Correlation of Peripheral Innervation Density and Dorsal Horn Map Scale

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Wang, Lei, Ronald Millecchia, and Paul B. Brown. Correlation of peripheral innervation density and dorsal horn map scale. J. Neurophysiol. 78: 689–702, 1997. Dorsal horn map scale and peripheral innervation density were compared to test a hypothesized linear relationship. In anesthetized cats, low-threshold mechanoreceptive peripheral nerve innervation fields (IFs) were measured by outlining areas of skin from which action potentials could be elicited in cutaneous nerves. The same nerves were processed histologically and used to count myelinated axons. Innervation density for each nerve was calculated as number of axons divided by IF area. Single units were recorded throughout the hindlimb representation, in laminae III and IV. These data, combined with single-unit data from other animals and with cell counts in laminae III and IV, permitted estimation of numbers of cells whose receptive field centers fell in contiguous 1-cm bands from tips of toes to proximal thigh. A similar estimate was performed with the use of the nerve innervation data, so that peripheral innervation densities and map scales for the different 1-cm bands of skin could be compared. Correlation between the two was quite high (r = 0.8), and highly significant (P = 2.5 × 10⁻⁷). These results are consistent with a proposed developmental model in which map scale, peripheral innervation density, and reciprocal of dorsal horn cell receptive field size are mutually proportional, as a result of developmental mechanisms that produce constant divergence and convergence between primary afferent axons and dorsal horn cells.

INTRODUCTION

In central somatosensory nuclei, peripheral innervation density [number of axon receptive fields (RFs) per unit area of skin] is positively correlated with map scale (number of central cell RFs per unit area of skin) and negatively correlated with RF size (e.g., Mountcastle 1957). Such correlations are of functional importance because they are believed to be related to tactile acuity, which is highest for the most densely innervated regions of skin (e.g., Weinstein 1968). They also provide clues to the organizational rules underlying development of the somatotopic map and assembly of RFs. We have suggested that a simple developmental rule would produce these correlations, namely invariance of primary afferent divergence (number of cells that can be excited by a single axon) regardless of afferent RF location, and invariance of dorsal horn cell convergence (number of axons that can excite a single cell) regardless of location of the cell in the dorsal horn map of the skin (Millecchia and Brown 1993). That is, although convergence may vary among cell types (e.g., spinocervical vs. spinothalamic cells) and divergence may vary among afferent types (e.g., slowly vs. rapidly adapting mechanoreceptors), neither would vary as a function of somatotopic location. One mechanism that can lead to invariant convergence and divergence is a competitive process such as Hebbian activity-dependent self-organization (Hebb 1949; Whitelaw and Cowan 1981; Willshaw and von der Malsburg 1976).

In our proposed sequence of developmental events (Brown et al. 1997), hindlimb primary afferents establish mediolateral (ML) gradients of somatotopy at their level of entry into the spinal cord by sorting their projections along the ML axis of the dorsal horn in the order of the distoproximal locations of their RFs on the skin. The slope of the presynaptic somatotopic gradient at each rostrocaudal (RC) level would therefore be determined by the relative abundance of RFs at different distances from the tips of the toes. We suggest that this presynaptic somatotopy is reflected in postsynaptic somatotopy of dorsal horn cell RFs established by initial axonal contacts of locally ingrowing primary afferent axons with dorsal horn cell dendrites (prototype RFs). This primary afferents grow collaterals along an RC course, connecting to cells within whose prototype RFs their own RFs are included. Finally, the RFs may be refined by a competitive mechanism that leads to constant divergence and convergence.

One piece of evidence we used to justify this hypothesized sequence of events was the apparent constancy of convergence and divergence in the adult (Brown et al. 1997). Constant convergence and divergence will produce the correlations among peripheral innervation density, map scale, and RF size that have been reported for somatosensory nuclei. With the use of indirect methods of calculation, we estimated the peripheral innervation density of the cat hindlimb low-threshold cutaneous mechanoreceptors from our sample of dorsal root ganglion cells and published cell counts in laminae III and IV, the laminae that receive almost exclusive input from cutaneous low-threshold mechanoreceptors (LTMRs) and in which the majority of terminals of these mechanoreceptive axons are found (e.g., Brown et al. 1977, 1978, 1980, 1981). There was a significant correlation between map scale and innervation density.

Because these were very indirect methods of calculation, based on data that were not gathered specifically for the purpose of testing this hypothesis, we wished to examine the relation between map scale and innervation density more directly with the use of more appropriate data. In this paper we report the results of such measurements. A preliminary report has been published elsewhere (Brown et al. 1994).
METHODS

Experimental design

Two sets of experiments were performed, designed to determine peripheral innervation density and dorsal horn map scale for different areas of skin. To correlate map scale with innervation density, it was necessary to calculate these two variables for corresponding sets of skin areas. We know from earlier studies that innervation density and map scale both vary as a function of distance from toes, and that there is a high correlation between the two when they are calculated indirectly for 1-cm circumferential bands of skin at 1-cm intervals along the distoproximal axis, from toes to hip (Brown et al. 1997). We therefore calculated innervation densities and map scales, with the use of more direct measures, for the same 1-cm bands of skin. The 1-cm bands are illustrated in Fig. 1.

To calculate innervation densities, we mapped the innervation fields (IFs) of the cutaneous nerves of the hindlimb and counted the number of myelinated axons in each nerve. This provides an approximation of the number of Aβ-LTMRs in each nerve. Innervation density for each nerve was calculated as the number of myelinated axons divided by the area of the IF. This was plotted as a function of distance of the center of the IF from the tips of the toes. For comparison with map scale, innervation densities for 1-cm bands of skin were calculated with the use of exponential smoothing (see APPENDIX for details of exponential smoothing).

Map scales were calculated by determining the numbers of dorsal horn cells whose RF centers fell in the different 1-cm bands on the leg and dividing by the areas of the 1-cm bands. Innervation densities were then estimated for the same 1-cm bands from the innervation density data for the peripheral nerves. Innervation density and map scale for each 1-cm band were plotted on a scattergram of map scale as a function of innervation density, and the linear correlation coefficient was calculated for these paired variables.

Experimental procedures

Subjects were adult cats (2–4 kg) of either sex. The experimental preparation was different for the two sets of experiments. We describe the procedures for nerve recording first, and then the differences in procedure for the dorsal horn cell recordings.

INNERVATION DENSITY STUDY. Animals were anesthetized with 35 mg/kg ip pentobarbital sodium. Tracheal, carotid, and external jugular cannulas were installed for artificial respiration, blood pressure monitoring, and infusion of liquids and drugs, respectively. Surgical anesthesia was maintained with supplements of barbiturate whenever nociceptive reflexes could be detected, the pupils were responsive to light, or the blood pressure responded to noxious stimuli. The fur of both hindlimbs was clipped to a length of ~1 mm. A rectal probe provided feedback control for a temperature-controlled heating pad and heating lamps, used to maintain core temperature at 38°C.

All of the major cutaneous nerves of the hindlimbs (Reighard and Jennings 1935) were mapped and their myelinated axons were counted. Nerves sampled included the following: anterior femoral cutaneous; lateral femoral cutaneous; posterior femoral cutaneous; saphenous; medial and lateral sural; medial and lateral plantar; and superficial peroneal. Several of these nerves have subdivisions that were also sampled to obtain innervation densities of smaller areas of skin. These included branches of saphenous, posterior femoral cutaneous, and dorsal proper digital nerves 2, 2-3, 3-4, 4-5, and 5, and plantar proper digital nerves 2, 2-3, 3-4, 4-5, and 5. At least two examples of each nerve were studied to obtain average innervation densities.

Each nerve selected for study was exposed at the bottom of a mineral oil pool and placed on bipolar hook electrodes attached to a differential amplifier (Grass, Quincy, MA). Signals were amplified 100,000 times, with band pass 300 Hz–3 kHz, visualized on an oscilloscope, and made audible with an audio monitor. This recording setup permitted resolution of single action potentials in single Aβ-axons, even in nerves with >1,000 Aβ-fibers (Brown et al. 1973). This allowed mapping the cutaneous nerve IF with a resolution of one afferent RF. Fortunately, for purposes of calculating innervation density, these cutaneous nerves have nonoverlapping LTMR IFs (Koerber and Brown 1982). IFs were mapped by gently probing the clipped mat of fur and the underlying skin with small hand-held probes, moving in and out of the area that elicited audible responses on the audio monitor and marking this boundary on the skin with a marker pen. In our experience, when different experimenters independently mapped IFs in this manner, boundaries were reproducible within ~1 mm.

The IF outlines on the skin were copied to standard leg drawings to demarcate their relative positions on the leg, with the use of bony landmarks as reference points. To obtain accurate measurements of IF areas, they were also traced onto flexible plastic wrap and traced from the flexible wrapping material onto transparency sheets. Areas were calculated by cutting out the IF tracings and weighing the cutouts, converting to units of cm² with the use of a set of calibration cutouts of known areas. The geometric center of each IF was determined by balancing the cutout on a pin.

As many nerves as possible were recorded in each animal, the main limitation being the damage to skin caused by the nerve exposures. After nerve recording was completed, 3- to 10-mm segments of recorded nerves were saved in 10% Formalin for ~1 wk. Nerves were embedded in paraffin, sectioned at 6–10 μm, and stained with hematoxylin/eosin and Fast Green (trichrome stain).
A single well-stained cross section of each nerve was viewed under high-magnification light microscopy, and its image was superimposed on the view of a sheet of paper with a camera lucida. Myelinated axons were marked on the paper in one pass and the nerve was scanned for unmyelinated myelin rings in a second pass. The marks were counted and marked again in a second color, and the camera lucida marks were checked to make sure all were double-marked in a second pass.

Peripheral innervation density was calculated as number of myelinated fibers, in units of nerve fibers per cm² of skin. This was plotted as a function of the distance of the IF geometric center from tips of toes.

MAP SCALE EXPERIMENTS. Procedures for the mapping experiments differed in the following respects.

The animal was mounted in a spinal frame and headholder, in traction, on a vibration isolation table. Spinal cord segments L₂–S₁ were exposed and covered with mineral oil. A bilateral pneumothorax was performed, and the animal was paralyzed with gallamine triethiodide (initial dose 20 mg, supplemented with 10 mg every half h). End-expiratory CO₂ was maintained at 3.5–4.0% with a respirator. During paralysis, pupillary responses to light and blood pressure responses to noxious stimuli were used to assess anesthetic level, and the paralyzing agent was allowed to wear off occasionally to test for withdrawal reflexes.

Single units were recorded in the dorsal horn with stainless steel microelectrodes (FHC, Brunswick, ME) attached to the high-impedance headstage of an electrometer. After AC amplification, action potentials were displayed on an oscilloscope and made audible with an audio monitor. The electrode was advanced by a micro-manipulator (Inchworm, Fishers, NY) mounted on a spinal arch (Robert Clark Instruments, Edinburgh, UK) during manual exploration of the skin of the hindlimb. When postsynaptic “hash” was detected, an attempt was made to isolate a single unit by moving the electrode up and down, seeking a single-unit potential that exceeded background noise. Units were studied if they had RFs larger than the RFs of primary afferents and responded to low-threshold cutaneous mecanorceptor input.

When an extracellular single unit was detected, the RF was mapped with the use of small probes to move the clipped hairs and to indent the skin gently. By moving the stimulus from within the excitatory response zone to the edges of the response zone and back, as judged by the sound of elicited action potentials on an audio monitor, the edges of the low-threshold excitatory RFs were determined with a precision of ~1 mm. No attempt was made to characterize mecanorceptor convergence or to map nociceptive or inhibitory RF components. After the RF was traced on the skin, it was copied to a standard leg drawing with the use of bony landmarks as reference points.

In each electrode track, only one single unit was recorded, at a depth corresponding to laminae III-V. A microlesion was produced by passing current through the microelectrode (~1 μA, 500/s square wave, 30 s). This produced microlesions 20–50 μm diam and left the recording capacity of the microelectrode unimpaired. Tracks were placed at segmental boundaries and at midsegment, in rows of tracks starting lateral to the dorsal root entry zone and spaced at intervals of 200 μm across the dorsal horn, on both sides of the spinal cord.

At the ends of experiments, animals were injected with 1,000 U heparin and perfused transcardially with 1 L 0.9% saline with 0.1% procaine hydrochloride and 0.1% NaNO₃ at 38°C, followed by 1 L 10% Formalin in saline at room temperature. Tissue was fixed for ~1 wk, embedded in paraffin, serially sectioned in the transverse plane at 25 μm, stained with Klüver-Barrera stain (Luxol Fast Blue and Cresyl Echt Violet), and scanned under dark field for microlesions. Reconstructed recording sites were recorded to the nearest 0.01 segment (e.g., L₇₋₅ = 1/4 segment caudal to the L₄–L₅ junction) and 0.01 dorsal horn width (e.g., 0.75 = 3/4 of the way from medial to lateral edge of dorsal horn).

IF outlines were digitized with the use of a graphics digitizer, and computer software was used to determine the area (cm²) and distance of the geometric center from tips of toes for each IF. A modified DORSALVIEW program (Brown 1986) was used to produce reconstructions of the electrode tracks and drawing maps in the horizontal plane.

Data from each animal were shifted rostrocaudally to provide the best fit with the “average animal” from these experiments and from earlier studies (Koerber and Brown 1995; Koerber et al. 1993). The average animal consists of a model of the somatotopy of the dorsal horn, mapping the distance of RFs’ distance from tips of toes, D, as a function of ML and RC location in the dorsal horn (RC, ML). This procedure, which minimizes the effect of random rostral and caudal shifts of the map from one animal to another, has been described in detail elsewhere (Koerber et al. 1993) and has been further refined for this study. The model map is now obtained by exponential smoothing of D (RC, ML). Exponential smoothing has the advantage of not requiring a choice of analytic function to which the data are to be fitted, a weakness of the earlier approach. Details are provided in the APPENDIX.

The shifted data were used to compute the probabilities of cells having RF centers within 1-cm bands on the leg, starting at the tips of the toes and progressing proximally in 1-cm steps. These probabilities were computed for a grid of locations in the plane of the map, in steps of 0.1 segment and 0.05 ML width of the dorsal horn. The probability in each grid square was determined as the fraction of cells whose RF centers fell within the 1-cm bands with the use of exponential weighting of all cells to compute the value for each grid square. Details are provided in the APPENDIX.

The stained sections of two of the animals in the cell recording study were used to count cells in laminae III and IV. Sections were selected at 10-section intervals, skipping to the nearest undamaged section in the case of sections damaged by electrode tracks. A camera lucida was used to draw outlines of these laminae at ×100 and to plot the locations of cells. Cells were examined at ×400 to determine whether they contained nucleoli. Cells were counted as neural somata if they had nucleoli and cytoplasm with Nissl substance. Locations of cells in each animal were shifted by the previously determined best shift for that animal.

Cells’ locations were entered into a data base according to RC, ML locations. These data were used to estimate cell densities in the grid squares, with the use of exponential smoothing, as described in the APPENDIX.

The number of neurons at each grid square that had RF centers in the 1-cm band of skin was then calculated by multiplying the probability by the density at each grid point. Summing over all grid squares, it was possible to solve for the number of cells with the use of exponential smoothing of the data for the different nerves (see APPENDIX). We then plotted map scale as a function of innervation density for each 1-cm band in a scattergram, and calculated the linear correlation coefficient.

RESULTS

Periperal innervation density

Fifty-three cutaneous nerves were characterized in six cats. No spontaneous activity was observed, nor were there

Correlation of map scale and innervation density

To correlate map scale with peripheral innervation density, we estimated innervation density for each 1-cm band with the use of exponential smoothing of the data for the different nerves (see APPENDIX). We then plotted map scale as a function of innervation density for each 1-cm band in a scattergram, and calculated the linear correlation coefficient.
any responses to muscle stretch, indicating that the nerves were purely cutaneous. Figure 2 graphs innervation density \( \rho_{\text{nerve}} \) as a function of \( D \), for all nerves characterized. The distribution of \( D \) is nonuniform. It is clear from inspection that large values of \( \rho_{\text{nerve}} \) are clustered between 0 and 7 cm (a little more than halfway up the foot), and smaller values are distributed from 7 cm to the proximal thigh. The peripheral innervation density is \( \sim 4 \) times higher at the toes than at the knee, with the steepest decline around the heel.

**Map scale**

A total of 96 dorsal horn cells was characterized and their recording sites were reconstructed in three animals. Combining these results with the normal cells of Koerber and Brown (1995) and Koerber et al. (1993), 357 single-unit recordings were available from segments L4 to S2 in 16 animals. These data were used for RC alignment of animals (see APPENDIX for a detailed description). Briefly, the data for all animals were combined to determine average (“predicted”) ML gradients of somatotopy in terms of RF distance from tips of toes (\( D \)). Then the data for each animal were shifted in trial shifts of 0.1 segment rostral and caudal to find the “best shift,” namely that shift at which observed \( D \) values for that animal had the least mean squared deviation from predicted \( D \) at the same ML, RC locations. The data from all animals were shifted according to each animal’s best shift and recombinated to obtain a new model of predicted \( D \). The process of finding best shifts, shifting and recombinating data were repeated until the best shift for each animal was zero. The shifts converged to zero in four iterations.

The reconstructed recording sites and low-threshold RF outlines of the 96 cells characterized in this study were extracted from the combined data set and used to construct the map of Fig. 3. This is a dorsal view of the left dorsal horn with rostral toward the top, lateral to the left. Each drawing is placed so the center of the single unit’s RF falls at the RC and ML location of the recording site reconstruction. RC locations are indicated as fractional distance from the rostral end of each segment, \( X \), to the caudal end, \( (X + 1)0.0 \). ML locations are indicated as fractional distance from medial to lateral edge of the lamina in which the recording site was found. Although a few cells were recorded in laminae V and VI, the great majority were in laminae III and IV, and none were recorded in lamina I or II.

After all animals had been shifted for least mean square deviation of \( D \), the exponential smoothing procedure was used to generate a model surface of \( D \) as a function of RC, ML. This surface is illustrated in Fig. 4. The “average map” represented by this model reproduces the findings of previous studies with regard to \( D \) as a function of RC, ML: at every RC, there is a monotonically increasing \( D \) as a function of ML, and \( D \) (ML) is different at different RC. Most notably, the foot representation is enlarged in medial L6 and L7, as reflected by the depression in this portion of the surface. This means that a disproportionately large portion of the map is occupied by cells whose \( D \) is \( < 12 \) cm.

The elevation of Fig. 4 is a plot of average \( D \) as a function of RC, ML. For purposes of calculating map scale, we need the probability of a cell having a \( D \) falling in specific 1-cm ranges (a measure of the fraction of cells in such ranges) as a function of RC and ML, \( P(D, \text{RC, ML}) \). These were estimated by exponential smoothing for a set of \( D \) ranges spanning the entire leg (See APPENDIX for details). Probability surfaces are plotted in Fig. 5 for each 1-cm band. Note that, although the peaks of these elevations are in good agreement with the average \( D \) map of Fig. 4, the RC, ML distribution of cells with a given \( D \) is relatively large, meaning the map is not a perfectly “focused” representation of \( D \). In other words, there is some variance of \( D \) (RC, ML).

To compute map scale for different portions of the leg, lamina III-IV cells were counted in two animals for which best shifts had been determined on the basis of single-unit RFs. These counts were shifted by the best shifts, combined, and plotted as a density map, where density of cells per grid square \( \rho_{\text{cells}} \) (RC, ML) is plotted as the elevation in a three-dimensional normalized dorsal view of the dorsal horn, in Fig. 6 (See APPENDIX for details) before and after shifting. Volume densities in the cross sections did not appear to vary as much as the plot indicates. The hump in the plot reflects a number of factors: 1) laminae III and IV together have a relatively flat lower boundary (IV-V boundary) and a convex upper (II-III) boundary, so there is a greater depth of tissue in the middle of the dorsal horn; 2) the dorsal horn is wider and deeper in L6 and L7, resulting in higher cell counts in those segments; and 3) the surface is not a plot of density per unit volume in the dorsal horn—it is a plot of cell density per unit RC, ML grid square, where the grid squares are constant fractions of segmental widths and lengths (i.e., segmental widths and lengths are normalized).

Having computed \( P(D, \text{RC, ML}) \) and \( \rho_{\text{cells}} \) (RC, ML), it was then possible to cross-multiply the surfaces to obtain the expected densities of cells in each range of \( D \) at each RC, ML, \( \rho_{\text{map}} \) (see APPENDIX for details). These were calculated for all \( D \) ranges and the surfaces are plotted in Fig. 7 for each 1-cm band. Although the detailed shapes of the surfaces differ from those of Fig. 5, the locations of the maxima are approximately the same.

Map scale for each 1-cm band on the leg could now be calculated by summing the values at all the grid points on the surfaces of expected cell numbers as a function of RC, ML and dividing by the areas of the bands of skin. These quotients are plotted as a function of \( D \), \( \rho_{\text{map}} (D) \), in Fig. 8.
8. Note that, as for the peripheral innervation densities, the highest values, in neurons per cm$^2$ of skin, are found in the range of $D = 0$–12 cm, and lower values are found at higher $D$.

**Correlation of innervation density and map scale**

The simplest way to compare $\rho_{\text{nerve}} (D)$ and $\rho_{\text{map}} (D)$ is to correlate the values of matched pairs at identical $D$s. Map scale $\rho_{\text{map}} (D)$ was obtained at uniform intervals of $D$, which would be advantageous in terms of avoiding bias in favor of any part of the leg, but $\rho_{\text{nerve}} (D)$ was obtained for nonuniformly distributed $D$ because the centers of the IFs were nonuniformly distributed. To obtain $\rho_{\text{nerve}} (D)$ at $D$ intervals corresponding to those for $\rho_{\text{map}} (D)$, values of $\rho_{\text{nerve}}$ were estimated for the same 1-cm bands by exponential smoothing (APPENDIX). The resulting values, obtained with $\lambda = 0.5$ cm, are plotted as a function of $D$ in Fig. 9A. The curve is qualitatively similar to $\rho_{\text{map}} (D)$.

Figure 9B is a scattergram of $\rho_{\text{map}} (D)$ as a function of $\rho_{\text{nerve}} (D)$, with the use of paired values from all $D$. The solid line is the linear regression. Correlation was quite high ($r = 0.80$) and very significant ($P = 2.5 \times 10^{-7}$). Therefore, at least for the 1-cm bands analyzed, there is a very close agreement between map scale and peripheral innervation density.
FIG. 5. Probabilities of cell RF centers falling in 1-cm bands starting at tips of toes ($D = 0-1$), as function of RC and ML position.
FIG. 5. (continued)

**DISCUSSION**

**Accuracy of the results**

The motivation for performing this analysis was to obtain data that provide more direct measures of relevant variables, and calculations that provide more accurate estimates, to test the hypothesis that map scale in the dorsal horn is determined by peripheral innervation density. When we presented this hypothesis, we tested it in a preliminary fashion by means of more indirect calculations based on data that had not originally been obtained for this purpose (Brown et al. 1997). In Brown et al. (1997), we estimated peripheral innervation density for 1-cm bands of skin on the leg by determining the percentage of dorsal root ganglion cells in an earlier study (Brown and Koerber 1978) that had RFs in those 1-cm bands, and multiplying those percentages by published cell counts for those ganglia. This method had the dual disadvantages that our original sample may have been biased (e.g., by undersampling cells at the edges of ganglia) and that not all ganglion cells are LTMRs or even cutaneous. Thus our estimates were really of the number of afferents per square centimeter of skin times some multiplier, which may vary across ganglia (i.e., different ganglia may have different fractions of cells that have cutaneous LTMRs).

Our previous estimates of dorsal horn cell map scale were known to have potentially serious sources of inaccuracies as well. The cell recordings were obtained in experiments in which it was not possible to compensate for pre- and post-fixation of the map by shifting the data, and there may have been bias in our sample (e.g., due to undersampling the edges of the dorsal horn).

Finally, the correlation of map scale and innervation density was further confounded by the fact that the innervation density data encompassed ganglia L₄–S₂, whereas the dorsal horn cell data encompassed only L₅–S₁. This probably introduced some error, in particular an underestimate of the proportion of dorsal horn cells with proximal RFs. The main discrepancy in the correlation was in fact for large D, where our estimates yielded higher estimated peripheral innervation density/map scale ratios.

In this study, we have been able to circumvent some of these problems, but we may have introduced other, albeit less serious,
sources of inaccuracy. Our estimates of peripheral innervation density are based on more direct measurements, because for each IF the innervation density must be the number of fibers divided by the area of skin. Also, in the cat, we have never seen any sign of axons that innervate deep structures in our cutaneous nerve recordings. However, the population counted does not exactly match the population of LTMRs. The myelinated fiber population includes some other types of afferents (e.g., thermal, nociceptive), particularly in the Aδ-class. However, if the Aδ-axons were excluded from the counts, a significant fraction of the LTMR population would be lost. We decided to count all myelinated axons, which means that our estimates of peripheral innervation density are accurate for myelinated axons but are overestimates of myelinated LTMR axons. It is not known whether LTMRs make up the same fraction of myelinated afferents in all cutaneous leg nerves. If they do, then the relative proportions of LTMR innervation densities should be fairly accurate for different parts of the hindlimb. However, it is particularly possible that some errors are introduced when comparing glabrous and hairy skin because they have different complements of receptor types.

Our estimates of dorsal horn cell map scale should be considerably more accurate than before, because of a number of factors. First, by shifting data from each animal by the best shift, our analysis is switched from the arbitrary “anatomic space,” which is known to vary physiologically from one animal to the next, to a more internally consistent and relevant “map space,” which is our best estimate not only of the average map, but of individual dorsal horn maps as well. This is the case because bilateral asymmetries and interanimal variations are more substantial than within-dorsal-horn variability (Koerber et al. 1993).

Our map scale estimates also compensate somewhat for sampling bias. Because the average map surface $D$ (RC, ML) is a smoothed fit to all data, holes in the sample are filled in and the sampling frequency for any part of the map does not influence the final results (e.g., the estimate of percentage or expected number of cells with RF centers at specific $D$). In fact, the variability of sampling frequency affects only the accuracy of estimation of $D$ as a function of RC, ML, which is inevitable. The use of exponential smoothing also represents a significant improvement over previous methods (Koerber et al. 1993) in that no attempt is made to fit the data to an arbitrary analytic function.

Our larger data base, in which all the cells have been shifted to their most likely positions in the physiologically defined map, also permits us to estimate the percentages of cells with different $D$ at each RC, ML location. This allows calculation of the expected number of cells with specific $D$ at each RC, ML location from cell counts, which were also shifted along with the recording sites. This should lead to a much more accurate estimate of the expected number of cells with RF centers in each $D$ than simply multiplying potentially biased proportions by total segment counts for unshifted data.

Finally, correlation of map scale and innervation density benefit not only from the improved estimates of both, but from the inclusion of data from the L4 segment.

**Innervation density**

As would be expected, our innervation density estimates are lower than those obtained in the earlier study, based on ganglion cell counts. In the previous study, estimates were known to be high, because in that study all ganglion cells were used and many cells in the dorsal root ganglia are other types of afferents (e.g., other types of cutaneous or noncutaneous afferent axons). Our estimates based on peripheral nerve recording and myelinated afferent counting give us numbers that are about one-third those obtained with the use of ganglion cell counts. Because some Aδ-fibers included in the current study were undoubtedly not LTMRs (e.g., thermoreceptors or nociceptors), the actual innervation density for myelinated LTMRs is somewhat lower.

**Map scale**

The probability surfaces of Fig. 5 and the expected density surfaces of Fig. 7 both provide a graphic representation of the distributions of cells responding to different bands of skin on the hindlimb. As is also evident from the $D$ surface of Fig. 4, the foot, especially the toes, is represented over a disproportionately large area of dorsal horn. However, the cell density is not notably different for this region. There are more cells in the segments in which foot and toes are represented, manifested as a larger dorsal horn, and reflected in the humped cell distribution, but there is no indication of a difference in cell density in the foot or toe region of the map.

Our method of representing map scale, as number of cells whose RF centers fall on a given area of skin, is not ideal. However, it does correspond to our peripheral innervation density measure, which can be interpreted as number of primary afferent RF centers per unit area of skin, so correlation of our innervation densities and map scales is appropriate. It would be better for some purposes to calculate the number of cells whose RFs overlap a unit area of skin, both for peripheral innervation density and map scale. However, this is computationally more difficult, and we have not yet perfected the software necessary for this purpose. Also, it would require accurate delineation of the small RFs of primary afferents, which vary for different afferent types and are problematic for multipoint RFs such as those of slowly adapting type I and II receptors. It will be well worth the effort, however, to model the population responses to tactile stimuli in studies of the neural correlates of spatial discriminations such as tactile localization and two-point discrimination.

**Correlation of innervation density and map scale**

Our new calculations yield a higher correlation between map scale and innervation density than our previous, less direct calculations. Such high correlations are generally taken as signs of causal relations. Either innervation density controls map scale during development and maintenance of dorsal horn cell RFs, or some common factor controls both. In the model that we have proposed, innervation density controls map scale by means of a competitive self-organization process. This hypothesis can be tested further by manipulating innervation densities and dorsal horn cell densities during development.
FIG. 7. Expected densities of cells in each range of $D$ at each RC, ML, $p_{in}$.
INNERVATION DENSITY AND MAP SCALE

FIG. 7. (continued)
Implications for maintenance and plasticity of the somatotopic map

In the somatosensory cortex of primates, innervation density may control map scale during development, but there is unquestionably an effect of experience on map scale (e.g., Recanzone et al. 1992). It would be of great interest to know whether the forelimb representation in dorsal horn manifests plastic changes of map scale as a result of experience. If such plastic changes do occur, then the dorsal horn changes may actually contribute to the cortical changes, or they may be a result of descending modulation by the cortex. In either case, the many instances of anatomic and physiological changes reported for partially deafferented dorsal horn may reflect continuing capacity for self-organization. A competitive self-organization process that maintains convergence/divergence ratios could be an important organizing mechanism in such plastic changes as collateral sprouting (e.g., LaMotte et al. 1989; Molander et al. 1988), consolidation of RFs after peripheral regeneration (e.g., Koerber and Mirnics 1996), changes of somatotopic organization (e.g., Basbaum and Wall 1976), and even the emergence of lamina III-IV cells devoid of any cutaneous RFs at all (e.g., Brown et al. 1983; Koerber and Brown 1995).

In principle, the relationship between innervation density and map scale can be examined for any part of the somatosensory system. The main requirement is that $D$, distance of IF center from tips of toes, can be plotted as a function of location in the plane of the map, and that some means is available for bringing data from different animals into register to combine data across animals. This may be easier in dorsal horn than elsewhere, because of the simplicity of the layout of the dorsal horn map. This approach can also be expanded to examine innervation density and map scale as a function of position in two dimensions on the surface of the leg, rather than one dimension along the length of the leg. Naturally, in areas where different layers may have different map scales (e.g., because of different cell densities) and different functional roles, it would be useful to keep data from different layers separate rather than combining them as we do here. Indeed, it will be of great interest to repeat these analyses for cells of origin of different spinal tracts, to determine whether they obey different convergence/divergence rules. The fact that there is no difference in RF sizes for cells in different laminae (Brown 1969) would suggest that they do.

APPENDIX

Peripheral innervation density

Peripheral innervation density was calculated as number of $A\beta$-fibers, $n_{\text{nerve}}$, divided by IF area, $A_{\text{nerve}}$, to obtain innervation density, $\rho_{\text{nerve}}$, for each nerve

$$\rho_{\text{nerve}} = \frac{n_{\text{nerve}}}{A_{\text{nerve}}}$$  \hspace{1cm} (1)

in units of nerve fibers per cm$^2$ of skin.

Exponential smoothing

In earlier work we have approximated the shapes of surfaces such as $D\ (RC, ML)$ as best-fitting analytic functions (e.g., Koerber et al. 1993). This had the disadvantage of not necessarily providing the best fit of all possible functions. To fit curves or surfaces to data without any assumption about underlying functions, we now use exponential smoothing. This is equivalent to a discrete form of a low-pass filter, where the spatial frequency cutoff is determined by a length constant similar to the time constant of a low-pass filter in time-varying functions. The length constant is the exponential decay rate of the low-pass filter’s impulse function. Low-pass filtering is accomplished by convolving the impulse function with the data points. Where data points are irregularly spaced, the values at regularly spaced grid points on a surface, or regularly spaced points on a one-dimensional function, can be estimated by exponential weighting of all data according to their distances from each point (e.g., Brown et al. 1982). The low-pass filter function also has the advantage of performing local averaging, reducing random noise to extract trends.
**Shifting data**

Data from each animal were shifted rostrocaudally to provide the best fit with the average animal. The ML and RC positions of all the cells and the distances of their RF centers from tips of toes (D) were entered in a data base. Exponential smoothing was then used to determine the best fit of $D'$ as a function of ML and RC (the model D), according to the following formula

$$D'_i = \frac{\sum_{h=1}^m w_{ih} D_h}{\sum_{h=1}^m w_{ih}}$$

(2)

where the data from each cell h of m cells is used to compute each $D'_i$, the model D at each grid point RC, ML, where

$$RC: \{ L_{10}, L_{11}, \cdots, S_{10}, S_{11} \}$$

ML: \{0, 0.05, \cdots, 0.95, 1.0\}

and

$$w_{ih} = \exp(-|RC - RC_h|/\lambda_{RC} - |ML - ML_h|/\lambda_{ML})$$

(3)

where RC.h and ML.h are the position of cell h, $\lambda_{RC} = 0.29$ segment, and $\lambda_{ML} = 0.13$ dorsal horn width (chosen as the best tradeoff between resolution and noise). For future reference, there were s RC grid lines (s = 51) and r ML grid lines (r = 21).

All the data from a given animal were shifted in 0.1-segment trial shifts to that level where the sum of squared deviations of observed D from model D is smallest in the least-squares sense. If the observed D of cell h is $D_h$

$$d_h = D'_i - D_h$$

(4)

where $d_h$ is the deviation of cell h’s observed D, $D_h$, from the model D at the cell’s RC, ML coordinates, $D'_i$, computed by exponential smoothing with the use of all the shifted cells’ data, $D'_i$, g: \{1 \cdots n\}, is

$$D'_i = \frac{\sum_{g=1}^n w_{ig} D_g}{\sum_{g=1}^n w_{ig}}$$

(5)

where

$$w_{ig} = \exp(-|RC_i - RC_h|/\lambda_{RC} - |ML_i - ML_h|/\lambda_{ML})$$

(6)

After the best shift for each animal was found, the model $D'$(ML, RC) was recalculated by exponential smoothing of the shifted data. This modeling and shifting procedure was repeated until the best shift for every animal was a zero shift. We have always found that this process converges within four iterations. The final model represented the best estimate of $D'$(ML, RC).

**Probability surface**

The shifted data were used to compute the probabilities of cells having RF centers within 1-cm bands on the leg, starting at the tips of the toes and progressing proximally in 1-cm steps. These probabilities were computed for each grid square according to the following formula

$$p(D_h, RC_h, ML_h) = \frac{\sum_{b=1}^i w_{eb}: b \leq D_h < b + 1}{\sum_{b=1}^i w_{eb}}$$

(7)

where $p(D_h, RC_h, ML_h)$ is the probability that D (RC_h, ML_h) falls in 1-cm band b, and $w_{eb}$ is the weight of cell h at grid square ef.

**Cell density surface**

Cells’ locations were entered into a data base according to RC, ML locations. These data were used to calculate the cell densities in the grid squares

$$\rho_{cell}(RC_h, ML_h) = 10C N_{cell}(RC_h, ML_h)$$

(9)

where $N_{cell}(RC_h, ML_h)$ is the number of cells located in grid square (e,f). The multiplication factor 10C is a correction for the sampling ratio (counting 1 of every 10 sections), and a correction for overlap of nucleoli overlapping adjacent sections, where

$$C = \frac{1}{(u + v)}$$

(10)

where u is section thickness and v is nucleolar diameter (Abercrombie 1946). For our purposes, u = 25 $\mu m$, v = 2.7 $\mu m$, and the nucleolar correction factor C = 0.91. Exponential smoothing was then used to estimate the averaged cell densities

$$\rho_{avg}(RC_h, ML_h) = \frac{\sum_{e=1}^{r-1} \sum_{f=1}^{s-1} w_{ef} \rho_{cell}}{\sum_{e=1}^{r-1} \sum_{f=1}^{s-1} w_{ef}}$$

(11)

in units of cells per grid square, where $\rho_{avg}(RC_h, ML_h)$ is the density of cells per grid square [not grid point, thus ($s-1) \times (r-1)$ squares], and $w_{ef}$ is the weight of each grid square relative to grid square ij

$$w_{ef} = \exp(-|RC_i - RC_h|/\lambda_{RC} - |ML_i - ML_h|/\lambda_{ML})$$

(12)

**Computing numbers of neurons with RFs in the 1-cm skin bands**

The expected number of neurons at each grid square that had RF centers in the interval $D_h \cdots D_h + 1$ cm from the toes could then be calculated from $p(D_h, RC_h, ML_h)$ and $\rho_{avg}(RC_h, ML_h)$ by multiplying the probability by the density at each grid point. This produced a mathematical surface of grid values $\rho_{eff}$ for each band of skin b

$$\rho_{eff} = \rho_{avg}(RC_h, ML_h) \cdot p(D_h, RC_h, ML_h)$$

(13)

in units of cells per grid square. Thus for each 1-cm band it was possible to plot the density of cells whose RF centers fall in that band as a function of location in the map.

By summing all the densities in all the grid squares, we calculated the total number of cells with RFs in each of the 1-cm bands. This quantity, divided by the area of the band $A_h$ (cm$^2$) is the map scale for that band, $\rho_{map}(D_h)$

$$\rho_{map}(D_h) = \frac{\sum_{i=1}^{r-1} \sum_{j=1}^{s-1} \rho_{eff}}{A_h}$$

(14)

in units of cells per cm$^2$ of skin.

**Correlation of map scale and innervation density**

To correlate map scale with peripheral innervation density, it is only necessary to plot $\rho_{map}(D_h)$ as a function of $\rho_{innervated}(D_h)$ in a scattergram and calculate the correlation coefficient. Innervation density data, originally computed as $\rho_{innervated}(D_{average})$, could not be used because IFs were not equivalent to the standard 1-cm bands chosen for comparison. The innervation densities of the 1-cm bands

\[\text{November 3, 2016} \quad \text{http://jn.physiology.org/ Downloaded from http://fn.physiology.org/}\]
were estimated from the innervation densities of the nerves as follows

\[ \rho_{\text{innervation}}(D_h) = \frac{\sum_{a=1}^{n} (\rho_{\text{nerv}a} \cdot w_{ab})}{\sum_{a=1}^{n} w_{ab}} \]  \hspace{1cm} (15)

where

\[ w_{ab} = \exp(-|D_a - D_b|/\lambda_0) \]  \hspace{1cm} (16)

\( a \) being a nerve and \( b \) being a skin band, and \( \lambda_0 = 0.5 \text{ cm} \).

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