by PCR was sequenced. The inserts were cloned into the Bam HI site of the CMV-neo-Bam vector (*31*) for expression in human cells.
21. For expression in *S. cerevisiae*, the coding regions of Cdc2 and Cdk2, mutant and wild-type and both tagged and untagged, were cloned into the Bam HI site of pMR438. The vector contains a *GAL1* promoter upstream of the inserted genes, a yeast 2μ origin of replication, and a *URA3* selectable marker. All plasmids were tested in *S. cerevisiae* strain 10083-5C (*MAT*a *GAL cdc28-4 gcn4 ura3*), and several were tested in K699 (*MAT*a *GAL cdc28-1N ade-1-1 can1-100 his3-11 leu2 trp1-1 ura3*). Plasmids were transformed into yeast by the lithium acetate method and selected on plates lacking uracil and containing 2% glucose. Transformants were tested on plates containing either 2% glucose or 2% galactose at the permissive temperature (25°C) or nonpermissive temperature (36°C) as described (*7*).
22. Cells were split 1:5 (Saos-2, C33A) or 1:8 (U2OS) and were transfected 20 hours later with calcium-phosphate precipitates of 20 to 26 μg of plasmid DNA for each 100-mm dish (*32*). After 16 hours, the cells were washed twice with phosphate-buffered saline (PBS) and incubated with fresh medium [Dulbecco's minimum essential medium (DMEM) with 10% fetal bovine serum (FBS)]. Forty-eight hours after the removal of DNA precipitates, cells were rinsed off the plates with PBS containing 0.1% EDTA, pelleted, and stained with 20 μl of a fluorescein isothiocyanate (FITC)–conjugated anti-CD20 monoclonal antibody, as described (*33*). Subsequently, cells were washed twice and fixed overnight in 80% ethanol. Before analysis, the cells were pelleted, washed once, and stained in a solution of 20 μg of propidium iodide and 250 μg of ribonuclease (RNase) A per milliliter. Flow cytometry analysis was performed on a Becton-Dickinson FACScan, and data from 80,000 cells per sample were analyzed with the CellFIT Cell Cycle Analysis software. A gate was set to select CD20-positive cells with FITC staining at least 20 times stronger than that in the negative untransfected cells. Other gates were selected for single cells within a normal size range. The propidium iodide signal was used as a measure for DNA content and hence cell cycle stage. The DNA histograms each contain data from 1000 to 5000 CD20-positive cells.
23. Associated cyclins were detected in re-immunoprecipitation experiments. We transfected U2OS cells with the tagged wild-type or mutant kinases and labeled them with $^{35}$S-methionine. The HA-tag immunoprecipitations were denatured and re-immunoprecipitated with different anticyclin monoclonal antibodies.
24. The DNA histograms of cells transfected with plasmids expressing the wild-type kinases were indistinguishable from controls transfected with the CMV vector.
25. Expression of the Cdk2-dn mutant resulted in a $G_1$ arrest in all cell lines tested, including U2OS and Saos-2 osteosarcoma cells, C33A cervical carcinoma cells, T98G glioblastoma cells, and 293 human adenovirus-transformed kidney cells. The Cdc2-dn effect was most prominent in U2OS cells, and similar effects were seen in Saos-2 and T98G cells. However, the cell cycle distribution of C33A cells was not affected by this mutant. This latter result stresses that absence of a dominant-negative phenotype in this assay does not exclude a cell cycle function for the wild-type kinase in normal cells. Transformed cells may be resistant to dominant-negative inhibition, for example, as a result of overexpression of cyclins, activating kinase mutations or loss of growth-control pathways.
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# Receptive Field Reorganization in Dorsal Column Nuclei During Temporary Denervation

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**Altered sensory input can result in the reorganization of somatosensory maps in the cerebral cortex and thalamus, but the extent to which reorganization occurs at lower levels of the somatosensory system is unknown. In cat dorsal column nuclei (DCN), the injection of local anesthetic into the receptive fields of DCN neurons resulted in the emergence of a new receptive field in all 13 neurons studied. New receptive fields emerged rapidly (within minutes), sometimes accompanied by changes in adaptation rates and stimulus selectivity, suggesting that the new fields arose from the unmasking of previously ineffective inputs. Receptive field reorganization was not imposed by descending cortical inputs to the DCN, because comparable results were obtained in 10 additional cells when the somatosensory and motor cortex were removed before recording. These results suggest that mechanisms underlying somatotopic reorganization exist at the earliest stages of somatosensory processing. Such mechanisms may participate in adaptive responses of the nervous system to injury or continuously changing sensory stimulation.**

Sensory maps in the cerebral cortex are maintained through dynamic processes. Modification of peripheral inputs to the central nervous system results in reorganization of cortical somatosensory maps in a number of species (*1*) including humans (*2*). The identification of the mechanisms that are involved in map reorganization has clinical implications for the treatment of peripheral nerve injury and phantom limb pain.

A critical issue that must be resolved before the mechanisms of reorganization can be uncovered is the extent to which reorganization at subcortical levels contributes to changes previously described in the cortex. Mapping studies suggest that peripheral nerve transection can result in map reorganization in the primate ventral posterior thalamic nucleus (*3*). Such reorganization has not been found in DCN or trigeminal nuclei (*4*), although it is difficult to detect reorganization in subcortical maps, which are three-dimensional and can exhibit large somatotopic shifts over relatively small distances. An alternative approach, which we have used in the present study, is to map a single neuron's receptive field, inject local anesthetic into the field to silence input from the receptive field temporarily, and then test for the appearance of a new receptive field. In thalamus (*5*) and cortex (*6*) this approach has been used to demonstrate that new receptive fields can emerge within minutes after the injection of lidocaine. Such changes in cat DCN neurons have not been investigated, although cold block of the dorsal columns has been reported to result in the emergence of new receptive fields in a small proportion of nucleus gracilis neurons (*7*).

To study subcortical reorganization in the present experiments, we recorded from 13 DCN neurons in six adult cats (*8*). Subcutaneous lidocaine injections into the original receptive field resulted in the rapid emergence of a new receptive field in every neuron tested (Table 1 and Fig. 1, A and B). However, the possibility remained that the primary site of reorganization was the cerebral cortex, and that subcortical reorganization was imposed by descending cortical inputs to the DCN. To test this possibility, we recorded from 10 neurons in four additional cats after the re-

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moval of cortical inputs to the DCN (9). Even in the absence of cortical inputs, subcutaneous lidocaine injections resulted in the rapid emergence of new receptive fields in every neuron tested (Table 1 and Fig. 1C). There were no evident differences in the receptive field reorganizations in these two sets of experiments, so the data have been combined for the following description.

Lidocaine injection resulted in the emergence of new receptive fields for every DCN neuron tested, even in eight neurons in which the blockade of the original receptive field was incomplete. Control injection of saline had no effect on receptive field organization or activity (*10*). In 16 of the 23 neurons, the new receptive fields remained throughout the entire recording period (up to 6 hours), even after responsiveness in the original receptive fields had returned. Neither recovery of responsiveness in the original receptive field nor stability of the new receptive field was related to neuronal response properties or to the size of the new receptive field.

In eight neurons the response class (hair or skin) or adaptation characteristics of the new receptive field differed from those of the original receptive field (Table 1 and Fig. 1B). In six other neurons new inhibitory receptive fields emerged after lidocaine injection. The appearance of these new response characteristics, which were not evident in the original receptive field, suggests that the new fields arose from peripheral inputs that were normally ineffective. These neurons were located in the core and rim of the cell cluster region of the middle cuneate nucleus (*11*) and in the rostral cuneate.

In contrast to the neurons described above, nine neurons showed a simpler type of receptive field reorganization: They responded selectively to stimulation of hair or skin in the original receptive field, and after lidocaine injection each began to respond to the same type of stimulation in a new field (Fig. 1A). These neurons were all located in the core of the cell cluster region.

Dynamic aspects of receptive field reorganization were particularly evident in three neurons in which the new receptive fields fluctuated in size 44 to 120 min after their initial appearance. The response threshold in one of these new receptive fields also fluctuated. The threshold decreased from 2.35 g at 52 min after lidocaine injection to 0.22 g at 83 min, increased to 0.44 g over the next 40 min, and by the end of the 6-hour recording period had returned to 2.35 g.

The present results suggest that (i) some inputs to DCN neurons are normally masked, (ii) masked inputs become effective when activity in the original receptive field is reduced, and (iii) unmasking does not depend on cortical inputs to the DCN. The anatomical substrate of masked inputs may involve the dendritic spread of DCN neurons (up to 500 μm) and the widespread terminal arborizations of primary afferents (*12, 13*). It has been estimated that as many as 300 primary afferents overlap at any point in the middle cuneate nucleus (*12*). The mechanisms of masking are not known. Calford and Tweedale (*14*) have suggested that tonic, inhibitory inputs might serve this function, and they have shown that silencing peripheral C fibers leads to receptive field expansion in cortical neurons. The influence of C fiber inputs on receptive field organization in the DCN has not been investigated.

The presence in the DCN of mechanisms that underlie receptive field reorganization raises the question of whether such mechanisms also exist at higher levels of the somatosensory system, or whether reorganization observed at higher levels is a reflection of changes in the DCN. A potential anatomical substrate for rapid reorganization in cerebral cortex is the widespread termination pattern of thalamocortical afferents (*15*). Direct evidence for receptive field reorganization in sensory cortex has been found in studies of retinal lesions, which result in immediate receptive field expansions in cortical (*16*), but not thalamic (*17*), neurons. Therefore, it appears that mechanisms underlying receptive field reorganization exist at multiple levels of sensory systems. After injury, such mechanisms might be involved in the development of referred sensations involving phantom limbs (*2*). In normal brain function, such mechanisms might help to adapt to a continuously changing sensory environment.

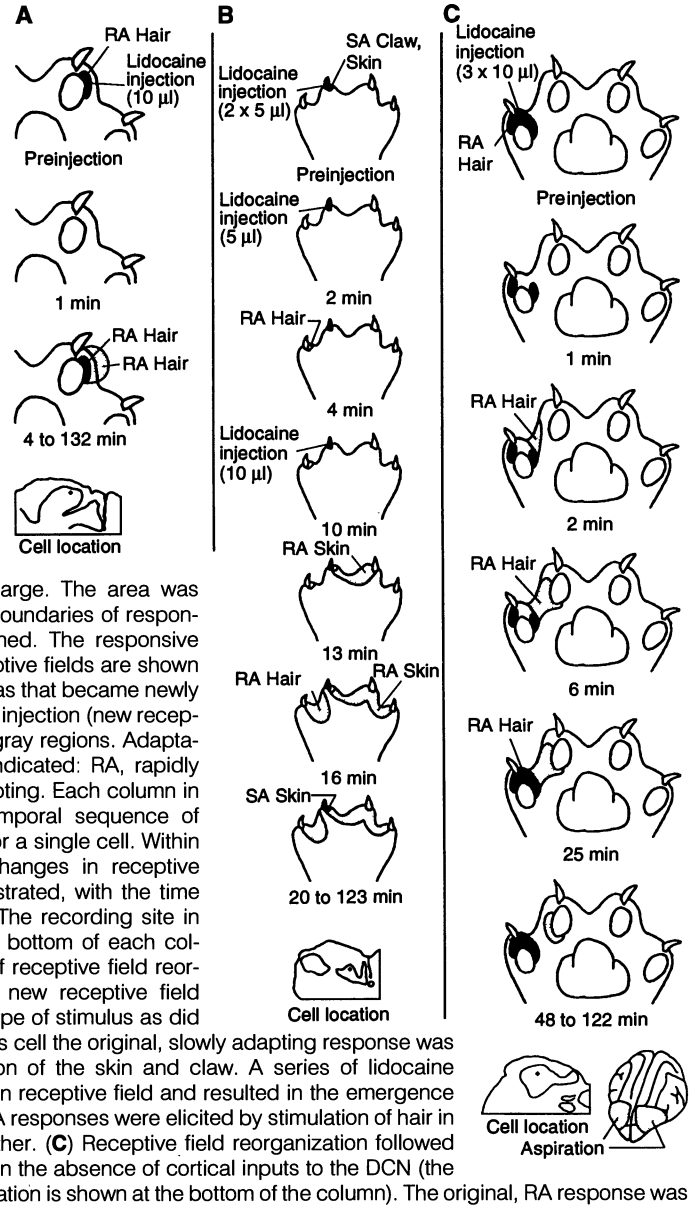


**Fig. 1.** Original receptive fields (black areas in top figures) mapped with the use of hand-held stimuli over periods of 10 to 30 min before inactivation to determine the stability of receptive field borders. No variations were seen over these periods in receptive field size or neuronal response properties. Temporary denervation was then produced by one to three subcutaneous injections of 2% lidocaine (4 to 30 μl each). Additional injections were made (for 10 of the 23 cells) if the receptive field was difficult to silence or was large. The area was again mapped, and the boundaries of responsive areas were determined. The responsive areas of the original receptive fields are shown as black regions, and areas that became newly responsive after lidocaine injection (new receptive fields) are shown as gray regions. Adaptation characteristics are indicated: RA, rapidly adapting; SA, slowly adapting. Each column in the figure shows the temporal sequence of receptive field changes for a single cell. Within a column, successive changes in receptive field organization are illustrated, with the time after injection indicated. The recording site in the DCN is shown at the bottom of each column. (**A**) A simple form of receptive field reorganization, in which the new receptive field responded to the same type of stimulus as did the original field. (**B**) In this cell the original, slowly adapting response was elicited only by stimulation of the skin and claw. A series of lidocaine injections silenced the skin receptive field and resulted in the emergence of new receptive fields; RA responses were elicited by stimulation of hair in one field and skin in another. (**C**) Receptive field reorganization followed lidocaine injections even in the absence of cortical inputs to the DCN (the extent of the cortical aspiration is shown at the bottom of the column). The original, RA response was elicited by stimulation of hair. Lidocaine injections silenced the central portion of the receptive field, and 2 to 6 min later a new receptive field with similar response properties emerged.

**Table 1.** Receptive field response properties of single DCN neurons before and after subcutaneous lidocaine injection. Receptive fields were mapped with hand-held stimuli [Semmes-Weinstein (Lafayette Instruments, Lafayette, IN) monofilaments, wooden probes, fine brushes, and light air puff]. Neurons were classified by adaptation characteristics (RA and SA) and response class (hair, skin, deep tissues); ipsi, ipsilateral; contra., contralateral. Spontaneous activity was measured over periods of 10 to 30 s before and after lidocaine injection. There was no significant effect of lidocaine injection on spontaneous activity. Cells were recorded in the cuneate (core, rim, rostral) and gracilis nuclei.

| Original receptive field | New receptive field | Size of new receptive field* | Spontaneous activity (Hz) | | Ratio† | Cell location | Observation time (min) |
|---|---|---|---|---|---|---|---|
| | | | Pre-inj. | Post-inj. | | | |
| RA, hair | RA, hair | 0.4 | 1 | 1 | 1 | Core | 20 |
| RA, hair | RA, hair | 2.2 | 0 | 0 | — | Core | 132 |
| RA, hair | RA, hair | 0.6 | 1 | 0 | 0 | Core | 79 |
| RA, hair | RA, hair | 0.1 | 0 | 2 | — | Core | 125 |
| RA, hair ipsi. forearm (−)‡ | RA, hair | 0.2 | 32 | 31 | 1 | Gracilis | 70 |
| RA, skin | RA, hair | 0.4 | 0 | 0 | — | Core | 69 |
| RA, hair, skin, hair (−) | hair (−), deep (−), ipsi. forepaw | 20.7 | 27 | 46 | 1.7 | Core | 150 |
| RA, hair, hair (−), ipsi. hindlimb (−) | RA, skin and hair | 2.5 | 16 | 5 | 0.3 | Core | 134 |
| SA, skin | SA and RA, hair | 6.2 | — | 0 | — | Rim | 125 |
| SA, skin and claw | RA, skin and hair, claw | — | — | — | — | Rim | 123 |
| SA, skin | SA, skin, hair, and deep | 1.8 | 89 | 0 | 0 | — | 117 |
| SA, hair and skin | SA, skin and claw (−) | 1.1 | 43 | 28 | 0.7 | Core | 133 |
| SA, claw and hair (−), ipsi. and contra. forepaw (−), ipsi. hindpaw (−) | hair (−) and deep (−) | — | 13 | 24 | 1.8 | Core | 106 |
| *Cells recorded after the removal of cortical inputs to the DCN* | | | | | | | |
| RA, hair | RA, hair | 2.6 | 29 | 27 | 0.9 | Core | 122 |
| RA, hair | RA, hair | 2.7 | 19 | 21 | 1.1 | Core | 100 |
| RA, hair | hair (−) | 25.7 | 9 | 16 | 1.8 | Core | 70 |
| RA, hair and hair (−) | hair (−) | 0.2 | 14 | 14 | 1 | Core | 82 |
| RA, hair | RA, skin and hair | 1.4 | 28 | — | — | Rostral | 364 |
| RA, skin, hair (−) and skin (−), ipsi. and contra. forepaw (−) | RA, skin | 0.5 | 6 | 13 | 2.2 | Rim | 168 |
| RA, skin, ipsi. forepaw (−) | RA, skin | 1.6 | — | 11 | — | Core | 18 |
| SA, skin | RA, skin | 0.5 | 21 | 23 | 1.1 | Rim | 84 |
| SA, claw and skin | SA, skin | — | 28 | 19 | 0.7 | Core | 119 |
| SA, skin and hair (−) | skin, skin (−), hair (−) | 1.0 | 14 | 11 | 0.8 | Core | 36 |

*Ratio of new receptive field size to original receptive field size. †Ratio of post-injection (post-inj.) spontaneous activity to pre-injection (pre-inj.) spontaneous activity. ‡Inputs from other limbs are listed according to the limb from which they were elicited. No injections were made into these fields. A minus sign denotes inhibitory fields.

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