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"Laminar Comparison of Somatosensory Cortical Plasticity"

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During tactile learning there is a transformation in the way the primary somatosensory cortex integrates, represents, and distributes information from the skin. To define this transformation, the site of earliest modification has been identified in rat somatosensory cortex after a change in sensory experience. Afferent activity was manipulated by clipping all except two whiskers on one side of the snout ("whisker pairing"), and the receptive fields of neurons at different cortical depths were mapped 24 hours later. Neurons in layer IV, the target of the primary thalamic pathway, were unaltered, whereas neurons located above and below layer IV showed significant changes. These changes were similar to those that occur in layer IV after longer periods of whisker pairing. The findings support the hypothesis that the layers of cortex contribute differently to plasticity. Neurons in the supragranular and infragranular layers respond rapidly to changes in sensory experience and may contribute to subsequent modification in layer IV.

The primary somatosensory cortex is reorganized during tactile learning (*1*). For example, among proficient Braille readers the fingertip used for reading elicits evoked potentials over a larger area of sensorimotor cortex than do fingertips not used for reading (*2*). The analogous observation has been made in experimental subjects. Adult owl monkeys were trained to discriminate between two frequencies of tactile stimulation of the fingertip (*3*). After several weeks of training, area 3b of the somatosensory cortex was explored with a microelectrode, and it was found that the cortical territory devoted to the stimulated skin area was expanded.

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Because of the precise somatotopy of the afferent sensory pathways, the rat whisker system is an ideal model for investigating how sensory experience influences the functional organization of cortex. Each whisker on the snout projects through the thalamus to a separate cortical "barrel," a cluster of neurons in layer IV, and the barrel forms the basis of a column of neurons extending through layers II to VI (*4*). We have previously shown that receptive fields in layer IV of column D2 shift as early as 65 hours after the onset of "whisker pairing" (*5*). Whisker pairing alters the pattern of afferent activity while leaving the peripheral nerve intact: All whiskers on the right side of the snout are clipped except D2 and one of its neighbors, either D1 or D3 (Fig. 1A). Here, we carried out a laminar comparison of plasticity after just 24 hours of whisker pairing to detect the site of earliest

2.

cortical adaptation. We hypothesize that some layers are particularly sensitive to changes in sensory experience and that initial modifications within these layers may subsequently influence the entire cortical column.

Subjects were adult male Long-Evans rats weighing 290 to 420 g. Experimental subjects with whiskers paired (n = 8) were compared to normal subjects with all whiskers intact (n = 11) (6). Rats were anesthetized with urethane (1.5 g per kilogram of body weight, intraperitoneally) and placed in a Narishige stereotaxic apparatus (7). All whiskers on the right side were trimmed to a length of 3 mm. Left somatosensory cortex was exposed, and a microelectrode was advanced into column D2. Histological analysis showed that penetration sites were distributed randomly across column D2 (Fig. 1B) (8). Within column D2, individual neurons at measured depths were studied. For every neuron, action potentials were counted in response to 50 computer-gated stimulus trials presented to right whisker D2 and to each of its immediate neighbors (D1, D3, C2, and E2) (9).

For analysis, every neuron was assigned to one of three groups (supragranular, granular, or infragranular) according to its distance from the cortical surface (10). Neurons in the supragranular and infragranular layers exhibited significant plasticity after a brief alteration in sensory experience, at a time when granular (layer IV) neurons showed no adaptation to the change in experience. Counting of the spikes evoked by deflection of whiskers D1 and D3 led the individual neurons in column D2 to be classified as having a greater response, or a "preference," for one or the other whisker. In control cases (Table 1), neurons as a group did not show a preference for either whisker D1 or D3 in any layer (supragranular: $\chi^2 = 0.154$, $P = 0.695$; granular: $\chi^2 = 0.00$, $P = 1.00$; infragranular: $\chi^2 = 0.615$, $P = 0.432$). In experimental cases (Table 2), the 24-hour period of whisker pairing induced a strong bias among supragranular neurons: 94% gave a greater response to "D-paired" (the intact whisker adjacent to D2) and only 6% gave a greater response to "D-cut" (the clipped whisker adjacent to D2). The preference for the paired whisker was significant ($\chi^2 = 27.457$, $P < 0.0005$). There was also a significant bias toward D-paired among infragranular neurons: 65% gave a greater response to D-paired and 27% gave a greater response to D-cut ($\chi^2 = 4.167$, $P < 0.05$). In contrast, whisker pairing failed to induce a bias among granular (layer IV) neurons: 44% gave a greater response to D-paired, 44% gave a greater response to D-cut, and 11% gave an equal response to the paired and cut neighbors of D2 ($\chi^2 = 0$, $P = 1$).

In control cases, neurons in column D2 responded most strongly to the anatomically defined principal input, whisker D2 (analysis of variance, $P < 0.005$ for all layers) and gave a weaker but significant response to surrounding whiskers (Fig. 2). The dominant response to whisker D2 was not altered by whisker pairing ($P < 0.005$); however, whisker pairing influenced the response of supragranular and infragranular neurons to the whiskers surrounding D2. Whereas in normal rats the response to whiskers D1 and D3 was equal (supragranular: $t = 0.767$, $P = 0.450$; granular: $t = 0.955$, $P = 0.343$; infragranular: $t = -1.682$, $P = 0.105$), after whisker pairing the response of supragranular neurons to D-paired was more than twice as large as the response to D-cut (29.5 and 13.8 spikes, respectively; $t = 5.992$, $P < 0.0005$). Among infragranular neurons, the response to D-paired was more than 50% larger than the response to D-cut (29.3 and 18.8 spikes, respectively; $t = 2.204$, $P < 0.05$). In contrast, granular (layer IV) neurons showed an unbiased response to D-paired and D-cut (16.09 and 17.02 spikes, respectively; $t = -0.486$, $P = 0.630$).

To characterize the degree of plasticity better in relation to laminar position, neu-

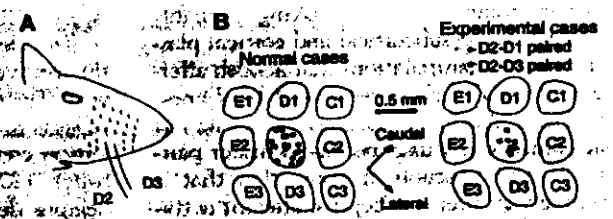

Fig. 1. (A) Each whisker of the rat snout is identified by row (A is most dorsal, E is most ventral) and by number. This drawing represents an experimental case in which whiskers D2 and D3 were paired. (B) Left side showing penetration sites (small circles) in normal cases. On the right side, penetration sites in experimental cases are coded according to the sensory experience of the rat: Four were from rats with whiskers D2 and D3 paired and four were from rats with whiskers D2 and D1 paired.

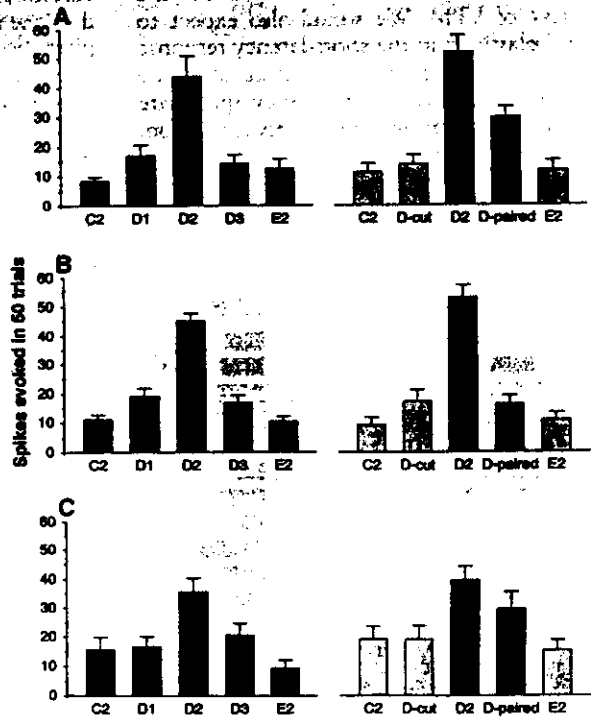

Fig. 2. Receptive fields of neurons in the (A) supragranular (layers II to III), (B) granular (layer IV), and (C) infragranular (layers V to VI) layers of column D2. Histogram bins are the average response evoked by stimulation of the principal whisker (D2) and four surrounding whiskers in normal cases (left side) and in experimental cases (right side) (whiskers paired for 24 hours). Solid bins, intact whisker; hollow bins, cut whisker. Bars indicate standard error of the mean.

Table 1. Bias of neurons (given in number of cells) in column D2 of normal subjects.

Layer	Preference		
	D1	D3	None
Supragranular	14	12	1
Granular	32	33	0
Infragranular	11	14	2

Table 2. Bias of neurons (given in number of cells) in column D2 of experimental subjects.

Layer	Preference		
	D-paired	D-cut	None
Supragranular	33	2	0
Granular	20	20	5
Infragranular	17	7	2

3.

rons were sorted and grouped into bins corresponding to 100-μm advances of the electrode, and the average response to deflection of the two whiskers adjacent to D2 was plotted (Fig. 3). In the normal cortex, neurons at all depths of column D2 gave a similar response to whiskers D1 and D3. After 24 hours of whisker pairing, neurons in the first four bins (0 to 400 μm) gave an enhanced response to D-paired. Neurons in the next four bins (401 to 800 μm) yielded, as in the normal brain, an equivalent response to D-paired and D-cut. In the final four bins (>800 μm), a moderate bias toward D-paired was evident.

The bias of nongranular neurons resulted from potentiation of the input from D-paired (with respect to the normal response to D1 and D3) rather than from depression of the input from D-cut (*11*). Among layer IV cells, there was neither potentiation of the input from D-paired nor depression of the input from D-cut. When the total evoked response in the 100 ms after the stimulus was subdivided into two intervals, 0 to 10 ms and 10 to 100 ms, it was found that the plasticity of neurons in the nongranular layers resulted exclusively from increased activity in the interval between 10 and 100 ms. There was no statistically significant change in the evoked activity occurring in the interval between 0 and 10 ms.

The receptive fields of neurons in layer IV of column D2 change progressively during a period of whisker pairing of 3 to 30 days (*5*). Our data show that the cortical column is not transformed as a homogenous unit: Rather, neurons in the supragranular and infragranular layers begin to adapt to whisker pairing within 24 hours, at a time when the receptive fields of layer IV neurons are not yet noticeably altered. The modifiability of neurons in the supragranular layers is particularly striking.

Although the plasticity stemmed from an increased response to D-paired, the enhancement probably is not explained by a release from intracortical inhibition in the supragranular and infragranular layers. Such a release would cause an elevated rate of spontaneous activity; in our sample, spontaneous activity did not increase in any layer. It is conceivable that specific intracortical circuits were selectively released, allowing enhanced spread of activity from the column of the D-paired whisker to the column of whisker D2 (*12*). Although this possibility cannot be ruled out, the fact that glutamic acid decarboxylase (GAD) staining, reflecting the synthesis of the inhibitory neurotransmitter α-aminobutyric acid (GABA), decreases in the barrels of cut whiskers but not in barrels of spared whiskers (*13*) opposes the hypothesis that potentiated responses result from decreased intracortical inhibition.

Another possibility is that plasticity occurred in the thalamic ventral posterior medial nucleus (VPM) and was relayed to cortex. Receptive fields in VPM shift within minutes after injection of lidocaine into the snout (*14*). Immediate unmasking of new receptive fields has been attributed to disinhibition due to silencing of nociceptive c fibers because the effect can be produced by injection of capsaicin, a selective c-fiber toxin (*15*). Unmasking probably contributes to subcortical and cortical plasticity after manipulations that abolish afferent activity (such as peripheral local anesthesia or nerve cut). Unmasking would not account for our data because whisker pairing is an innocuous manipulation that is unlikely to alter nociceptive afferent activity. Furthermore, if whisker pairing first induced modification in the sensory pathway that ascends through VPM, we would expect to find plasticity within layer IV, the target of VPM. We would also expect to find plasticity in the short-latency response of layer IV neurons (0 to 10 ms after the stimulus), because short-latency spikes are evoked by monosynaptic activation from VPM (*16*). Instead, plasticity of sensory responses was found only in the nongranular layers and only in the period from 10 to 100 ms after the stimulus.

The data are consistent with a second idea: Whisker pairing causes a rapid Hebbian (*17*) potentiation of the connection between the cortical columns of the two spared whiskers, D2 and D-paired. This interpretation agrees with the results of other studies that show strengthening of cortico-cortical connections after the pairing of sensory inputs. In raccoons, digits 3 and 4 were joined in syndactyly, and after 14 to 22 weeks neurons in the digit 3 subgyrus of somatosensory cortex had a stronger influence on neurons in the digit 4 subgyrus, as measured by intracellular recordings from the digit 4 subgyrus during electrical microstimulation of the "heterogenous zone" rostral to the digit 4 subgyrus (*18*). Our data add to the Hebbian model by showing different functions for the cortical layers. The response of neurons in column D2 to the whiskers surrounding D2 depends on convergence from surrounding columns (*19*), and these column-to-column circuits relay in the supragranular and infragranular layers (*12*). Therefore, synaptic modifications within the nongranular layers could be the first step in strengthening the linkage between columns.

The idea outlined above does not exclude the involvement of subcortical sensory pathways. The thalamic posterior complex (POm) projects to the nongranular layers of somatosensory cortex as well as to the septa between barrels (*20*). The sensory activity of POm neurons depends on descending projections from the infragranular layers of barrel cortex (*21*), so that POm could take part in a reverberating loop that regulates the strength of connection between cortical columns. Nor does the idea exclude longer term subcortical plasticity: After one week of whisker pairing, the projection from VPM to cortex becomes potentiated (*5*).

The striking plasticity of the supragranular layers may reflect a real contribution to tactile learning. A rat deprived of vision can learn to measure the gap between two platforms with its large facial whiskers before crossing to the opposite platform to collect a reward (*22*). If the supragranular layers overlying the barrels are lesioned after the "gap-cross" task has been learned, a rat can continue to perform the task (*23*). In contrast, if the supragranular layers are lesioned before the task has been learned, a rat is not able to learn the task; it remains on the "start" platform and does not cross to the "reward" platform even when it palpates the target with its whiskers.

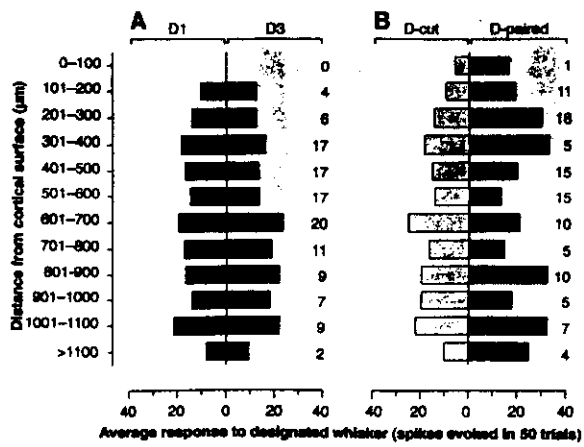

Fig. 3. Relation between laminar position and plasticity of the response to D-paired and D-cut in (**A**) normal and (**B**) experimental cases. The number of neurons in each depth range is given to the right of the bin.

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6. In experimental subjects, whiskers on the right side (except the paired whiskers) were clipped to a length of 2 to 3 mm; whiskers on the left side were not cut. The data from experimental subjects with whiskers D2 and D1 paired (four cases) and whiskers D2 and D3 paired (four cases) were combined because no significant differences were found when evaluated separately; also, see (*5*).
7. Body temperature was maintained at 36° to 37°C. Anesthesia was held at a consistent depth by maintaining the burst rate of layer V neurons at 2 to 4 Hz. When anesthetic depth decreased, a supplement of urethane was given (10% of the original dose).
8. The horizontal location of a recording site is critical because in normal rats the receptive-field symmetry of barrel D2 neurons is correlated with their horizontal location (*5*). After a microelectrode penetration was completed, an electrolytic lesion (positive direct current of 0.2 μA for 5 s) was made at a depth of 500 to 700 μm for visualization by cytochrome oxidase staining. At the end of the experiment, rats received pentobarbitol (50 mg/kg, intraperitoneally) and were perfused. The neocortex was separated from the white matter and flattened between glass slides. The slab was frozen and 50-μm tangential sections were processed for cytochrome oxidase activity. The points at which penetrations passed through layer IV of column D2 were projected onto the tangential barrel map.
9. Individual whiskers were deflected by a wire tip glued to a piezoelectric ceramic wafer positioned just below the whisker, 2 mm from the skin. The stimulus, delivered at 1 Hz, was a ~300-μm up-down movement of the tip with a total duration of 3 ms. Action potentials were recorded by carbon-fiber microelectrodes and isolated by a window discriminator. With the use of peristimulus time and latency histograms with 1-ms bins, the response to whisker deflection was measured on-line (CED 1401) and stored. To calculate the evoked sensory response, the number of spikes occurring in the first 100 ms after the stimulus was counted and adjusted by subtraction of the number of spikes occurring in the 50 ms preceding the whisker deflections (an estimate of spontaneous activity), multiplied by two.
10. In pilot experiments, small electrolytic lesions were made at measured depths and later examined in coronal, cytochrome-oxidase–stained sections. The granular layer (layer IV) could be reliably identified at the in vivo depth of 400 to 800 μm. Thus, 62 neurons (27 in normal cases, 35 in experimental cases) were classified as supragranular, 110 neurons (65 in normal cases, 45 in experimental cases) as granular, and 53 neurons (27 in normal cases, 26 in experimental cases) as infragranular. Layers II to III were grouped together, as were layers V to VI.
11. The response in the supragranular layers to D-paired increased ($t = 3.581$, $P < 0.005$), whereas the response to D-cut did not change ($t = 0.475$, $P = 0.636$). In the infragranular layers, the response to D-paired increased ($t = 2.032$, $P < 0.05$), whereas the response to D-cut did not change ($t = 0.100$, $P = 0.920$). In layer IV, neither the response to D-paired nor the response to D-cut changed ($t = -0.488$, $P = 0.626$ and $t = -0.192$, $P = 0.848$, respectively).
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5