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Experience-dependent plasticity in adult rat barrel cortex

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ABSTRACT **This study tested the hypothesis that the receptive fields (RFs) of neurons in the adult sensory cortex are shaped by the recent history of sensory experience. Sensory experience was altered by a brief period of "whisker pairing": whiskers D2 and either D1 or D3 were left intact, while all other whiskers on the right side of the face were trimmed close to the fur. The animals were anesthetized 64–66 h later and the responses of single neurons in contralateral cortical barrel D2 to stimulation of whisker D2 (the center RF) and the four neighboring whiskers (D1, D3, C2, and E2; the excitatory surround RF) were measured. Data from 79 cells in four rats with whiskers paired were compared to data from 52 cells in four rats with untrimmed whiskers (control cases). During the period of whisker pairing, the RFs of cells in barrel D2 changed in three ways: (*i*) the response to the center RF, whisker D2, increased by 39%, (*ii*) the response to the paired surround RF whisker increased by 85–100%, and (*iii*) the response to all clipped (unpaired) surround RF whiskers decreased by 9–42%. In the control condition, the response of barrel D2 cells to the two neighboring whiskers, D1 and D3, was equal. After whisker pairing, the response to the paired neighbor of D2 was more than twice as large as the response to the cut neighbor of D2. These findings indicate that a brief change in the pattern of sensory activity can alter the configuration of cortical RFs, even in adult animals.**

The purpose of this study is to examine the influence of sensory experience on the functional properties of neurons in the somatosensory cortex (SI). The rodent whisker sensory system offers several advantages for studying the mechanisms of experience-dependent cortical plasticity. The projection from the whisker follicles to the contralateral SI preserves the spatial organization of the sensory receptors, resulting in a somatotopic map of cortical columns (1, 2). Layer IV of each column contains a "barrel," a discrete cluster of closely packed cells that is readily identified by several histological markers (3, 4). The one-to-one correspondence between each whisker and its cortical barrel makes it possible to relate whisker-evoked single-unit cortical activity to distinct thalamocortical and corticocortical pathways. For example, cells in barrel D2 are excited quickly (6- to 10-ms latency) and powerfully (1–2 spikes per stimulus) by deflection of whisker D2 (5). Because this response depends upon direct inputs from the thalamic ventral posterior medial nucleus (6), it is convenient to call whisker D2 the "center receptive field" (CRF) of barrel D2. Deflection of neighboring whiskers (e.g., D1, D3, C2, or E2) excites cells in barrel D2 less strongly (approximately one spike every second or third stimulus) at a longer latency (20 ms on average). Since the response to these whiskers is generated in large part by a separate pathway—intracortical inputs from surrounding barrels (7)—the neighboring whiskers are referred to as the "excitatory surround receptive field" (SRF) of barrel D2.

In the present study, we manipulated the lengths of rats' whiskers on one side of the face to produce a temporary change in the pattern of afferent activity to barrel D2. The CRF, whisker D2, and one neighboring whisker, D1 or D3, were left intact while all others were clipped. Under this condition, activity from the CRF whisker is temporally correlated with activity from the "paired" SRF whisker and is temporally decorrelated with activity from all other SRF whiskers. The results indicate that, after only a few days of paired whisker activity, the RFs of barrel D2 cells were significantly biased: in comparison to control data, the response to both paired whiskers was elevated, whereas the response to the unpaired whiskers was depressed.

METHODS

Whisker Trimming. Experimental subjects were four adult male Long–Evans rats weighing 260–295 g. While the rats were carefully held immobile, all but two whiskers on the right side of the face were clipped to the level of the fur. Whisker D2 and either D1 (one case) or D3 (three cases) were spared (Fig. 1*A*). Whiskers on the left side of the face were left intact.

The subjects were in a cage with at least one normal littermate during the 65-h interval between whisker clipping and the start of the physiological recording session. They appeared to use the intact paired whiskers to palpate, explore, and "whisk" in the normal manner (8). At the start of the recording session, the two intact whiskers were trimmed to a length of 3–5 mm to match the length of the previously clipped whiskers.

Control experiments were carried out on four adult male rats (also caged with one normal littermate) whose whiskers were left intact prior to the recording session.

Preparation and Histology. Rats were anesthetized with urethane (1.5 g/kg, i.p.). Body temperature was maintained at 36–37°C. An opening was made in the skull to expose the whisker area of SI of the left hemisphere, and a small incision was made in the dura overlying barrel-column D2.

During the recording session the depth of anesthesia was held at a consistent level by maintaining the burst rate of layer V cortical neurons at 2–4 Hz, simulating a condition of slow-wave sleep (9). With any sign of decreasing depth of anesthesia, a supplement of urethane was given (10% of the original dose).

At the end of the experiment, the rat was given a lethal dose of Nembutal and perfused with a 0.1 M phosphate-buffered saline rinse followed by buffered 4% (wt/vol) paraformaldehyde. After being post-fixed in paraformaldehyde followed by 30% (wt/vol) sucrose, the neocortex was separated from the underlying white matter and flattened between glass slides (2). The slab was frozen, and 50-μm tangential sections were cut and processed for cytochrome oxidase activity (4).

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Abbreviations: RF, receptive field; CRF, center RF; SRF, surround RF; SI, somatosensory cortex; PSTH, peristimulus time histogram.

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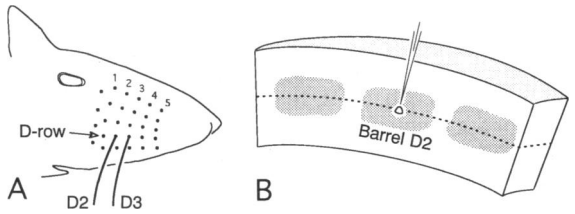

FIG. 1. Design of experiments. (*A*) Each of the major whiskers of the rat's face is identified by row (A–E) and arc (1–5). In this drawing, whiskers D2 and D3 are paired and all other whiskers are clipped. (*B*) Sixty-five hours later, a microelectrode records the activity of single cells in cortical barrel D2 in response to deflection of the CRF whisker D2 and SRF whiskers D3, D1, C2, and E2.

Whisker Stimulation. To deflect individual whiskers on the right side of the face, a piezoelectric ceramic wafer stimulator was positioned just below the shaft of the whisker, 2–4 mm from the skin. The wafer was deflected by a computer-gated electrical current. The stimulus was a 300-μm up–down movement of the wire tip with rise and fall times of 0.5 ms and a total stimulus duration of 3 ms. In each block of trials the stimulus was presented 50 times at 1 Hz. For every cell recorded in barrel D2, one block of stimulus trials was presented to whisker D2 and to each of its immediate neighbors (D1, D3, C2, and E2).

Recording and Data Analysis. Carbon fiber microelectrodes (10, 11) were used to record action potentials, which were isolated by a time–amplitude window discriminator (Bak Electronics, Rockville, MD). Accepted action potential waveforms were monitored on a digital storage oscilloscope (Nicolet) to ensure continued isolation of the same neuron.

By using raster plots, peristimulus time histograms (PSTHs), and latency histograms with 1-ms bins, the response to deflection of single whiskers was measured on-line (Cambridge Electronic Design 1401, Cambridge, England) and stored on a hard disk. The number of spikes occurring in the first 100 ms after stimulus was counted and adjusted by subtracting the number of spikes occurring in the 50 ms preceding the whisker deflections (an estimate of spontaneous activity), multiplied by 2. The resulting value was considered the evoked sensory response. The time of the first post-stimulus spike was measured for each trial and summated as a latency histogram. The bin with the peak value was the modal latency.

Cortical recording sites were marked by passing a direct current of 0.5–2 μA for 5–10 s (electrode tip, positive). This produced a spheroidal lesion roughly 50 μm in diameter that was easily seen in histological sections.

Identification of Recording Sites in Barrel D2. The design of this experiment required that all studied cells be located within cortical barrel D2 (Fig. 1*B*). A neuron was considered to be within barrel D2 if the recording site was later localized within the vertical and horizontal bounds of barrel D2 in cytochrome oxidase-stained tangential sections (Fig. 2). Penetrations localized in the septa between barrels were rejected. Although not every penetration was marked by an electrolytic lesion, those not marked were a short measured distance from a marked lesion, so that their location could be determined by geometric reconstruction.

In rats with normal sensory experience, the RF asymmetry of a cell in barrel D2 is correlated with the cell's location (12). For example, a neuron located in barrel D2 near the D2–D3 septum is likely to respond more strongly to whisker D3 than to whisker D1. Fig. 2 shows that the recording sites in these experiments were distributed throughout barrel D2, signifying that the RF asymmetries to be described below did not result from any bias in the location of recording sites.

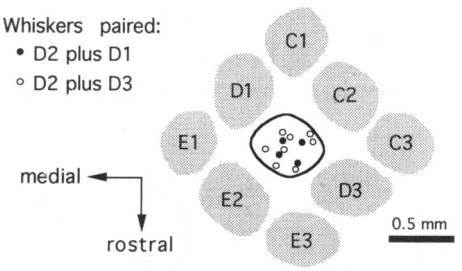

FIG. 2. Locations of microelectrode penetrations. A standardized barrel map was made from cytochrome oxidase-labeled tangential sections, and those penetrations marked by microlesions were projected onto the map. Other recording sites (not shown) were reconstructed from their coordinates relative to lesion sites. Penetrations are coded according to the sensory experience of the rat: eight sites were from rats with whiskers D2 and D3 paired and four sites were from rats with whiskers D2 and D1 paired.

RESULTS

The main finding was that a brief period of altered sensory experience led to significant changes in the functional properties of neurons in SI. This was evident from a comparison of the RF symmetry of neurons in barrel D2 in control cases (no whiskers trimmed) versus experimental cases (all whiskers except two trimmed). Of 52 cells recorded from barrel D2 in four control animals, 27 (52%) had a greater response to whisker D3 than to whisker D1, 24 (46%) had a greater response to whisker D1 than to whisker D3, and 1 (2%) had an equal response. Thus, the cell population in barrel D2 of control cases showed no "bias" in response toward one or the other neighboring same-row whisker ($z = 0.415$; $P < 0.68$). The 65-h period during which whisker D2 and one neighbor were paired produced a bias toward the paired neighbor: of 79 cells recorded from barrel D2 after whisker pairing, 62 (78%) gave a greater response to D-paired and only 16 (20%) gave a greater response to D-cut (D-paired and D-cut refer to the same-row neighbors of whisker D2). The preference for the paired whisker was greater than could be expected by chance ($P < 0.01$ versus control cases) and did not depend on whether the paired whisker was D1 or D3 ($\chi^2 = 0.037$; $P = 0.847$).

Fig. 3 provides a typical example of the RF shift in barrel D2 produced by whisker pairing. The left side of the figure shows PSTHs from a rat with normal sensory experience (case WP17). Whisker D2 evoked a vigorous response (52 spikes) at a short latency; whiskers in the SRF evoked weaker responses at modal latencies of >20 ms. In particular, note that whiskers D3 and D1 yielded similar response magnitudes (17 and 15 spikes, respectively) and that whiskers C2 and E2 yielded similar response magnitudes (13 and 11 spikes, respectively). The right side of the figure shows PSTHs from a rat with two whiskers paired for 64 h (case WP21) and illustrates three points: (*i*) whisker D2, the CRF, evoked a powerful response (89 spikes per 50 stimuli) at a short latency; (*ii*) whisker D3, the SRF whisker paired with D2, evoked a substantial response (27 spikes, see arrow); (*iii*) the SRF whiskers that had been clipped evoked much smaller responses (12, 5, and 6 spikes from whiskers D1, C2, and E2, respectively).

Mean whisker-evoked response magnitudes for control cases and the two experimental conditions are shown in Fig. 4. With normal sensory experience, the RF configuration was symmetrical. The CRF, whisker D2, evoked an average response of 44.6 spikes in 50 trials (significantly greater than all other whiskers; Wilcoxon signed rank, $P = 0.0001$); whiskers D1 and D3 evoked nearly equivalent levels of response, 16.5 and 14.9 spikes, respectively; and whiskers C2 and E2 evoked nearly equivalent levels of response, 11.2 and

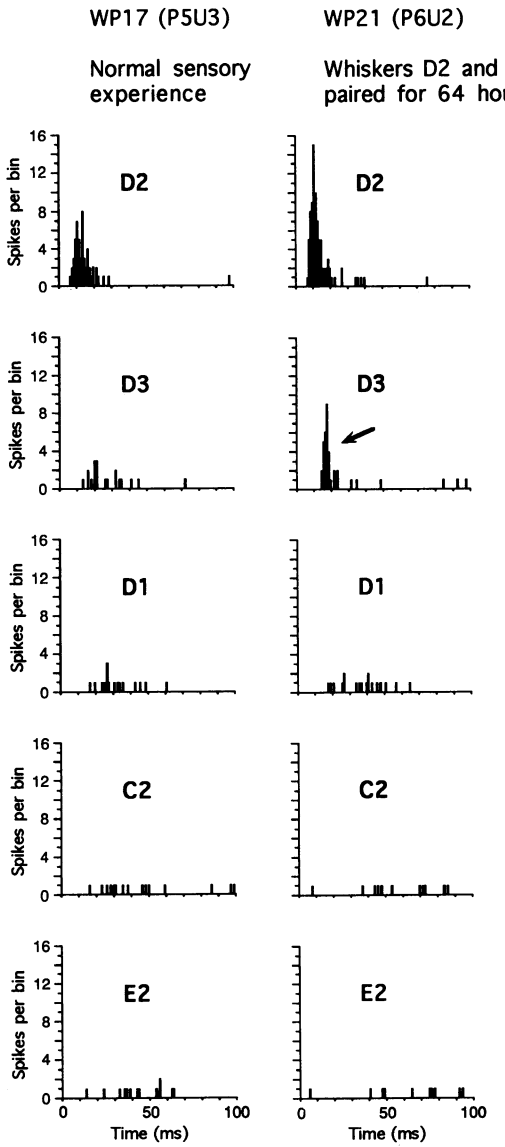

FIG. 3. Representative responses of barrel D2 cells in rats with differing sensory experience. Whisker deflection in each PSTH was at 0 ms. Cell P5U3 (*Left*) was recorded in a rat (WP17) with all whiskers intact. Note the vigorous response to the CRF whisker and the symmetry in the response to whiskers D1 and D3 and whiskers C2 and E2. This is in contrast to cell P6U2 (*Right*) that was recorded in a rat (WP21) with whiskers D2 and D3 paired during the preceding 64 h. Here, movement of whisker D3 yielded a stronger response (arrow) than the SRF whiskers that had been cut. The values of the cells' response magnitude given in the text were computed after subtraction of spontaneous activity (data not shown).

11.3 spikes, respectively. The above values will be referred to as the "control" responses.

After whiskers D2 and D1 were paired, the response to whisker D2 was significantly greater than it was in controls (64.1 versus 44.6 spikes; Mann–Whitney U test, $P = 0.004$); similarly, the response to whisker D1 was significantly greater than it was in controls (30.5 versus 16.5 spikes; $P = 0.003$). The response of barrel D2 cells to the clipped SRF whiskers (C2, D3, and E2) decreased, but the change was only modest (also see Fig. 5).

The outcome was analogous when whiskers D2 and D3 were paired: the response magnitude to whisker D2 increased with respect to its control value (61.2 versus 44.6 spikes; $P = 0.004$) as did the response to whisker D3 (29.8 versus 14.9 spikes; $P = 0.003$). Again, the response of barrel D2 cells to the clipped whiskers (C2, D1, and E2) decreased, but the change was irregular.

Considering the two experimental groups together (D2–D1 paired and D2–D3 paired), the average rate of spontaneous activity decreased from 1.5 spikes per second, in the control condition, to 1.1 spikes per second, after the whiskers were paired.

To determine which temporal component of the whisker-evoked response changed during the period of altered sensory experience, PSTHs were subdivided into 10-ms epochs and experimental data were compared to control data. This analysis showed that >90% of the enhanced response to the paired whiskers (D2 and its spared neighbor) occurred >10 ms after the stimulus. The relevance of this observation to mechanisms of cortical plasticity will be addressed in the *Discussion*.

Thus, the main finding presented in Fig. 4 is that, as a result of a brief period of altered sensory experience, the symmetrical RF of the D2 barrel cell population in the control condition changed to an RF biased to the paired SRF whisker. This shift can be appreciated by the ratio of the response to deflection of whiskers D1 and D3 when one was spared and the other was cut. With all whiskers intact (controls), the D1/D3 response ratio was 1.1; after pairing D1 with D2, the D1/D3 ratio became 2.2. By the same token, the D3/D1 response ratio increased from 0.9 in the control condition to 2.1 after whisker D3 was paired with D2. Summing the two experimental groups, the response ratio of D-paired divided by D-cut was 2.1 (i.e., 29.9 spikes/14.1 spikes).

The effect of the experimental manipulation is summarized in Fig. 5, which shows that clipping whiskers led to three distinct changes in the response of barrel D2 neurons. (*i*) There was a prominent increase in the response to the CRF, whisker D2. (*ii*) There was an even greater increase in response to the paired neighbor of whisker D2. (*iii*) There was a modest attenuation in the response to the SRF whiskers that were clipped.

DISCUSSION

Plasticity Evoked by Pairing Sensory Inputs. Our findings demonstrate that in adult rats a brief nonnociceptive change in the pattern of afferent sensory activity produces significant changes in SI. We view this experimental design as fundamentally different from studies involving peripheral nerve damage. The closest parallel to pairing whiskers may be "syndactyly," the surgical fusion of two adjacent digits of the owl monkey (13). After syndactyly, many of the recorded cell clusters in SI responded to stimulation of both "paired" digits, whereas in the control condition cell clusters responded to only a single digit. The experimental design differed from ours in that cortical receptive fields were mapped months, rather than days, after the onset of the sensory manipulation, and they were assessed qualitatively rather than quantitatively, so that the exact degree of increase in response to the SRF digit could not be determined. Despite methodological differences, their result is analogous with the present findings. If we raised the threshold for what was accepted as a "response," we could conclude that after whisker pairing many cells in barrel D2 responded to stimulation of both whisker D2 and its paired neighbor, whereas at the same threshold barrel D2 cells in the control condition would be considered to respond only to whisker D2 (Fig. 3).

Hypotheses of Neuronal Plasticity. The principle of experience-dependent synaptic modification (14, 15) has been incorporated into the Bienenstock, Cooper, and Munro theory (16), a mathematical model that calculates changes in synaptic strength based on a sliding threshold. The position of the threshold for synaptic modification is set at any given time by the recent history of postsynaptic activity. The efficacy of the various inputs to a neuron can change inde-

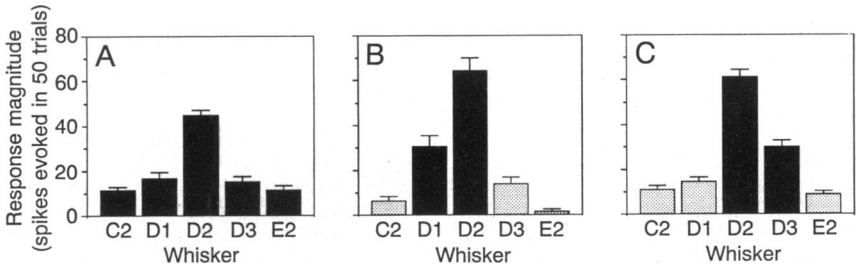

FIG. 4. Average response magnitude of barrel D2 cells to stimulation of the CRF whisker (D2) and four SRF whiskers (C2, D1, D3, and E2). Bars indicate the SEM. The RF was symmetrical for the cell population in rats with normal sensory experience (52 cells in four rats). When whiskers D2 and D1 were paired (20 cells in one rat) or when whiskers D2 and D3 were paired (59 cells in three rats), there was an increased response both to whisker D2 and to its paired neighbor. Histogram bins: solid, intact whisker; stippled, cut whisker. (*A*) Normal sensory experience. (*B*) Whiskers D2 plus D1 paired. (*C*) Whiskers D2 plus D3 paired.

pendently, provided their anatomical pathways to the neuron are separate. Thus, the model has been applied to left-eye/right-eye inputs to the developing cat visual cortex, where it simulated the shift in ocular dominance induced by monocular eyelid suture and other types of activity-dependent modification (17).

In the present experiment, we propose that leaving whisker D2 and one neighbor intact, while clipping all others, sets up conditions where evoked afferent activity to barrel D2 from the intact SRF whisker (D1 or D3) occurs frequently and often in synchrony with activity from the CRF, whisker D2. In contrast, evoked activity from the clipped SRF whiskers occurs only infrequently and rarely in synchrony with activity from the CRF whisker. By using these conditions as parameters in the Bienenstock, Cooper, and Munro equations, a circuit model of the barrel cortex simulates the empirical data of this paper (Lubica Benuskova, personal communication). Such mathematical formulations and computer simulations may provide testable predictions about cortical modification by sensory experience and, thus, lead to insights into the mechanisms of plasticity.

Neuronal Substrates of Cortical Plasticity. What synaptic mechanisms might underlie experience-dependent cortical plasticity? Several lines of evidence indicate that the response of barrel D2 cells to both their CRF and SRF depends to a large degree on intracortical pathways—intrabarrel or interbarrel circuits, respectively (6, 18). We therefore suggest that changes in the efficacy of CRF and SRF input to barrel D2 are due in large part to modification of corticocortical, rather than subcortical, synapses. Since monosynaptic thalamic input activates barrel cells <10 ms after a stimulus (5, 6, 12, 18), the fact that whisker pairing affected the magnitude of cortical response almost exclusively in epochs after the first 10 ms is consistent with this suggestion.

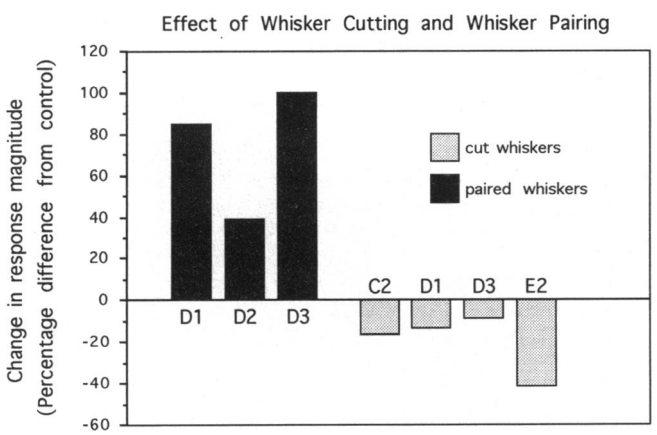

FIG. 5. Summary of cortical plasticity produced by a brief period of altered afferent activity. Whiskers that had been spared yielded increased responses from barrel D2 cells in comparison to control data. The enhancement was greater for the paired SRF whisker (D1 or D3) than for the CRF whisker (D2). All increases were significant at the level of $P < 0.005$ (Mann–Whitney U test). SRF whiskers that had been trimmed yielded decreased responses. Only whisker E2, however, yielded a statistically significantly depressed response in comparison to its control value ($P = 0.002$). (Whiskers D1 and D3 are represented twice because they were studied under two conditions, cut and paired, in different animals.)

Experience-dependent cortical plasticity probably involves a number of types of intracortical modification, and at this point no possibility can be definitively proved or eliminated. One hypothesis is that clipping whiskers causes a greater response to the paired whiskers through a change in the balance of intracortical inhibition. However, the finding that the average rate of spontaneous activity decreased from 1.5 spikes per second in control animals to 1.1 spikes per second in animals with 64–66 h of whisker-pairing experience argues that there was not an overall release of the cortex from inhibition. Clipping whiskers is known to lead to down-regulation of glutamate decarboxylase (GAD) (the synthetic enzyme for the inhibitory neurotransmitter γ-aminobutyric acid), but GAD is down-regulated only in the barrels corresponding to the clipped whiskers (19). The change in GAD activity could be expected to decrease the amount of intracortical inhibition evoked by deflection of whiskers that had been clipped while maintaining the normal amount of inhibition evoked by deflection of whiskers that had been spared. Thus, our experimental observations—a significant increase in response to deflection of spared whiskers and a modest decrease in response to deflection of clipped whiskers (Fig. 5)—were opposite to what would be predicted if changes in intracortical inhibition were the major or the only player.

We therefore favor the view that excitatory synapses mediated by glutamate receptors of the *N*-methyl-D-aspartate (NMDA) type are regulated by sensory experience. Due to their voltage dependence, NMDA receptors possess the ideal characteristics for modulating synaptic strength as a function of the temporal correlation of presynaptic and postsynaptic activity (20), and they are known to mediate a high proportion of within-barrel and between-barrel communication (18). The communication between barrels with "paired" afferent input may be potentiated through a mechanism similar to that operating in *in vitro* slices from adult rat SI (21). Paired—but not unpaired—4-Hz electrical stimulation at two sites in layer VI, separated by ≈1 mm in the horizontal dimension, potentiated the response at a layer III recording site located between them. Additional studies may be able to clarify the contribution of NMDA receptor-mediated synaptic changes to experience-dependent plasticity.

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