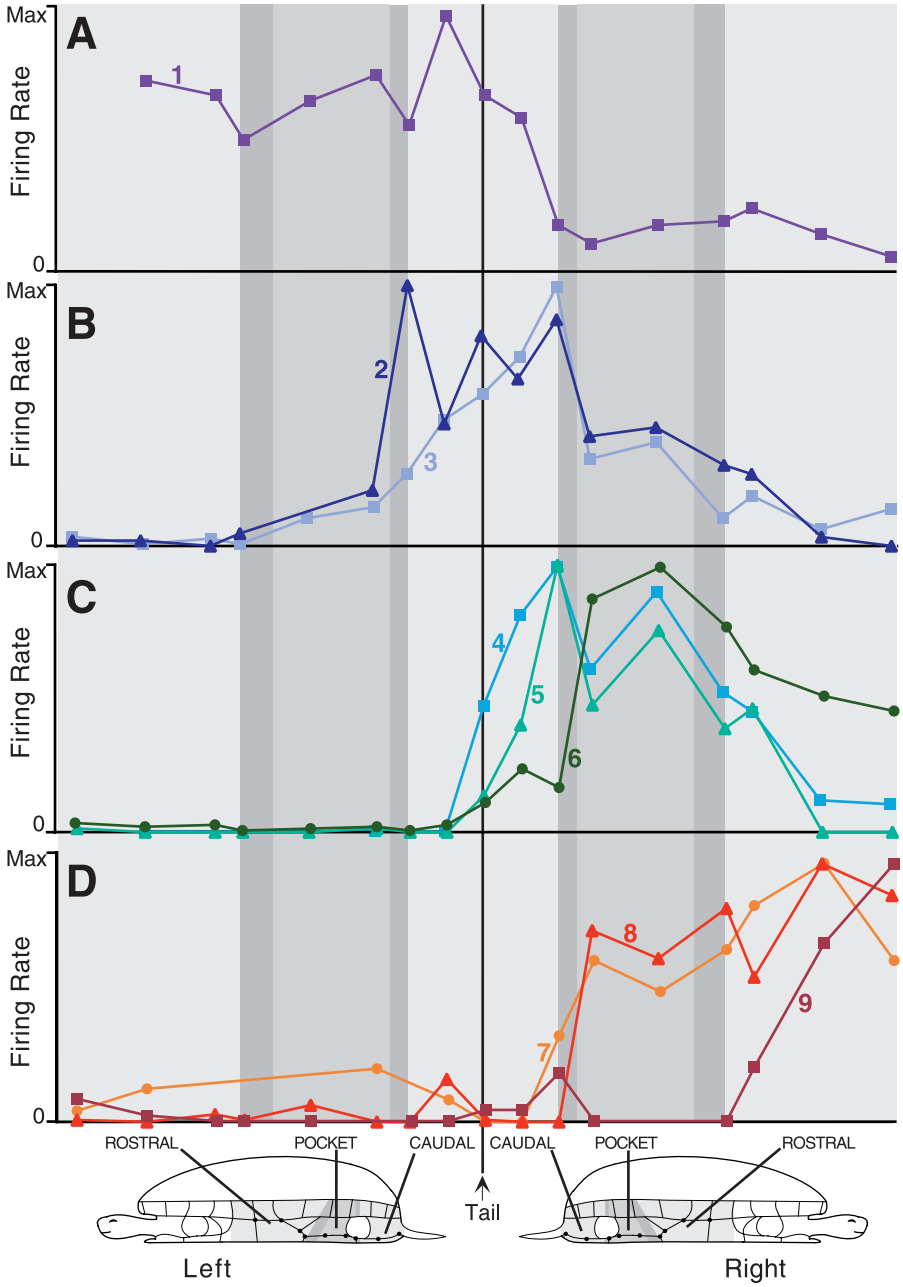


FIG. 3. Tuning curves for 9 broadly tuned neurons all recorded from the D_9 segment of a single animal. Tuning curves are numbered, colored, and arranged to illustrate the spectrum of tuning locations observed, from caudal (violet/blue) to rostral (red). *A*: caudal-tuned neuron (also activated by contralateral stimulation). *B*: 2 caudal-tuned neurons. *C*: 2 caudal/pocket-tuned neurons (*4* and *5*) and 1 pocket-tuned neuron (*6*). *D*: 2 pocket/rostral-tuned neurons (*7* and *8*) and 1 rostral-tuned neuron (*9*). Each curve was normalized to the maximum firing rate exhibited by the neuron (calculated as the average firing rate over all acceptable cycles at a given site of stimulation). *Curve 2* in *B* is from the neuron illustrated in Fig. 2. Vertical shading indicates receptive fields for each form of scratching as well as transition zones.

ambiguity about the segment of the recording. To compare the electrode depth measurements and laterality estimates to the results of histological localization, three electrolytic lesions were made at predetermined depths and lateralities in the D_9 segment of one additional animal (Fig. 5). Figure 5 suggests that the method of localizing the depth and laterality of recording sites was sufficiently precise to provide meaningful information on these locations, especially regarding depth.

The recording locations for all the broadly tuned and sharply tuned neurons are plotted in Figs. 6 and 7, with color-coded symbols that indicate the region of the body surface to which each neuron was tuned. The electrode depth and laterality of each recording is plotted; these plots are superimposed on schematic spinal cord cross sections to provide approximate orientation. The vast majority, if not all, of the neurons recorded are likely to be interneurons, rather than motoneurons, because recordings were made only at depths of $<1{,}200$ μm and neurons were only recorded at depths >900 μm if the electrode penetration was at $\leq 60\%$ laterality; turtle motoneuron cell bodies are generally located further ventrolaterally (Berkowitz and Stein 1994c and unpublished observations). The set of neurons recorded from each of the five spinal cord segments of the hindlimb enlargement included neurons tuned to a wide variety of regions of the body surface (Fig. 6). For example, both caudal-tuned and rostral-tuned neurons were found in every spinal segment, except D_{10} (from which only 3 tuned neurons were recorded). Caudal-tuned neurons were common in the D_8 and D_9 segments (the most anterior segments of the hindlimb enlargement) despite the fact that the caudal scratch region is innervated by the S_2 and more poste-

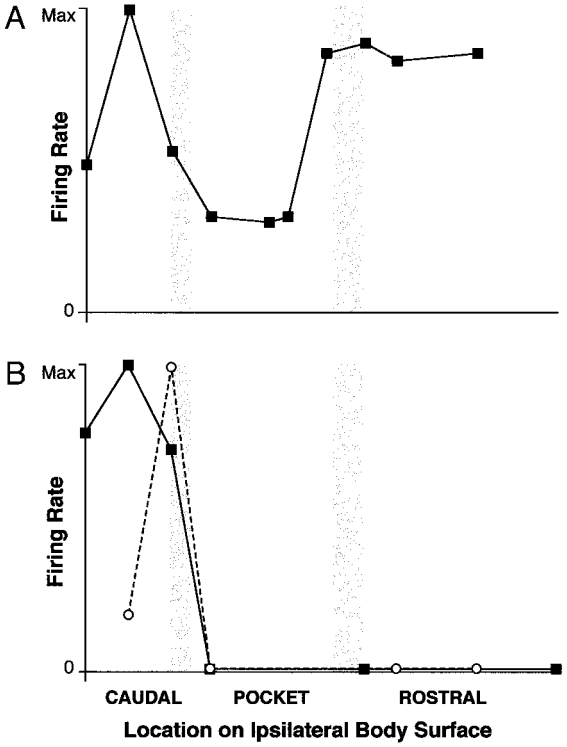


FIG. 4. Exceptional types of tuning. *A*: tuning curve of a neuron that was bimodally tuned to the caudal and rostral scratch regions. *B*: tuning curves of both sharply tuned neurons recorded; both were tuned to the caudal scratch region.

rior spinal cord segments (Mortin and Stein 1990), which are at least three segments away.

Figure 7*A* shows the depth and laterality of all single-unit recordings from tuned neurons, regardless of spinal segment, on a single plot. There appears to be no relationship between either the laterality or the depth of the recording and the region of the body surface to which the neuron was tuned. This is especially evident if one examines the average depth and laterality for all neurons tuned to a particular region (Fig. 7*B*); these averages overlap extensively and show no clear arrangement according to region of tuning. Electrode penetrations that were <30% lateral or more than 75% lateral recorded only very small action potentials, presumably from axons; these small spikes were not easily discriminated, and such units were not studied.

DISCUSSION

The central finding of this study is that many spinal neurons are broadly tuned to the caudal scratch region of the turtle's body surface; other spinal neurons are broadly tuned to the pocket scratch or the rostral scratch region instead. Spinal neurons tuned to each region are found in each animal. This finding extends previous research showing that many descending, propriospinal neurons are broadly tuned to either the pocket scratch or the rostral scratch region; these previous experiments were performed using a more reduced spinal cord preparation that did not include the spinal segments that provide sensory innervation to the caudal scratch region (Berkowitz and Stein 1994a). One cannot be certain, of course, that any one of the neurons recorded actively contributes to selection or generation of scratching motor patterns. The large proportion of spinal neurons that are broadly tuned to a scratch region, however, makes it likely that at least some of these broadly tuned neurons do contribute to selection and generation of scratch motor patterns. The current findings add support to the hypothesis that the turtle spinal cord selects an appropriate form of scratching for each stimulated site via the activity of large populations of broadly tuned neurons rather than via the activity of a small number of sharply tuned neurons (Berkowitz and Stein 1994a,b). This suggests that populations of broadly tuned neurons can mediate selection among several discrete behavioral choices, just as they have been shown to mediate the selection of continuously varying behaviors in other systems (Georgopoulos et al. 1986; Heiligenberg 1987; Kristan and Shaw 1997; Lee et al. 1988; Levi and Camhi 2000; Lewis and Kristan 1998; Liebenthal et al. 1994; Lockery and Kristan 1990; Miller et al. 1991; Ritzmann and Pollack 1988; Sparks et al. 1976, 1997; Westin et al. 1988). This same set of neurons may also mediate generation of the appropriate scratch synergy for each form of scratching (Berkowitz 2001).

Two of the 56 spinal neurons studied were sharply tuned; both of these were tuned to the caudal scratch region. Thus it is conceivable that the spinal cord relies on a small number of sharply tuned spinal neurons to select caudal scratching and that broadly tuned neurons are not necessary for caudal scratch selection. This seems unlikely, however, given that the vast majority of recorded spinal neurons activated by stimulation in the caudal scratch region were broadly tuned. It is intriguing, however, that the only neurons found to be sharply tuned to a scratch receptive field were both tuned to the caudal scratch region; this may relate to the fact that the spinal segments innervating the caudal scratch region are separated from the spinal segments innervating the rostral and pocket scratch regions by intervening spinal segments innervating the hindlimb (see INTRODUCTION) (Mortin and Stein 1990). Given this anatomical constraint, it is remarkable that so many of the neurons recorded were activated to some degree by stimulation

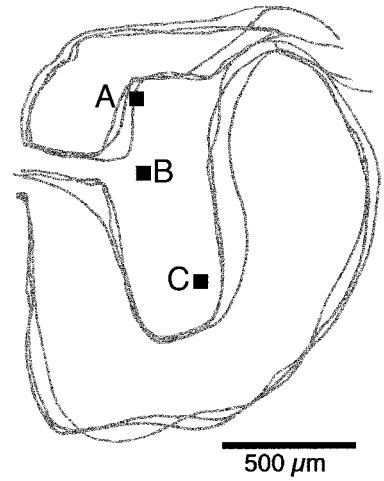
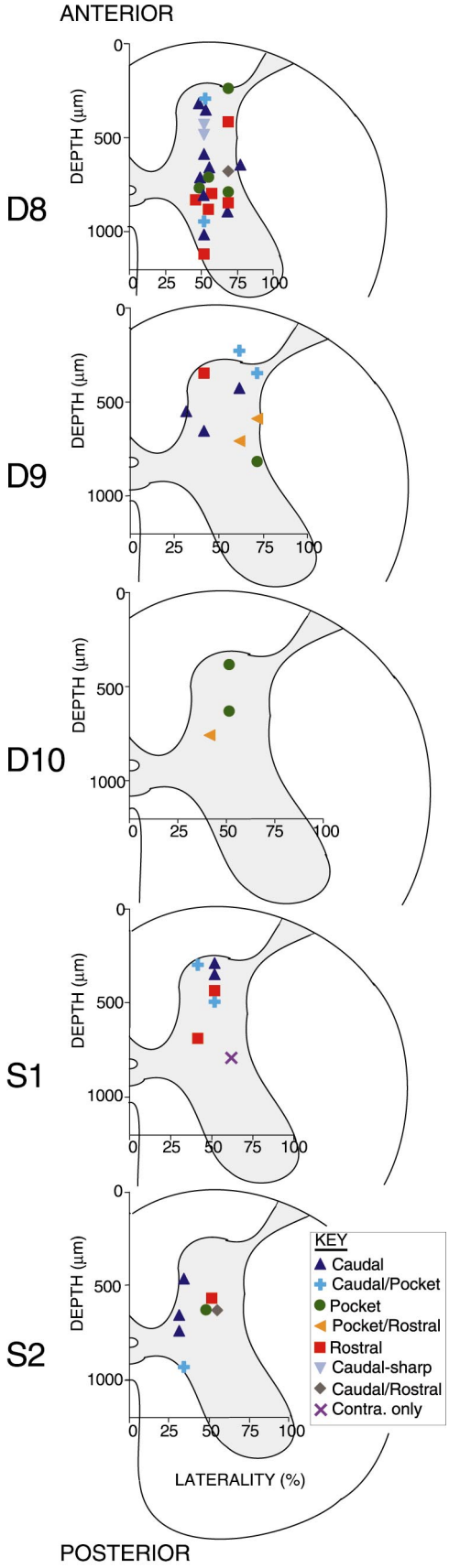


FIG. 5. Comparison of methods for localization of recording sites. The depth and laterality of each microelectrode were monitored as in other experiments (see METHODS). Electrolytic lesions were made at predetermined depths and lateralities in the D_9 segment of a single animal, and these sites were later examined histologically. Superimposed tracings of sections show 3 lesion sites (■) made at the following intended locations: *A*) 300 μm down, 30% lateral; *B*) 700 μm down, 50% lateral; *C*) 1,100 μm down, 70% lateral.



in both the caudal scratch region and the pocket scratch and/or rostral scratch regions. These broadly tuned spinal neurons must receive convergent input over long distances (directly or indirectly) from a large number of primary afferents, which themselves have very small receptive fields (Currie and Stein 1990). It is known that both turtle primary afferents (Kunzle and Woodson 1983) and turtle descending propriospinal neurons (Berkowitz and Stein 1994c) often have axons that project through several spinal cord segments.

A second finding of this study is that the spinal neurons activated during caudal, pocket, or rostral fictive scratching do not appear to be segregated according to the region of the body surface to which they are tuned; that is, the recorded cells do not appear to be somatopically arranged within the spinal gray matter. It is possible that some of these gray matter recordings were from dendrites or axons, rather than cell bodies. These electrodes recorded only very small spikes from the putative white matter, however, suggesting that the larger spikes of the units studied are unlikely to be from axons.

The companion paper (Berkowitz 2001) shows that the rhythmicity of this same set of units is clearly correlated with electrode depth; this suggests that the lack of organization of neurons according to region of maximal response is a reflection of the actual layout of the spinal cord, rather than a reflection of insufficient precision in the localization methods employed. This finding adds to previous research that showed that the descending axons of propriospinal neurons activated during pocket and rostral fictive scratching do not appear to be somatopically arranged within the spinal white matter (Berkowitz and Stein 1994a). If such neurons indeed contribute to selection of a form of scratching, these two studies together suggest that the spinal cord interneuronal circuitry used to select and generate distinct behaviors may be located in overlapping or identical spinal cord regions, rather than being segregated into a separate spinal cord region for each behavior or for each region of the body surface. The distribution of spinal neurons activated during turtle fictive scratching in this study is also consistent with the distributions of spinal neurons activated during cat fictive scratching in previous studies (Barajon et al. 1992; Berkinblit et al. 1978).

The finding that neurons tuned to different scratch receptive fields may not be segregated in distinct zones of the spinal gray matter appears to conflict with findings that activation of any one of a small number of anatomical zones in the frog spinal cord generates a convergent force field that would move the ipsilateral hindlimb to a particular location in the workspace (Bizzi et al. 1995; Saltiel et al. 1998). In addition to the species difference, however, there are a number of differences in

FIG. 6. Recording locations of all broadly tuned and sharply tuned units. For each spinal segment of the hindlimb enlargement (D_8, D_9, D_{10}, S_1, and S_2), the depth and laterality of the electrode tip is plotted for all single units recorded in that segment in all animals (see METHODS); these plots are superimposed on a schematic cross section of the right side of the corresponding spinal cord segment to provide approximate orientation. Symbols indicate the region to which each neuron was tuned and whether it was broadly tuned or sharply tuned; the key is displayed on the S_2 cross section. Caudal/Pocket, Pocket/Rostral, and Caudal/Rostral refer to neurons that were tuned to a transition zone between scratch receptive fields or were bimodally tuned (see METHODS). Caudal-sharp neurons were the only sharply tuned neurons; all others shown were broadly tuned. Note that neurons tuned to any particular region of the body surface were recorded in a wide variety of locations in the spinal cord. All D_9 units were recorded from a single animal; their tuning curves are shown in Fig. 3.

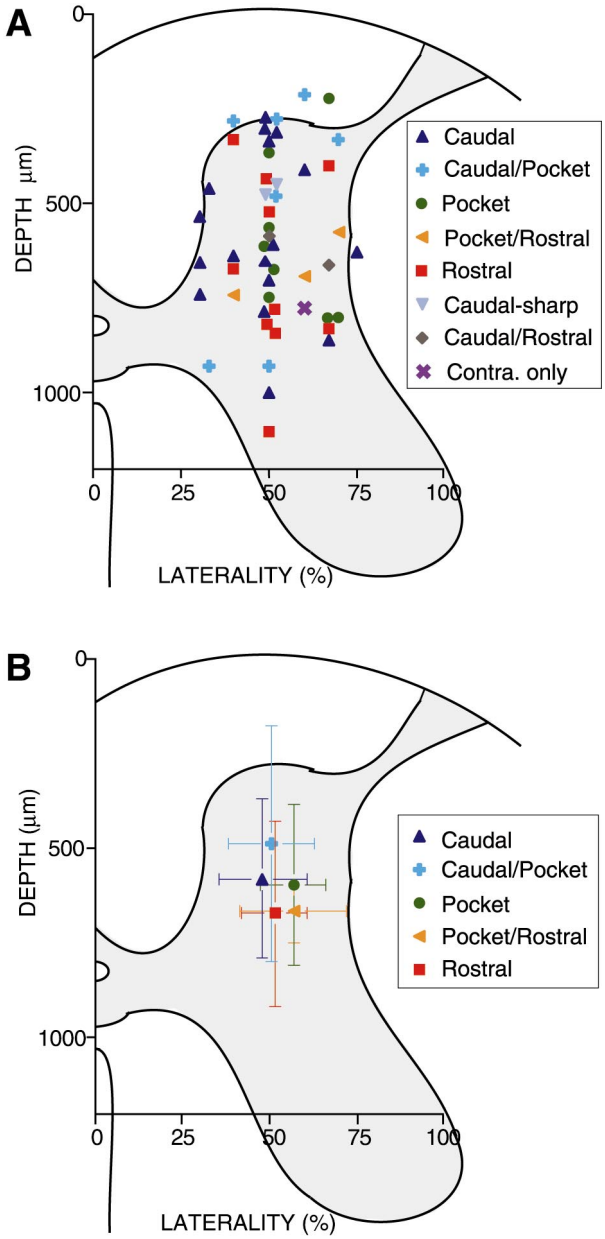


FIG. 7. Summary of recording locations. *A*: recording locations for all broadly tuned and sharply tuned units, regardless of spinal cord segment, are displayed on a single plot of depth vs. laterality, superimposed on a schematic cross section of the right D_9 segment to provide approximate orientation. *B*: average depth and laterality of recording locations for each of the 5 major types of broadly tuned neurons. Error bars indicate the standard deviation. Note that the averages are near each other and show no clear arrangement according to their tuning; error bars overlap extensively.

methods that might account for this apparent conflict. The frog experiments used electrical (Bizzi et al. 1995) or chemical (Saltiel et al. 1998) stimulation of a spinal cord region to generate types of movement that may or may not normally occur; the current experiments used adequate stimulation in the scratch receptive fields to generate fictive scratching. It is not clear how the population of neurons activated by electrical or chemical stimulation of a region relates to the population of neurons activated when the animal moves its limb in a particular direction during a natural behavior.

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