Structure of the Excitatory Receptive Fields of Infragranular Forelimb Neurons in the Rat Primary Somatosensory Cortex Responding To Touch

We quantitatively studied the excitatory receptive fields of 297 neurons recorded from the forelimb infragranular somatosensory cortex of the rat while touch stimuli were applied to discrete locations on the forelimbs. Receptive fields were highly heterogeneous, but they were regulated, on average, by an underlying spatio-temporal structure. We found the following. (i) Neurons responded with decreasing magnitude and increasing latency when the stimulus was moved from the primary location to secondary locations and to far ipsilateral locations of their excitatory receptive fields, displaying smooth transitions from the primary location to secondary locations. (ii) Receptive field patterns revealed functional connectivity between the digits and ventral palm, which did not depend on whether the digits were stimulated dorsally or ventrally. (iii) The structure of the receptive fields (i.e. the neural responses to stimulation of secondary locations compared to the neural responses to stimulation of the primary location), reflected cortical (rather than body) distances. (iv) There was a functional separation between the forepaw and the rest of the forelimb. Namely: if the primary location was in the digits or palm, secondary locations were biased toward the digits and palm; if the primary location was in rest of the forelimb, secondary locations appeared equally distributed over forelimb, digits and palm. (v) More than 40% of neurons extended their receptive field to the ipsilateral forelimb, without any evident spatial organization. Overall, the stimuli evoked ~3 times more spikes from secondary responses than from primary responses. These results suggest that a rich repertoire of spatio-temporal responses is available for encoding tactile information. This highly distributed receptive field structure provides the electrophysiological architecture for studying organization and plasticity of cortical somatosensory processing.

Keywords: tactile, plasticity, forelimb, multi-electrode, cortical

Introduction

The primary sensory cortex is organized in columns, which are subserved by a layered structure within each column (Staiger et al., 2000). Cells in the different layers of a single column have different receptive field properties. This structure provides the spatial architecture for processing sensory information (Mountcastle, 1957, 1997; Grossberg and Seitz, 2003; Linden and Schreiner, 2003; Lund et al., 2003; Panzeri et al., 2003) and supports a hierarchical processing scheme for the information arriving from the thalamus (HubeI and Wiesel, 1962, 1968; Raizada and Grossberg, 2003; Thomson and Bannister, 2003). Thalamic signals first excite the input layer (layer IV) whose cells have small receptive fields (Killackey and Ebner, 1972). The signal then passes to the supra-granular layers (layer II/III) whose cells have larger receptive fields (McKenna et al., 1984; Chapin, 1986). Finally, the signal is transmitted to the infragra-
stimulation of their primary location, secondary locations and far ipsilateral locations. By simultaneously recording ensembles of neurons from 32 separate electrodes equally distributed in the left and right hemispheres, and by presenting the same standardized stimuli at 20 different locations on the forelimbs, we were able to objectively assess the relative strength of responses in different cortical columns/segemnts, to quantify the activation of neurons from stimuli delivered to multiple forelimb locations, and to quantitatively evaluate the spatial and temporal structure of the excitatory contralateral and ipsilateral receptive fields of forelimb infragranular neurons in the rat primary somatosensory cortex responding to touch stimuli.

Materials and Methods

Recordings were made from seven Long-Evans rats (240–300 g) and all procedures were approved by the Institutional Animal Care and Use Committee at Drexel University and followed NIH Guidelines. To record from large numbers of neurons simultaneously and to minimize the effect of anesthesia on the somatosensory responses of the cells, two arrays of 16 microwire electrodes each, one for each hemisphere of the brain, were chronically implanted under Nembutal anesthesia (50 mg/kg). Animals were allowed to recover (approximately one week) and then the responses of neurons to touch stimulation of the forepaw and forelimb were quantified under light Nembutal anesthesia (induction dose: 35 mg/kg, stage III-2; Friedberg et al., 1999).

Surgical Procedures to Implant Microelectrodes

Animals were anesthetized and placed in a stereotaxic apparatus for surgery (Cartesian Research, Sandy, OR). Rectangular shaped craniotomies with coordinates 0.5 mm anterior to bregma, 3.5 and 4.5 mm lateral to the activation of neurons from stimuli delivered to multiple forelimb locations, and to quantitatively evaluate the spatial and temporal structure of the excitatory contralateral and ipsilateral receptive fields of forelimb infragranular neurons in the rat primary somatosensory cortex responding to touch stimuli.

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ipsilateral side of the body where the neuron showed a significant excitatory response were defined as far ipsilateral locations. A n additional parameter, the normalized response magnitude, was calculated for every neuron and for every location as the ratio between the response magnitude and the response magnitude of the primary location. Therefore, the normalized response magnitude of the primary location is equal to 1.0 by definition. The discrete receptive field size was calculated as the total number of locations where a neuron exhibited a significant response. Note that this discrete definition of receptive field size (number of locations) is dependent on the stimulation protocol and is comparable with the discrete definition typically used in whisker studies (number of whiskers) but not with the continuous definition conventionally employed in non-whisker studies (mm² of skin). To reduce errors due to the finite number of locations stimulated, neurons were included in the analyses only if the response magnitudes to stimulation of their primary location were strong enough to allow a reliable estimation of the parameters for the primary and secondary locations. Based on preliminary analyses, a threshold for the primary response magnitude was set at 0.2 spikes/stimulus. Once this minimal response magnitude for stimulation of the primary location was respected, responses to stimulation of secondary locations were considered significant according to the three criteria above, without additional thresholds (i.e. secondary response magnitudes were allowed to be <0.2 spikes/stimulus).

Comparison of Primary Location, Secondary Locations and Far Ipsilateral Locations

In order to compare the responses to stimulation of the primary location, secondary locations and far ipsilateral locations of the neurons’ receptive fields, and to test whether there were differences between cells whose primary location was in the glabrous surface and cells whose primary location was in the hairy surface of the forelimb, we used a two-way independent-measures analysis of variance (ANOVA) that was separately performed for each of the following variables: response magnitude, peak response, first bin latency and peak latency. Each significant response was conservatively considered as an independent sample. The first factor of the ANOVA was the position of the stimulus within the receptive field, with three levels: primary location, secondary locations or far ipsilateral locations. The second factor was the identity of the cell’s primary location, with two levels: glabrous skin (i.e. grouping digits 1, 2, 5 and ventral palm together) or hairy skin (i.e. grouping digits 3, 4, dorsal paw, wrist, distal forelimb and proximal forelimb together). Sheffe’s test was employed for post-hoc comparisons.

Transitions from the Primary Location to the Secondary Locations

In order to test whether receptive fields were characterized by an abrupt or smooth transition of response magnitudes and latencies when stimuli were moved from the primary location to the secondary...
locations, two analyses were performed. The transition of response magnitudes from the primary location to the secondary locations was examined by quantifying the discrete receptive field size as a cumulative function of the normalized response magnitude, i.e. counting the total number of locations where a neuron exhibited a significant response with normalized response magnitude greater than a given value (Nicolelis and Chapin, 1994). The transition of response latencies from the primary location to the secondary locations was examined by quantifying the first bin latency of the responses as a function of the normalized response magnitude. The dependence between the discrete receptive field size of neurons and the location of their primary location was tested through a one-way ANOVA. The main factor was the identity of the primary location, the dependent variable was the discrete receptive field size and each neuron was considered as an independent sample. A non-paired t-test was also used when the identity of the primary location was categorized into glabrous versus hairy skin.

Receptive Field Patterns
Receptive field patterns were characterized by assigning, for each neuron, a 1 to each location eliciting a significant response when stimulated in a 0 to the remaining locations. The receptive field pattern of a neuron was then defined by the combination of locations that elicited significant responses when stimulated. Neurons were therefore considered to possess the same receptive field pattern if they significantly responded to stimulation of the same locations.

Receptive Field Structure
To evaluate the structure of the receptive fields, neurons were grouped based on their primary location. Every secondary location for each group of neurons with the same primary location was characterized by two parameters: (i) the response likelihood, defined by counting the number of neurons responding to that secondary location divided by the number of neurons with the same primary location; and (ii) the average normalized response magnitude for that secondary location. Note that both the response likelihood and the average normalized response magnitude are numbers between 0 and 1, and that the primary location by definition corresponds to the point [1,1]. A quantitative evaluation of these spatial properties was performed by clustering the responses, as defined by response likelihood and average normalized response magnitude, based on the average Euclidean distance between pairs of objects in different clusters weighted by the number of objects in the cluster (Sneath and Sokal, 1973; weighted pair-group average in Statistica). This is identical to the centroid method since the space defined by the system is two-dimensional with limits of 0 and 1 in both dimensions. An ANOVA was performed on each of the clusters to evaluate the most significant clustering for each primary location (i.e. the grouping of responses by stimulus location that generated the greatest F-value) using Statistica (Statsoft, Tulsa OK).

Throughout the text, results are means ± SD.

Histology
To examine the position of the electrode, post-mortem brain slices were Nissl stained to mark cell bodies. After the final recording session, rats were anesthetized and a stimulating current was placed down the electrode to mark the electrode tip (60 μA for 45 s). Each rat was transcardially perfused with 0.1 M phosphate buffer then 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was removed from the skull and electrodes were extracted from the brain. The brain was stored in 4% paraformaldehyde in 0.1 M phosphate buffer overnight then transferred to a 30% sucrose solution. After 5 days, the brain was removed from solution, embedded in Tek solution and frozen. The brains were cut into 40-μm-thick coronal sections using a cryostat and collected on slides. The positions of the tips of the electrodes were easily visualized due to the hole created by the microstimulation (Fig. 1C).

Results
We analyzed 297 neurons that responded to touch stimulation of the forelimb, recorded from seven rats bilaterally implanted in the forepaw somatosensory cortex. Figure 2 shows typical neurophysiological responses of 6 representative neurons simultaneously recorded from one rat, with raster plots and peri-stimulus time histograms (PSTHs) corresponding to all 10 locations stimulated on the contralateral forelimb and three representative locations stimulated on the ipsilateral forelimb. The results described in the next sections are derived from the quantitative analysis of 5940 PSTHs (297 neurons × 20 locations), of which 1794 presented significant responses.

Distribution of Receptive Field Primary Locations
The forepaw region was targeted and, accordingly, the primary location for most neurons was in the ventral palm or the digits, with fewer neurons having primary locations in dorsal paw, wrist, distal forelimb or proximal forelimb (Table 1, Fig. 3). More precisely, 77% of the cells had their receptive field primary location on the glabrous surface of the forepaw (digits 1, 2, 5 or ventral palm), whereas 23% of the cells had their primary location on the hairy surface of the forepaw (digits 3, 4, dorsal paw, wrist, proximal forelimb and distal forelimb). The number of cells sharing the same primary location was well correlated between the two sides of the brain (Spearman: $R = 0.81$, $P = 0.0044$, $n = 10$; see Table 1), confirming the experimental symmetry of our implants and the functional symmetry of the distribution of receptive field primary locations. There was no positive correlation (Spearman, significance level: $P < 0.01$; see Table 1) between the number of cells sharing the same primary location and the number of cells sharing any other primary location, which was not surprisingly given the unpredictability of the final electrode position using microwires. The number of cells sharing the same primary location was instead well correlated with the total number of significant responses (primary or secondary) elicited at that location (Spearman: $R = 0.95$, $P = 0.00003$, $n = 10$; data not shown).

Comparison of Primary Location, Secondary Locations and Far Ipsilateral Locations
Response Magnitudes
We quantified for each neuron the response magnitude, (i.e. probability of spike per stimulus) and the peak response, (i.e. maximum probability of spike per bin at 1ms binsize) of every significant response (Table 2). As expected, there was a significant difference between responses to stimulation of the primary location, secondary locations or far ipsilateral locations, using either response magnitude or peak response (ANOVA: $P < 0.000001$). Neurons responded to stimulation of their primary location ($n = 297$ responses), secondary locations ($n = 1295$ responses) and far ipsilateral locations ($n = 202$ responses) with a response magnitude of $0.74 ± 0.69$, $0.47 ± 0.38$ and $0.29 ± 0.29$ spikes respectively, and with a peak response of $0.10 ± 0.07$, $0.07 ± 0.04$ and $0.04 ± 0.04$ spikes/bin × stimulus. No significant differences were found between the responses of neurons whose primary location was on the glabrous surface of the forelimb and the responses of neurons whose primary location was on the hairy surface of the forelimb, using either response magnitudes ($P > 0.49$) or peak responses ($P > 0.27$). There was an overall significant correlation between response magnitude and peak response to stimulation of either the primary location (Pearson: $r = 0.78$, $P < 0.0001$, $n = 297$), the secondary locations ($r = 0.87$, $P < 0.0001$, $n = 1295$), or the far ipsilateral locations ($r = 0.88$, $P < 0.001$, $n = 202$), which suggested that these two parameters were equivalent measures.
Figure 2. Neurophysiological responses of neurons in the infragranular layers of the primary somatosensory cortex to punctuate tactile stimuli. The figure shows raster plots and peri-stimulus time histograms (PSTHs) of a representative subset of six neurons (one per column) simultaneously recorded from one rat (rat 5, right hemisphere) responding to stimuli delivered to 10 locations on the contralateral forelimb (top ten rows) and three locations on ipsilateral forelimb (bottom three rows). Despite the sparseness of the locations stimulated, neurons responded with remarkable consistency to ventral palm (PLM) and to the five digits, even though D1, D2 and D5 were stimulated ventrally whereas D3 and D4 were stimulated dorsally. The responses were less consistent when the stimuli were delivered to dorsal paw (PAW), wrist (WR), distal forelimb (DFL) and proximal forelimb (PFL). Significant responses were also observed when stimuli were delivered to the ipsilateral forelimb.
Receptive Field Structure of Rat Forelimb Neurons

Proximal forelimb (PFL). The size and shape of the receptive fields for each of these cells is presented in Figure 7.

From primary responses (297 responses ~ 202 responses ~ 3 spikes/stimulus) than the primary location to the secondary locations. However, this was not the case. The transition of a contralateral response depended strongly on the position of the stimulus within the neuron’s excitatory receptive field.

Transitions from the Primary Location to the Secondary Locations

Given our sparse stimulation protocol, one might expect that the response magnitude of a neuron would abruptly decrease and the response latency would abruptly increase when a stimulus is moved from the primary location to the secondary locations. However, this was not the case. The transition of response magnitude from the primary location to the secondary locations was quantified by examining the discrete receptive field size (i.e. the number of locations where a neuron exhibited a significant response) as a cumulative function of the normalized response magnitude. As the normalized response magnitude was allowed to decrease from 1 (i.e. the primary location) to 0 (i.e. all locations), the discrete receptive field size smoothly, not abruptly, increased (linear fit: \( r = -0.997, P < 0.0001, n = 11 \); see Fig. 4A). This occurred despite the sparseness of the cell’s response. Because the response magnitude represented the total response probability of the cell to the stimulus, we used this measure, rather than peak response, for subsequent analyses. Overall, in the 297 neurons recorded, the stimuli evoked ~3 times more spikes from secondary responses [(1295 responses × 0.47 spikes/stimulus + 202 responses × 0.29 spikes/stimulus) × 100 stimuli/response = 66723 spikes] than from primary responses (297 responses × 0.74 spikes/stimulus × 100 stimuli/response = 21978 spikes).

Response Latencies

To compare the temporal properties of the neural responses, for every neuron we quantified the first bin latency (i.e, latency of the first significant bin of the response) and the peak latency (i.e, latency of the response peak) of every significant response (Table 2). There was a significant difference between responses to stimulation of the primary location, secondary locations or far ipsilateral locations, using either first bin latency or peak latency (ANOVA: \( P < 0.00001 \)). Neurons responded to stimulation of their primary location, secondary locations and far ipsilateral locations with a first bin latency of \( 14 \pm 5, 17 \pm 7 \) and \( 23 \pm 12 \) ms, respectively, and with a peak latency of \( 22 \pm 6, 23 \pm 8 \) and \( 28 \pm 12 \) ms. No significant differences in response latencies were found between cells whose primary location was on the hairy skin and cells whose primary location was on the glabrous skin when the primary location or the secondary locations were stimulated (Sheffe: \( P > 0.7 \)). These results suggest that the latency of a contralateral response depended strongly on the position of the stimulus within the neuron’s excitatory receptive field.

Table 1

Distribution of the primary locations

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<td>10</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>45</td>
</tr>
</tbody>
</table>

Values represent the number of neurons whose primary location was the one indicated by the column headings. Rows labeled left and right represent the side of the body contacted; rows labeled Total represent the total number of cells whose primary location was the one indicated by the column headings. The column labeled Total represents the total number of cells for each side of the brain and the total number of cells combined for both sides of the brain. Values are given for all animals together and for each individual animal. The symmetry in the distribution of the primary locations between the left and right side of the body was tested through Spearman correlation between the first two rows of the table (excluding the Total column). The presence of relations between locations in the distribution of the primary locations was tested through Spearman correlation considering the 14 arrays as single samples and the number of cells corresponding to each primary location as variables. \( 1 = \text{digit 1 (D1); 2 = digit 2 (D2); 3 = digit 3 (D3); 4 = digit 4 (D4); 5 = digit 5 (D5); 6 = dorsal paw (PAW); 7 = ventral palm (PLM); 8 = wrist (WR); 9 = distal forelimb (DFL); 10 = proximal forelimb (PFL).} \|
locations stimulated in this study. In other words, there was a linear relationship between normalized response magnitude and discrete receptive field size. This suggests that the response magnitude of cells to stimulation of any location in their excitatory receptive field is uniformly distributed between 0 and the maximal response (i.e. the response to stimulation of the primary location). The average discrete receptive field size considering all significant responses was $6.0 \pm 3.4$ locations, with some cells displaying small receptive fields of one or two locations and the majority of them having large receptive fields that included several secondary locations on the contralateral forelimb and often extended to the far ipsilateral locations. The discrete receptive field size did not depend on the identity of the primary location (ANOVA, $P = 0.22$). In particular, the discrete receptive field size of cells whose primary location was on the glabrous skin (digits 1, 2, 5, ventral palm) was not significantly different (unpaired t-test, $P = 0.94$) from the discrete receptive field size of cells whose primary location was on the hairy skin (digits 3, 4, dorsal paw, wrist, distal forelimb, proximal forelimb) (Fig. 4B). On average, 40.7% of the neurons had significant responses to stimulation of at least one far ipsilateral location.

The transition of response latency from the primary location to the secondary locations was quantified by examining the first bin latency as a function of the normalized response magnitude (Fig. 4C). In agreement with previous studies, on average the primary location had the shortest response latency. In addition, there was a significant negative correlation between normalized response magnitude and first bin latency (Pearson: $r = -0.32$, $P < 0.001$, $n = 1794$; see Fig. 4C). In other words, as the normalized response magnitude was allowed to decrease from 1 to 0, the first bin latency smoothly, not abruptly, increased.

**Receptive Field Patterns**

Figure 5 shows the receptive fields of all neurons recorded from a representative animal (Rat 1). This animal had cells whose primary locations were predominantly on digit 5 (12 cells) and on the ventral palm (13 cells). All of the neurons whose primary location was on digit 5 also responded to stimulation of the ventral palm, and eight of the neurons whose primary location was on the palm also responded to stimulation of digit 5, suggesting a possible functional connectivity between digit 5 and the ventral palm, which is consistent with the adjacency between the two locations on the cortical map (Fig. 3). It is

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**Figure 3.** Geographical distributions of the receptive field primary locations with respect to electrode arrangement in the cortex, estimated from the neurophysiological responses. (A) Somatotopic arrangement of the rat primary somatosensory cortex (layer IV), as described by Chapin and Lin (1984). The cortical regions corresponding to the forelimb locations stimulated are labeled as follows D1 = digit 1; D2 = digit 2; D3 = digit 3; D4 = digit 4; D5 = digit 5; PAW = dorsal paw; PLM = ventral palm; WR = wrist; DFL = distal forelimb; PFL = proximal forelimb. (B-D) Enlargement of the forelimb area for three representative animals (rats 1, 4 and 5). Gray and black numbers superimposed to the map represent infragranular cells whose primary location of the excitatory receptive field corresponded to the related map location. Each number indicates the specific electrode from which the neuron was recorded. Numbers 1-16 (in gray) correspond to electrodes of the array implanted in the left hemisphere. Numbers 17-32 (in black) correspond to electrodes of the array implanted in the right hemisphere. Electrodes that had more that one neuron appear multiple times, whereas electrodes that did not have any responsive neuron are not shown. Note that the final placement of the electrode tips cannot be controlled using microwires. Therefore, the figure was constructed based on electrophysiological data and the exact placements of the numbers/electrodes within the locations of the map are arbitrary.
important to note that virtually none of the receptive fields were duplicated: of the 13 cells whose primary location was on the ventral palm and of the 12 cells whose primary location was on digit 5, every cell’s receptive field had a unique pattern of response. This suggests that receptive fields were highly heterogeneous across cells, even from the same rat and even for cells with the same primary location.

In order to quantify this heterogeneity in our entire dataset, we defined the receptive field pattern for each neuron simply as the combination of locations that elicited significant responses when stimulated. Neurons were therefore considered to possess the same receptive field pattern if they significantly responded to stimulation of the same locations, thus without considering the variability in response magnitude. Still, the 297 neurons analyzed exhibited 121 different receptive field patterns (Fig. 6). Considering only contralateral responses, the most frequent pattern was found in 28 cells (9.4% of the sample), which responded to all contralateral locations except proximal forelimb (i.e. [1--9]). The subsequent most frequent patterns were small variations of the above pattern. Namely:

### Table 2

| Response magnitude (spikes/stimulus) | 0.53 ± 0.34 | 0.61 ± 0.68 | 0.79 ± 0.60 | 0.57 ± 0.33 | 0.68 ± 0.60 | 0.76 ± 1.02 | 0.93 ± 0.76 | 0.79 ± 0.49 | 0.64 ± 0.58 |
| Peak response (spikes/bin × stimulus) | 0.10 ± 0.06 | 0.08 ± 0.08 | 0.11 ± 0.08 | 0.08 ± 0.04 | 0.10 ± 0.07 | 0.10 ± 0.09 | 0.12 ± 0.07 | 0.13 ± 0.12 | 0.04 ± 0.02 |
| First bin latency (ms) | 15.1 ± 4.6 | 13.7 ± 4.6 | 14.9 ± 5.4 | 13.8 ± 5.1 | 14.0 ± 4.2 | 19.4 ± 5.3 | 13.6 ± 7.5 | 16.8 ± 4.8 | 18.7 ± 11.8 |
| Peak latency (ms) | 20.4 ± 5.3 | 22.8 ± 6.7 | 22.8 ± 5.2 | 21.3 ± 5.0 | 21.3 ± 3.6 | 22.0 ± 5.1 | 21.2 ± 7.6 | 21.7 ± 4.8 | 25.6 ± 12.1 |
| Discrete receptive field size (number of locations) | 5.3 ± 3.2 | 5.7 ± 2.1 | 6.3 ± 2.5 | 5.5 ± 2.5 | 5.4 ± 3.1 | 5.2 ± 4.4 | 6.6 ± 3.3 | 7.3 ± 3.2 | 5.3 ± 2.2 |

Values represent the average and the SD of each of the variables listed in the first column. The response magnitude is the total number of spikes in response to a stimulus, in spikes per stimulus. The peak response is the number of spikes in the largest post-stimulus bin, in spikes per (stimulus × bin). The first bin latency and the peak latency are, respectively, the latency to the first significant bin and the latency to the peak of the response in millisecond (ms). The discrete receptive field size is the number of locations that generated an excitatory response. The columns represent the location contacted: 1 = digit 1 (D1); 2 = digit 2 (D2); 3 = digit 3 (D3); 4 = digit 4 (D4); 5 = digit 5 (D5); 6 = dorsal paw (PAW); 7 = ventral palm (PLM); 8 = wrist (WR); 9 = distal forelimb (DFL).
cells responded to [1--7], 22 other cells responded to [1--5, 7] and 17 cells responded to [1--8]. Overall, 115 neurons (39% of the sample) exhibited significant responses at least to all five digits and the ventral palm (i.e. [1--5, 7]). Importantly, only nine neurons responded to digits 1, 2 and 5 (stimulated ventrally) without responding to digits 3 and 4 (stimulated dorsally), and only five neurons responded to digits 3 and 4 without responding to digits 1, 2 and 5. These results (i) display the high heterogeneity of receptive field patterns across cells and (ii) suggest the presence of a general non-specific connectivity between the digits and ventral palm that does not depend on whether the digits are stimulated dorsally or ventrally.

Figure 5. Cartoons of the rat forelimb showing size and shape of neurons recorded simultaneously from a single animal (rat 1). The template (upper left corner) indicates the forelimb locations stimulated, namely: D1 = digit 1; D2 = digit 2; D3 = digit 3; D4 = digit 4; D5 = digit 5; PAW = dorsal paw; PLM = ventral palm; WR = wrist; DFL = distal forelimb; PFL = proximal forelimb. The color scale is coded from black (maximal normalized response magnitude, NRM = 1, i.e. the primary location) to white (NRM = 0, i.e. no significant response). Neurons are sorted by the primary location of their receptive field. The side of the brain where the neurons were recorded, either left (L) or right (R), is also indicated.
Receptive Field Structure

To quantitatively examine whether the above patterns depended on the overall receptive field structure, neurons were grouped across rats based on their primary location. For each population of neurons with the same primary location, two measures were employed to define the overall response at each secondary location: (i) the average normalized response magnitude, i.e. the normalized response magnitude averaged over the neurons that exhibited a significant response to stimulation of that secondary location; and (ii) the response likelihood, i.e. the fraction of neurons that exhibited a significant response to stimulation of that secondary location. Average normalized response magnitude and response likelihood were significantly correlated (Pearson: $r = 0.63$, $P < 0.001$), and were studied together with a cluster analysis. The detailed receptive fields for all 297 individual cells are shown in Figure 7, which provided the data for the quantitative cluster analysis shown in Figure 8.

For populations whose primary location was in the ventral palm or in the digits, the responses to stimulation of secondary locations could be separated into two significant clusters: responses to stimulation of the digits or palm clustered together and were significantly greater than the responses to the other forelimb locations stimulated (ANOVA, $P < 0.05$). More explicitly, if the primary location was in the ventral palm or in the digits, then both the average normalized response magnitudes and response likelihoods were quite large (typically $>0.5$, Fig. 8A) when the stimuli were delivered to secondary locations in the digits or ventral palm, and much smaller (typically $<0.5$, Fig. 8A) when the stimuli were delivered to secondary locations in the dorsal paw, wrist, distal forelimb and proximal forelimb. The receptive field structure did not appear to consistently discriminate between the median nerve innervated glabrous skin (digits 1 and 2, Figs 7A and 8A), the ulnar territory (digit 5, Figs 7B and 8A), the glabrous palm (ventral palm, Figs 7C and 8A) and the hairy skin (digits 3 and 4, Figs 7D and 8A). Of particular note, for cells whose primary location was in digits 1, 2, 5 or in the ventral palm, digits 3 and 4 — which were contacted on the dorsal hairy surface — clustered with the other digits and the palm — which were contacted on the ventral glabrous surface — and not with the other dorsal locations (Fig. 8A). Reciprocally, neurons whose primary location was in digits 3 or 4 exhibited greater responses to stimulation of the ventral palm (average normalized response magnitude $= 0.58 \pm 0.29$ and $0.77 \pm 0.13$ respectively; response likelihood $= 0.68$ and 0.73) than to the dorsal paw (average normalized response magnitude $= 0.34 \pm 0.18$ and $0.45 \pm 0.19$; response likelihood $= 0.42$ and 0.27). These results show that the receptive field structure does not have a direct relation with the peripheral distribution of somatosensory nerves, but does exhibit a more consistent relation with the cortical distribution of receptive field primary locations. In fact, according to the cortical map in Figure 3, digits 3 and 4 are closer to the other digits and to the ventral palm compared to the dorsal paw (in the map there is no separation between the dorsal and ventral representations of the digits). More generally, for neurons whose primary location was in the digits or in the ventral palm, the two closest secondary locations in the (average normalized response magnitude--response likelihood) plane were, on average, the closest adjacent locations in the cortical plane (with one exception; see below) (Fig. 8A). Namely, digit 2 and the ventral palm were the two closest secondary locations for cells whose primary location was in digit 1; digit 1 and the ventral palm for cells whose primary location was in digit 2; digits 2 and 4 for cells whose primary location was in digit 3; digit 3 and the ventral palm for cells whose primary location was in digit 4; ventral palm and digit 1 (exception) for cells whose primary location was in digit 5; and digits 1 and 5 for cells whose primary location was in the ventral palm. According to the same principle, both the average normalized response magnitude and the response likelihood to stimulation of the dorsal paw, wrist, distal forelimb and proximal forelimb tended to decrease as the cortical distance from the cell’s primary location increased. In other words, locations that are represented in progressively more distant regions in the brain are progressively less likely to be represented in the receptive field structure. Therefore, despite the heterogeneity of receptive field patterns, the average receptive field structure of forelimb infragranular neurons is consistent with the somatotopic organization of the primary somatosensory cortex.

For populations of cells whose primary location was in the dorsal paw, wrist or distal forelimb, the two clusters above could...
not be found and these neurons were equally likely to respond to all secondary locations (Fig. 8B). Only for cells whose primary location was in the dorsal paw, the two closest secondary locations were the closest adjacent locations in the cortical plane, namely digit 2 and ventral palm (Fig. 8B). It should be noted that few cells had their primary location in the dorsal paw ($n = 5$), wrist ($n = 13$) or distal forelimb ($n = 7$), but the two clusters were not found even when these cells were combined together ($n = 25$, data not shown). These results suggest two important differences when comparing secondary responses to stimuli delivered to the forepaw (digits and palm), with secondary responses to stimuli delivered to the rest of the forelimb (from the dorsal paw to the shoulder). First, more cells in the somatosensory cortex exhibit secondary responses, and
second, each individual cell has a greater probability of responding when a stimulus is delivered to the forepaw compared to the rest of the forelimb.

For reasons of symmetry, one might expect that a neuron would have the highest probability of exhibiting a significant response to the far ipsilateral locations when the homologous location of the contralateral primary location is stimulated on the ipsilateral side. On the contrary, the identity of the cell's primary location did not influence the organization of its response to stimulation of far ipsilateral locations. In fact, these responses were distributed across all locations stimulated (Fig. 9A) and in only 12% of the cases — which is approximately what one would expect by chance — the location of an ipsilateral response coincided with the location of the contralateral primary location (Fig. 9B).

Discussion
We quantitatively studied the excitatory receptive field structure of forelimb neurons in the infragranular somatosensory cortex of the rat in response to touch. Receptive fields were large and their patterns were highly heterogeneous across neurons, even for cells with the same primary location. However, the quantitative evaluation of magnitudes and latencies of the responses of neurons to stimulation of their primary location, secondary locations and far ipsilateral locations revealed an overall receptive field organization. Neurons responded with decreasing magnitude and increasing latency when the stimulus was moved away from the primary location to the secondary locations and to the far ipsilateral locations. Despite the discreteness of the stimulation protocol, these transitions of response magnitudes and latencies from the primary location to the secondary locations were smooth. In addition, the identity of the primary location did not influence the discrete receptive field size of neurons, but did affect the structure of the receptive field, suggesting a functional separation between the forepaw and the rest of the forelimb. Namely: if the primary location was in the digits or in the ventral palm, the secondary locations were biased toward the digits and ventral palm, which clustered together; if the primary location was in the rest of the forelimb, the secondary locations appeared equally distributed over the entire forelimb, including digits and palm. On average, the structure of the receptive field within individual neurons was largely consistent with the overall somatotopic organization of the somatosensory cortex (i.e. the neural responses to stimulation of a secondary location in the receptive field decreased when the cortical distance between that secondary location and the primary location increased), suggesting that the receptive fields themselves are somatotopically organized. Finally, >40% of the recorded cells extended their receptive field to the ipsilateral forelimb, without any evident spatial organization. Taken together, these results suggest (i) that even a light punctuate stimulus can robustly activate large regions of the forelimb infragranular somatosensory cortex; (ii) that the majority of spikes sent by the infragranular cortex to intracortical and subcortical structures originates by secondary — rather than primary — responses; and (iii) that there is a rich repertoire of spatio-temporal responses distributed across the somatosensory cortex which is available for encoding information for tactile discrimination.

Studying Excitatory Receptive Fields
This work is focused on the excitatory responses to touch. Therefore, we did not explicitly consider inhibition, which is difficult to study using extracellular recordings when the
background firing rates of neurons are very low. Our results are therefore exclusively related to the excitatory receptive fields of forelimb neurons in the rat infragranular somatosensory cortex and their full functional interpretation should assume the presence of spatial and temporal inhibition (Laskin and Spencer, 1979; Simons, 1985; Brumberg et al., 1996; Xing and Gerstein, 1996; DiCarlo and Johnson, 2002; Derdikman et al., 2003; Wirth and Luscher, 2004).

Figure 7. Continued.
Using a Sparse Stimulation Protocol

In this study we adopted a sparse stimulation protocol, delivering punctuate stimuli to 20 locations bilaterally distributed on the forelimbs. The rationale for this choice has three main bases: (i) to parallel as much as possible the discrete stimulation protocol necessarily employed when studying the whisker cortex (Ghazanfar and Nicolelis, 1999; Panzeri et al., 2001; Foffani and Moxon, 2004); (ii) to sample the entire likely extent of the excitatory receptive field of infragranular forelimb neurons (Chapin, 1986; Foffani et al., 2004); and (iii) to guarantee that cortical distances (rather than body distances) between adjacent locations were qualitatively similar and comparable to cortical distances between adjacent whiskers. Importantly, these inter-location cortical distances are consistent with the distances observed between columns/seggregates in the forelimb somatosensory cortex of cats and monkeys (Favorov et al., 1987; Favorov and Whitsel, 1988a,b; Favorov and Diamond, 1990), and between forepaw barrels in the somatosensory cortex of the rat (Waters et al., 1995). With these premises, our estimates of magnitudes and latencies of responses to stimulation of the receptive field secondary locations relative to responses to stimulation of the receptive field primary location are dependent on the assumption that the identity of the primary location does not significantly change.
when stimuli are delivered within a skin area corresponding to the same cortical segregate, which was experimentally verified in the cat (Favorov and Diamond, 1990). Still, in some cells we may have missed the true primary location due to the limited number of locations stimulated.

Nonetheless, our measure of discrete receptive field size should be dimensionally interpreted in the discrete cortical space (i.e. number of segregates) rather than in the continuous body space (i.e. mm² of skin). For example, a discrete receptive field size of six locations means that a neuron is excited by afferent input that primarily project to (at least) six cortical segregates. Our result that the discrete receptive field size does not depend on the identity of the primary location, therefore, is not in contradiction with the classical concept of magnification (Mountcastle, 1957; Wang et al., 1997; Brown et al., 2004) and suggests that at the infragranular level each cortical segregate receives an approximately equal amount of convergent input from the periphery, which is consistent with informational and energetic optimization principles regulating the development of cortical somatotopic maps (Durbin and Mitchison, 1990; Montague, 1996; Van Essen, 1997; Plumbley, 1999). Our result that the receptive field structure does depend on the identity of the primary location, suggests that the equal amount of convergent input can be differently distributed in different segregated, according to functional needs. The smooth transitions in response magnitudes and latencies from the primary location,

Figure 8. Receptive field structure. Cells were grouped across animals based on the primary location of their excitatory receptive field. For every population, the average normalized response magnitude (i.e. response magnitude divided by the response magnitude to stimulation of the primary location, x-axes) and response likelihood (i.e. fraction of neurons exhibiting a significant response, y-axes) to stimulation of each secondary location was calculated. By definition, the value of both the average normalized response magnitude and response likelihood is 1 for the primary location (upper right corner in every subplot). For every population, the average normalized response magnitude was significantly correlated with the response likelihood. (A) For populations whose primary location was in the palm or the digits, the responses to stimuli delivered to secondary locations could be separated into two significant clusters: response to the palm and other digits (black) or responses to the other locations stimulated (red). This was true regardless of whether the digits were stimulated on the ventral glabrous skin (digits 1, 2 and 5) or on the dorsal hairy skin (digits 3, 4). Notably, the two closest secondary locations in the (average normalized response magnitude — response likelihood) plane typically coincided with the two closest adjacent locations in the cortical map (see Fig. 3). (B) For populations of cells whose primary location was in the wrist, dorsal paw or distal forelimb, the two clusters could not be found and, in fact, these cells were equally likely to respond to all secondary locations regardless of their primary location. 1 = digit 1; 2 = digit 2; 3 = digit 3; 4 = digit 4; 5 = digit 5; 6 = dorsal paw; 7 = ventral palm; 8 = wrist; 9 = distal forelimb; 10 = proximal forelimb. Numbers in parenthesis are the total number of cells represented in the graph (see Table 1).
the glabrous skin of the forepaw and fewer neurons had their primary location in the hairy skin. This bias could be due to two reasons, which are not mutually exclusive: (i) the specific location targeted during surgery (i.e. the forepaw) and (ii) the differences in tactile mechanoreceptors density across the forelimb. Interestingly, the latter possibility is consistent with the distal-to-proximal gradient of peripheral innervation density described in cats in classical studies (Mountcastle, 1957) and more recently by Brown and co-workers (Wang et al., 1997; Brown et al., 2004), and with the differences in the distribution of cutaneous mechanoreceptors observed in humans by Edin and co-workers (Edin, 1990, 1992; Edin and Abbs, 1991; Essick and Edin, 1995). The receptive field structure is therefore likely to reflect, at least in part, these differences in the distribution of cutaneous mechanoreceptors. Also the different physical characteristics of the skin area tested could have influenced our results. However, the overall excitability and discrete receptive field size of neurons did not depend on whether their primary location was on the glabrous or hairy surface of the forelimb, and in both the receptive field patterns and the receptive field structure digits 3 and 4 clustered with the glabrous forepaw locations (digits 1, 2, 5 and ventral palm) even though they were contacted on the hairy surface of the skin. From a functional point of view, this clustering is not surprising because in rodents the dorsal regions of the digits are likely to be stimulated together with the glabrous digit tips when the animal manipulates objects such as food or explores its environment with its forepaws. From the point of view of cortical anatomy, the above clustering is not surprising either. In fact, the cortical representation of the digits does not have a clear dorsal-ventral separation and, accordingly, in the cortical map digits 3 and 4 are closer to the other digits and to the ventral palm compared to the dorsal paw (see Fig. 3). More generally, our findings suggest that the receptive field structure of infragranular neurons in the primary somatosensory cortex reflects cortical rather than body distances, which indirectly relates our results to the distribution of cutaneous mechanoreceptors through the well-known proportionality between central magnification and peripheral innervation density (Mountcastle, 1957; Wang et al., 1997; Brown et al., 2004). Because cortical distances were based on the map of the input layer (layer IV) (Chapin and Lin, 1984), our results are also consistent with the columnar organization of the neocortex (Mountcastle, 1997). However, it should be remarked that other kinds of stimuli and different attentional states (e.g. awake versus anesthetized) will likely produce different response patterns and that our results are specific to the infragranular layers of the rat primary somatosensory cortex. In this regard, a recent study in the whisker cortex confirmed the layer specificity in the awake state and showed that the anesthetized state is a good model for passive unexpected stimuli (Krupa et al., 2004).

**Comparison with the Trigeminal System**

Despite the evident differences in the organization of peripheral receptors between the forelimb and the whisker pad, a number of similarities can be found when comparing our data with the results obtained in the infragranular whisker cortex. The distribution of surround responses, latencies and even discrete receptive field sizes are remarkably similar (Simons, 1985; Simons and Carvell, 1989; Armstrong-James et al., 1992; Moore and Nelson, 1998; Ghazanfar and Nicolelis, 1999;...

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**Activating Touch Mechanoreceptors**

Stimuli were optimized for activating touch mechanoreceptors. Accordingly, the majority of cells had their primary location in

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**Figure 9.** Distribution of responses to stimulation of the far ipsilateral locations. (A) All ipsilateral locations, including the distal forelimb, elicited significant responses. The $y$-axis represents the percentage of the total ipsilateral responses (202 total responses) elicited when each location was stimulated ($x$-axis). (B) The identity of the primary location ($x$-axes) did not influence the identity of the ipsilateral location stimulated. Neurons with any primary location had a significant probability to exhibit an ipsilateral response ($y$-axis, circles). Approximately 12% of the times, the ipsilateral location was the same as the contralateral primary location ($x$-axis, squares), similar to the percentage that would be expected by chance.

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location to the secondary locations revealed by our discrete stimulation protocol suggest that the intra-segregate heterogeneity of receptive field patterns (Favorov et al., 1987; Favorov and Whitsett, 1988a,b; Favorov and Diamond, 1990) possesses an underlying spatio-temporal organization that could be critical to guarantee spike-timing precision for distributed strategies of cortical somatosensory processing (Foffani et al., 2004). Finally, the sparse stimulation protocol is conservative for the finding that most responses in the infragranular somatosensory cortex arise from stimulation of secondary locations, in the sense that the large inter-location distances would at most bias the data toward an underestimation of the overall contribution of secondary responses. All the above considerations support the conclusion that the entirety of the receptive field — not only the primary location — plays an essential role for the organization of cortical somatosensory processing.
Zhu and Connors, 1999). Moreover, the ipsilateral responses we observed in the forelimb, consistent with previous studies (Zarei and Stephenson, 1996), are also found in the whisker cortex (Shuler et al., 2002; Manns et al., 2004). It is not clear whether the ipsilateral responses observed in this paper originate below the thalamus (Erzurumlu and Killackey, 1980), from thalamocortical inputs or from callosal projections, either from secondary somatosensory cortex (Wise and Jones, 1976; Killackey and Belford, 1979) or directly from the opposite primary somatosensory cortex through the septa (Olavarria et al., 1984; Shuler et al., 2001; Fox et al., 2003). Whatever pathway is involved, our results show that the transmission of somatosensory information to the forelimb ipsilateral cortex is slower than the transmission to the contralateral cortex.

Ipsilateral responses also provided a remarkable difference between the forelimb system and the whisker system. Namely, contralateral and ipsilateral receptive fields are spatially homologous in the rat whisker system (Pidoux and Verley, 1979), whereas we did not find any spatial correspondence between the contralateral primary location of the receptive field and the locations that elicited forepaw, or even forelimb, ipsilateral responses. The specificity of different cortical layers likely plays a critical role in the organization of ipsilateral responses. Nevertheless, the fact that ipsilateral receptive fields are present in both the forelimb and whisker cortices suggests that they might play important roles in bilateral integration of somatosensory information (Shuler et al., 2001, 2002).

The matrix organization of the whisker pad is present at the cortical level so that external distances are geometrically transformed, although distorted, in the cortical map. The forelimb cortex has the geometrically more complex task of transforming a non-Euclidean body surface into an almost-Euclidean cortical map. As previously discussed, this transformation has been described in terms of segments (Favorov et al., 1987; Favorov and Whitisel, 1988a,b; Favorov and Diamond, 1990) and forepaw barrels (in layer IV; Waters et al., 1995), which define discrete transitions between receptive fields when moving across the somatosensory cortex. The whisker system and the forelimb system, therefore, share a discrete architecture of somatosensory processing at the cortical level, which represents the somatotopic reference for defining the spatio-temporal structure of the receptive fields. Neurons responding to stimulation of their primary location on the body also respond to stimuli from secondary locations, much like neurons responding to stimulation of their principal whiskers respond to stimuli from surrounding whiskers (Ghazanfar and Nicolelis, 1999, 2001; Petersen and Diamond, 2000). Even more specifically, cells whose primary location is on dorsal regions of the forelimb can be recruited to respond to stimulation of the glabrous skin, suggesting that these neurons are available to assist with discrimination of objects manipulated by the paw, similarly to the recruitment of cells whose principal whisker is a caudal whisker to assist with discrimination when the rostral whiskers are stimulated (Ghazanfar and Nicolelis, 1999, 2001). Overall, despite the differences in the structure of ipsilateral responses, which likely have a behavioral origin (whisker movements are mostly symmetrical), our results are consistent with the idea that the same basic principles of somatosensory processing are shared by the forelimb system and the whisker system in the infragranular primary somatosensory cortex of the rat (Foffani et al., 2004).

**Implications for Somatosensory Plasticity**

The structure of the excitatory forelimb receptive fields is also important for studying cortical reorganization and plasticity after peripheral or spinal injury. Our results suggest that the cortical responses observed after digit removal (Kelahan et al., 1981; Merzenich et al., 1983a,b; Byrne and Calford, 1991) are likely due to the previously existing responses from secondary locations of the excitatory receptive field that are providing the primary neural activity recorded after the lesion. Similarly, our results explain why the amputation of an entire limb renders silent the cortical region corresponding to the distal paw (Lane et al., 1997; Cusick, 1996; Stojic et al., 1998), as it is normally unlikely to respond to the remaining stump or shoulder. The size and structure of the bilateral receptive fields will likely be important for studying long-term changes after injury and be crucial for understanding the bilateral effects of lesions and the mechanisms for plastic compensatory reorganization (Kaas et al., 1983; McKinley et al., 1987; Florence et al., 1998; Jones and Pons, 1998; Dupont et al., 2001), especially after spinal cord injury (Jain et al., 2003; Kaas and Collins, 2003). In conclusion, our study suggests that even small and spatially confined stimuli in the forepaw activate large areas of the infragranular somatosensory cortex with a spatio-temporally organized receptive field structure, which represents the electrophysiological architecture for studying cortical somatosensory function and dysfunction.

**Notes**

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