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Modulation of spike timing by sensory deprivation during induction of cortical map plasticity

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Abstract

Deprivation-induced plasticity of sensory cortical maps involves long-term potentiation (LTP) and depression (LTD) of cortical synapses, but how sensory deprivation triggers LTP and LTD *in vivo* is unknown. Here we tested whether spike timing–dependent forms of LTP and LTD are involved in this process. We measured spike trains from neurons in layer 4 (L4) and layers 2 and 3 (L2/3) of rat somatosensory cortex before and after acute whisker deprivation, a manipulation that induces whisker map plasticity involving LTD at L4-to-L2/3 (L4–L2/3) synapses. Whisker deprivation caused an immediate reversal of firing order for most L4 and L2/3 neurons and a substantial decorrelation of spike trains, changes known to drive timing-dependent LTD at L4–L2/3 synapses *in vitro*. In contrast, spike rate changed only modestly. Thus, whisker deprivation is likely to drive map plasticity by spike timing–dependent mechanisms.

Sensory deprivation induces long-lasting changes in sensory cortical maps that reflect the importance of experience in guiding cortical development and adult function^{1,2}. According to a dominant model, such map plasticity involves LTP and LTD at cortical synapses, driven by sensory-evoked and spontaneous firing patterns within the cortical circuit^{2–5}.

Considerable evidence now confirms that LTP and LTD or similar processes are induced at specific cortical synapses during map plasticity^{6–9}, but how deprivation alters firing patterns to drive LTP, LTD and overall map plasticity remains largely unknown.

In vitro, LTP and LTD can be induced by either modulation of pre- and postsynaptic firing rates^{10,11} or modulation of the precise, millisecond-scale timing of pre- and postsynaptic action potentials, which is relatively independent of firing rate^{12–14}. In rate-dependent plasticity, LTP and LTD are induced by high- (~10 Hz or greater) and low-frequency (0.5–10 Hz) presynaptic firing, respectively^{4,10,11,15,16} or by changes in covariance of pre- and postsynaptic firing rates on the ~50 ms time scale^{2,5}. In spike timing–dependent plasticity (STDP), LTP is induced when presynaptic cells consistently fire 0–20 ms before postsynaptic cells, and LTD is induced either when the firing order is reversed or when pre- and postsynaptic firing become uncorrelated on the ~100 ms time scale^{12–14,17}. Both rate-dependent and timing-dependent modes of induction occur in primary sensory cortex *in vitro*^{2,4,12,18}, and theoretical and modeling studies indicate that both can explain Hebbian-

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like receptive field and map plasticity *in vivo*^{4,5,19–21}. It is not known, however, which mode actually contributes to deprivation-induced plasticity.

We studied whether STDP or rate-dependent plasticity contribute to deprivation-induced receptive field plasticity in the whisker region of the rat primary somatosensory cortex (S1). Neurons in S1 normally respond most strongly to deflection of the whisker corresponding to their cortical column, termed the principal whisker (PW), and less strongly to surround whiskers (SW), resulting in an orderly whisker map^{22,23}. Plucking or trimming whiskers causes rapid, long-lasting receptive field plasticity that includes the activity-dependent depression of responses to deprived whiskers, termed principal whisker response depression^{24,25}. In adolescent rats, principal whisker response depression occurs most rapidly in L2/3, is delayed or absent in L4, and involves induction of LTD or LTD-like synaptic depression at feedforward, excitatory L4–L2/3 synapses in deprived columns^{7,24}. Such deprivation-induced LTD has been directly observed in S1 following whisker deprivation⁷, and in V1 following monocular deprivation⁸. L4–L2/3 synapses show rate-dependent and spike timing-dependent LTD *in vitro*^{7,17,18}, but how deprivation drives LTD at these synapses *in vivo* is unknown.

We characterized the spiking patterns of L4 and L2/3 neurons *in vivo* during normal sensory use and acute whisker deprivation, to determine whether deprivation induces LTD at L4–L2/3 synapses by altering spike rate, spike timing, or both. Whisker deprivation produced immediate, major changes in spike timing that were appropriate to drive spike timing-dependent LTD, but only modest changes in spike rate that seemed insufficient to drive rate-dependent LTD, assuming similar learning rules *in vivo* and *in vitro*. Thus, we propose that whisker deprivation drives cortical plasticity by modulating spike timing and inducing STDP at S1 synapses.

RESULTS

Acute modulation of firing rate by whisker trimming

To determine whether deprivation alters mean firing rates at L4–L2/3 synapses, we measured spike trains of single L4 and L2/3 neurons using chronically implanted electrode arrays (Fig. 1a) in awake, behaving rats during free exploration. The animal's behavior was videotaped and classified into five whisker-related behavioral states (Fig. 1b,d). Mean firing rate was calculated for each behavioral state following sham whisker cut (control, all whiskers intact) and, for the same neurons, immediately after trimming the PW corresponding to the recorded column (Fig. 1c). Deprivation of a single or several whiskers drives receptive field plasticity^{24,26} and LTD at L4–L2/3 synapses⁷ in deprived whisker column(s).

When all whiskers were intact, mean single-unit firing rates, independent of behavior, were 2.7 ± 0.4 Hz (mean $\pm$ s.d.) for L4 ($n = 39$ units) and 2.1 ± 0.3 Hz for L2/3 ($n = 44$ units). These low firing rates are consistent with recent findings that whisker S1 (refs. 27,28) and other areas (ref. 29) show substantially lower firing rates to optimal stimuli than classically described for primary sensory cortex^{30,31}. Trimming the PW reduced overall firing rates, as expected from previous studies³², but did so only modestly, to 2.1 ± 0.4 Hz for L4 and 1.7 ± 0.2 Hz for L2/3 ($P < 0.05$, paired *t*-test). Significant reductions in firing rate occurred during most whisker-related behaviors (Fig. 1d). However, these changes in mean firing rate appear insufficient to drive rate-dependent LTD as defined at L4–L2/3 synapses in V1 (refs. 4,17) and Schaffer collateral–CA1 synapses¹⁶ *in vitro*, where 2–3 Hz spiking elicits identical, near-maximal LTD. This indicates that another feature of spike trains, perhaps spike timing, drives LTD in response to whisker trimming.

Acute modulation of spike timing by whisker deprivation

To measure spike timing at L4–L2/3 synapses, we recorded spike trains simultaneously from L4 and L2/3 neurons in single S1 columns. Anesthetized animals were used so that precisely timed whisker deflections could be delivered. Recordings were made with a linear electrode array from sites in L4 and either L2 or L3, defined by depth criteria and confirmed by latency analysis and lesion recovery in a subset of experiments (see Methods and Fig. 2a,b). For each L4–L2 or L4–L3 cell pair, responses were first recorded to simultaneous deflection of all contralateral whiskers via a moveable lightweight mesh (multi-whisker stimulation), intended to mimic near-synchronous whisker motion during exploratory whisking.

L4, L3 and L2 neurons responded to multiwhisker stimulation with sparse, precisely timed spikes (Fig. 2c–e). Single L4 neurons ($n = 40$) generated a single spike in only 24% of trials, and >1 spike in only 8% of trials (mean $\pm$ s.e.m.: 0.66 ± 0.15 spikes/stimulus). Single L2 or L3 neurons ($n = 44$) generated a single spike in only 20% of trials, and >1 spike in only 7% of trials (mean: 0.39 ± 0.07 spikes/stimulus). Thus, spiking was infrequent. In addition, spike timing was precise: post-stimulus time histograms (PSTHs) had a mean width of 13 ± 9 and 15 ± 8 ms for L4 and L2/3 (Fig. 2c), and the coefficients of variation for spike times within single units were 0.39 (L4, mean across 40 units), 0.48 (L3, $n = 23$) and 0.28 (L2, $n = 21$). Mean response latency was 8.6 ± 1.0 ms for L4, 10.2 ± 1.5 ms for L3, and 13.0 ± 3.0 ms for L2, consistent with feedforward relay of information from L4 to L3 and L2 (Fig. 2c,d). Thus, multiwhisker responses typically consisted of a single precisely timed spike elicited serially from L4 and L2/3 neurons, as reported for responses to PW deflection alone^{33,34}.

After recording multiwhisker responses for a given cell pair, the effect of acute PW deprivation was assayed by trimming the PW to just escape the mesh, thereby allowing all whiskers to be deflected except the PW (PW cut). Figure 3 shows the effect of PW cut on a representative L4–L2 cell pair. During multiwhisker stimulation, these L4 and L2 units responded strongly with mean onset latencies of 8 and 12 ms, respectively (Fig. 3a,b, left, ‘all whiskers’), and individual L4 spikes preceded L2 spikes by ~ 2 –10 ms, as revealed by the joint peristimulus time histogram (JPSTH), which shows the time, relative to stimulus onset, of all L4 and L2 spikes that co-occurred in the same sweep³⁵ (Fig. 3c, left). This pattern of L4-leading-L2 firing was also evident in the cross-correlogram (CCG) derived from the JPSTH data, which showed a modal delay (Δt) of $+7$ ms (positive values indicate L4 leading L2/3), and in which 80.2% of spike pairs exhibited L4-before-L2 spike order (Fig. 3d, left).

PW cut produced an immediate reversal in firing order, which was evident from the JPSTH and the shift in the modal Δt of the CCG to -8 ms (L2 leading L4) (Fig. 3, middle, ‘PW cut’). The change in CCG shape was highly significant ($P < 0.001$, t -test comparing Δt distributions before and after PW cut), with 68.3% of spike pairs showing L2-before-L4 spike order after PW cut (Fig. 3d, middle). Spike timing recovered immediately when the mesh was advanced slightly to once again deflect all whiskers including the PW (Fig. 3, right, ‘Recovery’). In addition to altering spike timing, PW cut also reduced the number of whisker-evoked spikes for this and most cell pairs (Supplementary Fig. 1 online), as expected from the behaving animal results (Fig. 1).

A similar, but smaller, effect was observed for L4–L3 pairs, as shown by the example in Figure 4. During multiwhisker stimulation, response latency was shorter for L4 than L3 (Fig. 4a, left), and individual L4 spikes preceded L3 spikes by a mean Δt of 1.05 ms and a modal Δt of 2 ms, with 67.3% of spike pairs showing L4-leading-L3 firing order (Fig. 4c, left). This small L4 lead is consistent with the short axonal path length from L4 to L3 and the presence of direct thalamo-cortical input to portions of L3. After PW cut, spike timing changed modestly but significantly ($P < 0.001$, t -test comparing Δt distributions before and

after PW cut), so that only 54.1% of spike pairs had an L4-leading-L3 firing order, and the modal and mean Δt were -1 ms and -4 ms, respectively (L2 leading L4) (Fig. 4a–c, middle column).

Across all L4–L2 cell pairs ($n = 46$), modal Δt values from CCGs shifted from $+5.4 \pm 1.9$ ms (L4-leading-L2) during multiwhisker stimulation to -2.5 ± 4 ms after PW cut, which represents a reversal in average firing order (Fig. 5a). Of these cell pairs, 62% showed complete reversals in firing order (CCG peak shifted from L4-leading to >0.5 ms L2-leading), and another 15% of pairs shifted from L4-leading to firing that was synchronous on average (CCG peak of -0.5 to 0.5 ms). For L4–L3 pairs ($n = 128$), modal Δt values shifted from $+1.3 \pm 1.6$ ms during multiwhisker stimulation to -1 ± 2.1 ms after PW cut. We found that 53% of L4–L3 pairs had complete reversals in mean firing order, and another 28% switched from L4 leading to mean synchronous firing. Combining across layers, almost 80% of cell pairs showed changes in firing order from L4-leading to either L2/3-leading or synchronous firing, with L4–L2 pairs showing the largest effects (Fig. 5a). These changes were not due to time-dependent instability of recording, as cross-correlation peaks and other features of whisker-evoked responses remained constant over comparable recording durations without PW cut (Supplementary Fig. 2 online). The changes in firing order after PW cut are appropriate to predict spike timing-dependent LTD at L4–L2/3 synapses¹⁷.

Changes in spike timing reflect altered response latency

The changes in spike timing were due to systematic changes in response latency after PW cut. During multiwhisker stimulation, mean onset latency was shortest in L4, and progressively longer in L3 and L2 (Table 1), consistent with feedforward relay of information from L4 to L3 and L2 (ref. 34). With PW cut, mean onset latency increased by 4.2 ms in L4 ($P < 0.001$, paired t -test) and by 2.0 ms in L3 ($P < 0.02$), but actually decreased in L2 by 2.2 ms ($P < 0.03$) (Fig. 5b and Table 1). As a result, L4 onset latency became longer than L2 or L3 onset latency after PW cut. These changes in onset latency account for almost 90% of the change in modal Δt of the CCG observed with PW cut. Furthermore, changes in modal latency, which reflects average timing of all spikes in the response, completely explain the changes in modal Δt of the CCG (Table 1). These latency changes are apparent from simple PSTHs (Supplementary Fig. 3 online), are not due to temporal instability of recording (Supplementary Fig. 2 and Supplementary Methods online), and can largely be explained by known S1 circuits mediating principal and surround whisker responses (see Discussion).

Decorrelation of spike trains during PW deprivation

Timing-dependent LTD is induced at L4–L2/3 synapses *in vitro* not only by consistent, post-leading-pre firing order, but also by decorrelation of pre- and postsynaptic spike trains, as a result of a bias toward LTD in the STDP learning rule¹⁷. To determine whether PW deprivation reduced overall firing correlations between L4 and L2/3 spike trains *in vivo*, we calculated mean CCGs representing the average shape of cross-correlation for all cell pairs within a given stimulus condition (Fig. 6). For multiwhisker stimulation, mean CCGs contained a sharp peak of highly temporally correlated spikes (gray) superimposed on a background of broadly uncorrelated firing (black). The peak was centered at $+4$ ms and $+2$ ms (L4 leading) for L4–L2 ($n = 46$) and L4–L3 ($n = 128$) cell pairs, respectively (Fig. 6a, left).

The fraction of highly temporally correlated spikes (within ± 10 ms of the peak of the mean CCG) fell from 52% and 44% during multi-whisker stimulation to 32% and 30% after PW cut for L4–L2 and L4–L3 cell pairs, respectively. This represents a 32–39% average reduction in correlation strength. Moreover, 89% of individual L4–L3 pairs and 85% of L4–

L2 pairs showed a reduction in firing correlation after PW cut (Fig. 6b). Thus, PW cut reduced overall firing correlations between L4 and L2/3 neurons, an effect that is known to drive spike timing–dependent LTD *in vitro*¹⁷. This finding suggests that both decorrelation and reversal of mean firing order may drive timing-dependent LTD *in vivo* during whisker deprivation.

One mechanism by which PW cut may decorrelate spike trains is simply by reducing the number of whisker-evoked spikes, which are highly correlated between neurons (Figs. 2–4). This would increase the proportion of spontaneous spikes, which may be more poorly correlated, in L4 and L2/3 spike trains¹⁷. PW cut does reduce whisker-evoked responses (Supplementary Fig. 1 online). Consistent with this model, spontaneous spiking, measured in long-duration recordings from 219 L4–L2/3 cell pairs, was almost completely uncorrelated on the 100-ms time scale (Fig. 6c). Quantitatively, highly correlated spikes ($\Delta t = 0 \pm 10$ ms) accounted for only 14.9% of all spike pairs with $\Delta t < \pm 100$ ms, only slightly more than predicted for completely uncorrelated spiking (10%). This observation contrasts with reports of highly correlated spontaneous firing across cortical columns on longer time scales³⁶ and suggests that spontaneous spiking, because of its correlational structure, may powerfully drive spike timing–dependent LTD.

Spike timing changes are appropriate to drive LTD

To determine whether the observed spike timing changes were quantitatively appropriate to drive spike timing–dependent LTD at L4–L2/3 synapses, we developed a simple model to predict STDP induction by multiple spike pairs with specific spike timing distributions. The model incorporates two main assumptions: (i) that each L4–L2/3 spike pair, separated by a delay Δt , induces an amount of LTP or LTD determined from the measured STDP rule for L4–L2/3 synapses *in vitro*¹⁷; and (ii) all possible L4–L2/3 spike pairs within a train with $\Delta t \leq \pm 100$ ms contribute linearly toward total net plasticity. The second assumption reflects experimental data that STDP sums linearly at low frequency and departs significantly from linearity only at high frequency (>10 – 20 Hz)^{14,37,38}. S1 firing rates fall into the linear summation regime, with mean firing rates in behaving animals of 2.7 Hz for L4 neurons and 2.2 Hz for L2/3 neurons (Fig. 1), and only ~36% and ~20% of spikes having >10 and >20 Hz instantaneous frequency, respectively. Given these assumptions, the predicted net STDP induced by measured L4 and L2/3 spike trains was calculated as the dot (inner) product of the CCG for those spike trains and the STDP learning rule.

To increase model accuracy, we improved the sampling of the published STDP learning rule at L4–L2/3 synapses *in vitro*¹⁷ for Δt values near 0 ms. Using whole-cell recording from L2/3 pyramidal cells in S1 slices (ages: postnatal days (P)17–21) and extracellular stimulation of afferents in L4, we repeatedly paired single pre- and postsynaptic spikes at specific Δt values and measured the resulting LTP or LTD (methods as in ref. 17). When these data were combined with the original data set¹⁷, it was apparent that Δt values of 0 to 50 ms post-leading-pre elicited LTD, and Δt values of 3–15 ms pre-leading-post elicited LTP. The crossover between LTP and LTD occurred between 0 and 3 ms pre-leading-post (Fig. 7a, inset). The STDP learning rule was approximated in the model by empirically fit curves (Fig. 7a).

The model was used to predict the amount of STDP that would be induced by the spike timing distribution (CCG) measured for each cell pair under each stimulus condition (Fig. 7). For representative L4–L2 and L4–L3 cell pairs (Fig. 7b), as for the entire population (Fig. 7c), the distribution of spike timing measured during multiwhisker stimulation predicted little or no net synaptic plasticity—that is, the amount of LTP induced by L4-leading-L2/3 spike pairs was counterbalanced by an equal amount of LTD induced by L2/3-leading-L4 spikes. In contrast, the spike timing distribution measured with PW cut predicted

significant LTD (on average for L4–L2 pairs, –9% in the first 100 pairings). This analysis indicates that the spike timing changes measured during PW deprivation were quantitatively appropriate to drive spike timing–dependent LTD at L4–L2/3 synapses, assuming similar STDP rules *in vivo* and *in vitro*.

DISCUSSION

STDP has emerged as an attractive candidate mechanism for experience-dependent plasticity, owing to its powerful Hebbian properties, existence at multiple neocortical synapses, and physiologically realistic induction requirements^{12,13}. It was shown recently that precisely timed sensory stimuli can drive receptive field plasticity via STDP-like mechanisms^{39,40}. However, whether STDP contributes to forms of plasticity not involving precise control of stimulus timing, including classical, deprivation-induced map plasticity, is unclear.

The present data indicate that simple sensory use and deprivation, manipulations that robustly drive experience-dependent cortical plasticity^{1,2}, cause immediate changes in spike timing of L4 and L2/3 neurons in S1 cortex that are appropriate to drive STDP *in vivo*. Multiwhisker stimulation, intended to mimic normal sensory use, evoked spikes that were highly temporally correlated between L4 and L2/3 neurons, with L4 spikes occurring several ms before L3 and L2 spikes. Stimulation of all whiskers but the PW, meant to mimic principal-whisker deprivation, caused an immediate reversal in firing order for most neurons, and a reduction in overall firing correlations. Thus, sensory use correlated L4 and L2/3 firing, and deprivation reversed and decorrelated this firing. The measured firing correlations were quantitatively appropriate to predict stability of synaptic strength during multiwhisker stimulation, and significant spike timing–dependent LTD during principal-whisker deprivation. Thus, these changes in spike timing can explain how whisker deprivation leads to LTD at L4–L2/3 synapses⁷.

Mechanisms for spike-timing reversal

The stimulus-dependent changes in firing order observed here can be explained by known properties of S1 circuits. PW responses are largely mediated by feedforward excitation from thalamus to L4 and from L4 to L2 and L3 within the active column, followed by disynaptic inhibition to constrain responses to a single, precisely timed spike^{34,41}. Multiwhisker stimulation, which includes PW deflection, elicited single spikes at short, serially increasing latencies in L4, L3 and L2, consistent with activation of this feedforward, intracolumnar pathway.

In contrast, SW responses are mediated in large part by convergent, cross-columnar inputs to L4 and L2/3 neurons^{32,39} (but see ref. 41). Cross-columnar spread of excitation is functionally rapid in L2/3 (refs. 28,34), but slow in L4 (refs. 23,34), paralleling the relative density of cross-columnar projections in these layers^{28,42,43}. As a result, deflection of a single SW causes simultaneous responses in L2, L3 and L4 (ref. 34). Deflection of multiple SWs together, which comprised the stimulus during PW deprivation, caused most L3 and L4 neurons to respond simultaneously, consistent with this pattern of network activation. In L2, PW deprivation had the additional effect of reducing response latency compared with multiwhisker stimulation, which led to the reversal in firing order for L4–L2 cell pairs (Table 1, Fig. 5c and Supplementary Fig. 3 online). We hypothesize that this effect reflects the loss of normal, PW-evoked inhibition in surround columns⁴¹, resulting in faster cross-columnar relay of SW input to L2 of the deprived column.

Thus, because L4 and L2/3 neurons receive functionally and temporally distinct inputs representing PW and SW whiskers, PW use or disuse can dynamically modulate spike

timing of these neurons. We hypothesize that these stimulus-dependent changes in spike timing drive the rapid synaptic^{7,8} and receptive-field plasticity^{24,25,44} that occur in L2/3 with altered sensory experience.

Rate versus timing for plasticity *in vivo*

S1 neurons have low firing rates, with optimal stimuli eliciting only ~1 precisely timed spike from activated neurons^{23,27,28,39}, and with spike timing carrying most information about stimulus location⁴⁵. Other cortical areas²⁹, but not all^{31,46}, also seem to use a low-rate, sparse coding strategy. We suggest that sparse or infrequent spiking constrains mechanisms of activity-dependent plasticity and may favor use of timing-dependent, rather than rate-dependent, LTP and LTD. One reason for this may be that the high spike rates required for LTP (>10 or 20 Hz^{4,15,16}) are rare in such networks (in our data, only ~36% and ~20% of spikes had >10 and >20 Hz instantaneous frequency, respectively, in behaving animals). In addition, low mean firing rates during normal use indicate that deprivation can further reduce firing rates only modestly, and perhaps insufficiently to drive LTD. This was the case for PW deprivation in awake behaving rats (Fig. 1). Whether LTD may be driven in S1 by rate covariance on a short (50 ms) time scale is less clear and is likely to be highly model dependent^{5,19}. However, because co-columnar L4 and L2/3 neurons have similar receptive fields and positively correlated firing even after PW cut, covariance-induced LTD may be minimal (Figs. 3–7).

In contrast, conditions appear suitable for STDP in sparsely spiking networks, particularly in sensory cortex. Cortical networks use precise spike timing and spike-timing correlations to encode stimulus features^{30,45,47}, and many cortical synapses show STDP^{12,13,17}. Furthermore, as shown here, sensory use and deprivation modulate spike timing *in vivo* in a manner appropriate to drive STDP. Thus, although both rate and timing cooperate to guide synaptic plasticity *in vitro*^{14,38}, endogenous spiking patterns *in vivo* may make STDP most relevant for plasticity in sparsely spiking networks, including S1 cortex, and rate-dependent plasticity most relevant in networks with higher sustained firing rates⁴⁶.

METHODS

Surgical preparation for *in vivo* recording

All procedures were approved by the UCSD IACUC. Long-Evans rats (P30–41) were anesthetized with either ketamine and xylazine (150 and 60 mg/kg injected intraperitoneally (i.p.)) for chronic recording experiments or ethyl carbamate (Urethane, Sigma, 1.5 g/kg, i.p.) for acute experiments. Atropine sulfate (1 mg/kg) and lactated Ringers (3 ml) were administered, and a small head bolt was mounted to the skull. A craniotomy was made over left S1, and the dura was removed. Body temperature was maintained at $37.0 \pm 0.1^\circ\text{C}$ for recording.

Chronic recordings in behaving animals

The barrel column of interest (C₁, D₁ or D₂) was located by receptive-field mapping using a glass-insulated carbon fiber electrode (7 μm fiber, 0.8–1 M Ω at 1 kHz). Two pairs of twisted 20 μm diameter microwires (0.4–0.6 M Ω at 1 kHz, 110–120 μm total outer diameter), vertically offset by 500–600 μm (Fig. 1a), were inserted perpendicular to the pia within a single barrel column, and mounted to the skull with dental cement. The lower pair of microwires was positioned 800 μm below the pia, in L4 (see below), and the upper pair in L2/3 of the same column (Fig. 1a). Laminar and column identity was confirmed using computer-controlled, calibrated single-whisker deflections applied by a piezoelectric actuator. After 3–7 d recovery, recordings were made by a custom-made, differential on-head amplifier (1,000 $\times$ gain). Signals were band-pass filtered (0.5–6.0 kHz), further

amplified (5×), and sampled at 32 kHz from 2–4 electrodes simultaneously, using custom Igor routines (Wavemetrics). Spike sorting was performed off-line using a published algorithm⁴⁸ implemented in Matlab (Mathworks) by S. Mehta and D. Kleinfeld (Physics Department, UCSD). Single units with peak amplitude >3 s.d. above background noise were accepted for analysis. Sham and real whisker cut (trimming to the level of the fur) were performed during gentle restraint in the awake animal. Recording depths were verified by recovery of electrolytic lesions (3–4 μ A DC current for 10 s, tip negative) in cytochrome oxidase–stained coronal sections²⁸.

Spike data were analyzed with respect to specific whisker-related behaviors, as determined by video recording (30 frames/s) synchronized with electrophysiological data acquisition. Behavior was classified off-line by trained, blinded observers as either (i) exploratory whisking, (ii) whisking in air, (iii) whisker twitching, (iv) grooming of face and whiskers, (v) whisker movement during sniffing or (vi) none of the above. Behaviors (i) and (ii) were defined as large-amplitude, low-frequency whisker deflections with (i) or without (ii) locomotion⁴⁹. Behavior (iii) was low-amplitude, high-frequency deflection in air⁴⁹.

Acute electrophysiology

After initial mapping to locate the column of interest (C₁₋₃, D₁₋₃, E₁₋₃), a linear array of 16 recording pads spaced 50 μ m apart (“Michigan Probe”, University of Michigan Center for Neural Communication Technology) was placed radially in the target column. The craniotomy was filled with 2% low-melting point agarose. Recording pads spanned cortical depths (read from the microdrive) from 50 to 850 μ m below the pia, corresponding to L1 to L4 (see below) (Fig. 2a). Signals were pre-amplified (1,000×), band-pass filtered (0.5–6.0 kHz), further amplified (5×) and digitized at 32 kHz in 600–700 ms sweeps. Whisker deflection was performed using a computer-controlled piezoelectric bimorph actuator carrying a lightweight plastic mesh positioned 4–5 mm from the face, into which whiskers (trimmed to 6 mm length) were inserted. Whisker deflections (250 μ m [2.8–3.3°] ramp-and-hold deflection, 200 ms duration, delivered 200 ms after sweep onset, rise/fall time 1 or 4 ms) were delivered at 1 Hz. For each stimulus condition, 900 trials were collected. Only onset responses were studied. Spike counts and interspike intervals were calculated within 50 ms of stimulus onset. All reported error values are s.e.m. unless stated otherwise. Anesthesia was maintained by additional urethane (10% of original dose, i.p.) at a level that suppressed whisker movements and corneal and limb withdrawal reflexes and maintained breathing and heart rates at <2 Hz and 200–300 b.p.m.

Laminar identification of recording sites

In a pilot study, we determined the correspondence between microdrive depth and laminar identity. PW responses were recorded in three radial penetrations (3 rats) at 100 μ m depth increments between 0–1,400 μ m below the pia. Onset latencies were minimal at 750–900 μ m (7.7 ± 0.2 ms (mean $\pm$ s.e.m.), $n = 27$ neurons), consistent with L4 (refs. 34,50), and lesions made at these depths (800–850 μ m) were recovered in L4 (Fig. 2b). Onset latencies were longer at 350–700 μ m depth (9.5 ± 0.5 ms, $n = 10$), consistent with lower L2/3, and even longer at 0–300 μ m depth (15.1 ± 2.7 ms, $n = 14$), consistent with upper L2/3. Lesions made at 150–300 μ m were recovered in the upper half of L2/3 (Fig. 2b). Deeper layers had longer latencies (1,050–1,400 μ m, 12.1 ± 0.5 ms, $n = 63$), and lesions made at 1,050–1,100 μ m were recovered in upper L5 (Fig. 2b). Therefore, for the main study, upper L2/3 (termed L2 for brevity), lower L2/3 (termed L3), and L4 were identified by recording depths of 150–250, 450–550 and 800–850 μ m, respectively.

Cross-correlation, JPSTH and latency calculation

JPSTHs (1 ms bin size) were calculated over a ± 100 ms range of Δt and were not corrected for firing rate. Cross-correlograms (CCGs; 1 ms bin size) were calculated as the sum of JPSTH pixel values for the paradiagonal corresponding to each Δt , divided by the number of pixels in that paradiagonal³⁵. CCGs were calculated for all possible pairs of simultaneously recorded neurons, with a single neuron contributing to multiple cell pairs. CCG data was accepted for analysis only if the CCG contained at least 45 spikes (in ± 100 ms) and contained significant sampling of both peak and flanks (peak bin required to contain 5–50% of total CCG area). JPSTH and CCG data represent raw correlations, uncorrected for firing rate covariance. Mean CCGs were calculated from normalized CCGs (peak scaled to 1.0) of all cell pairs within a given stimulus condition. Onset latency was defined as the first of three contiguous 1-ms PSTH bins with firing ≥ 3 s.d. above mean pre-stimulus firing.

Model

CCGs for individual cell pairs were normalized to an area of 1.0 to allow prediction of plasticity due to the shape of the Δt distribution alone. Net predicted STDP was calculated for each cell pair and stimulus condition as the dot (inner) product of the normalized CCG with a vector representing the STDP learning rule. The STDP rule was approximated by (i) a quadratic fit $((-3.7 \times 10^6)x^2 - 0.0019x + 0.77)$ for $-250 \leq \Delta t \leq 0$ ms (LTD component), (ii) a cubic fit $((-4.7 \times 10^{-7})x^3 + 0.00028x^2 - 0.022x + 1.4)$ for $+3 < \Delta t \leq +32$ ms (LTP component), (iii) a linear interpolation for $0 < \Delta t \leq +3$ ms (LTD to LTP transition) and (iv) $y = 0$ for $\Delta t > +32$ ms. The scalar output of the model has units of fractional increase or decrease in synaptic strength with 100 pre–post spike pairs. How much additional plasticity may occur with >100 pairings is not known.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Principal whisker deprivation reduces mean firing rates in behaving rats. (a) Chronic probe for simultaneous recording of spikes from L4 and L2/3 neurons in a single column in a behaving rat. R, reference electrode for differential recording. G, ground. (b) System for simultaneous electrophysiological and behavioral recording during free exploration. (c) Experimental design. After the rat was placed in the exploration environment, the PW was sham-cut to provide a control for animal handling, then after 30 min, it was actually cut. Spiking and behavioral data were collected for 30 min after sham and actual whisker cut. (d) Mean firing rates for L4 ($n = 39$) and L2/3 ($n = 44$) neurons for each classified behavior, and for all spikes irrespective of behavior. Error bars indicate standard deviation (s.d.). Black bars, all whiskers intact. White bars, PW cut.

Figure 2. Spike trains elicited by multiwhisker stimulation in L4 and L2/3 of a single S1 column. **(a)** Placement of recording array in a single S1 column. **(b)** Confirmation of depth criteria for cortical laminae. Left, Lesions made at microdrive depths of 150 and 1,050 μm below pia, recovered in upper L2/3 and L5 in a cytochrome oxidase-stained coronal section. Barrels are visible in L4. Right, location of all recovered lesions, showing that microdrive depth criteria can be used to accurately target specific layers (see Methods). **(c)** Representative simultaneous recordings from L4 and L2 during whisker deflection. **(d)** Raster plot and peristimulus time histogram (PSTH) of spikes recorded simultaneously from L4 (gray) and L2 (black) single units, in response to 100 whisker deflections (stimulus onset, time 0). **(e)** Number of spikes evoked by multiwhisker stimulation across all L4 ($n = 40$) and L2/3 ($n = 44$) neurons.

Figure 3.

Reversal of firing order by principal whisker deprivation for a representative L4-L2 cell pair. **(a)** Representative spike trains recorded during multiwhisker deflection (all whiskers), deflection of all whiskers except the PW (PW cut), and subsequent multiwhisker stimulation (recovery). Consecutive trials containing at least one stimulus-evoked spike from each cell are shown. Dashed line, whisker deflection onset. **(b)** PSTHs for all 900 trials for each stimulus condition. Stimulus onset, 0 ms. **(c)** Joint PSTH (JPSTH) showing time relative to stimulus onset of all L4 and L2 spikes that co-occurred in the same sweep. Bin width, 1 ms. Pixel color indicates number of coincident spikes in 900 trials. Pixels above the diagonal indicate L4 spikes that preceded L2 spikes; pixels below the diagonal indicate L2 spikes that preceded L4 spikes. PW cut caused a reversal in firing order from L4-leading to L2-leading. **(d)** Cross-correlograms (CCGs) derived from the JPSTH data.

Figure 4.

Shift toward synchronized firing by principal whisker deprivation for a representative L4–L3 cell pair. **(a)** PSTHs for responses to multiwhisker deflection (all whiskers), deflection of all whiskers except the PW (PW cut), and subsequent multiwhisker deflection (recovery). **(b)** JPSTH for each condition. During multiwhisker stimulation, most spike pairs occurred with L4- leading-L3 order. After PW cut, more L3- leading spike pairs occurred (pixels below the diagonal). **(c)** CCGs derived from the JPSTHs. PW cut significantly shifted the Δt distribution.

Figure 5.

Effect on mean spike timing across all cell pairs. **(a)** Distribution of CCG peaks (modal Δt values) for all L4–L2 (gray, $n = 46$) and L4–L3 (black, $n = 128$) pairs. During multiwhisker stimulation, most cell pairs showed L4-leading–L2/3 firing. With PW cut, 77% of L4–L2 pairs and 81% of L4–L3 pairs showed immediate reversal of firing order (modal $\Delta t > 1$ ms L2-leading) or a shift to mean synchronous firing (modal $\Delta t < \pm 0.5$ ms). **(b)** Onset latency for all neurons to whisker stimulation. Triangles are means for each layer. L4 latency increased substantially with PW cut, whereas L3 latency increased slightly and L2 latency actually decreased.

Figure 6.

Decorrelation of L4 and L2/3 spike trains by principal whisker deprivation. **(a)** Mean normalized CCGs from all L4–L2 and L4–L3 cell pairs for each stimulus condition. Gray, highly correlated spikes (within ± 10 ms of the mean CCG peak). After PW cut, highly correlated spikes became a smaller fraction of the overall CCG. Arrow, shoulder reflecting cell pairs that showed large reversals in spike timing. **(b)** Effect of PW deprivation on firing correlation for each cell pair. Firing correlation is defined as the fraction of spike pairs within ± 10 ms of the CCG peak, relative to all spike pairs within the CCG (± 100 ms). Each dot represents one cell pair (L4–L2, $n = 46$; L4–L3, $n = 128$). Dashed lines, correlation values for random (uncorrelated) firing. Dotted lines, mean correlation during spontaneous spiking. **(c)** Mean CCG for spontaneous firing ($n = 219$ cell pairs, 45 min recording, 1,000–2,500 spikes per cell). Long-duration recordings were necessary for this measurement because spontaneous firing rates were low (L4: 1.3 ± 1.3 Hz, $n = 21$; L2/3: 0.8 ± 0.8 Hz, $n = 20$). Inset, enlargement of peak showing tendency for L4 spikes to lead L2/3 spikes during spontaneous firing.

Figure 7.

Quantitative model of STDP from spike timing data measured *in vivo*. **(a)** STDP learning rule used in the model. Experimental data points show fractional change in EPSP initial slope induced by 100 spike pairs at a single Δt value. Dots are from ref. 17, and squares are from the present study. Positive values on the ordinate indicate LTP and negative values indicate LTD. Curve, fit to experimental data used in model (see text). Inset shows expanded view of sharp crossover from LTP to LTD. **(b)** Area-normalized CCGs for representative L4–L2 (top) and L4–L3 (bottom) cell pairs used as input to the model. Numbers indicate the fractional change in synaptic strength predicted by the model to result from 100 spike pairs with the spike timing distribution represented by the CCG. Positive values, LTP; negative, LTD. **(c)** Model results showing predicted magnitude of plasticity (mean $\pm$ s.e.m.) for all cell pairs. * $P < 0.05$, paired t -test.

Table 1

Response latencies for S1 neurons

Layer	# cells	Onset latency ^a	Difference from L4 ^b	Modal latency ^c	Difference from L4 ^b	Average modal Δ from CCG ^{b,d}
Multiwhisker stimulation (all whiskers intact)						
L2	21	13.0 ± 2.6	4.4	15.5 ± 3.0	3.5	5.4
L3	23	10.2 ± 1.5	1.6	14.2 ± 1.6	2.2	1.3
L4	40	8.6 ± 1.0	—	12.0 ± 1.6	—	—
Principal whisker cut						
L2	21	10.8 ± 2.3 [*]	-2.0	15.3 ± 2.6	-2.7	-2.5
L3	23	12.2 ± 2.6 ^{**}	-0.6	16.2 ± 2.1 [*]	-1.8	-1.0
L4	40	12.8 ± 4.3 ^{**}	—	18.0 ± 4.7 [*]	—	—

All values are mean ± s.d., in ms.

^{*} $P < 0.05$ and

^{**} $P < 0.005$ relative to multiwhisker stimulation, paired t -test.

^a First of three consecutive 1-ms PSTH bins containing >3 standard deviations over mean background firing rate.

^b Positive values indicate L4 leading L2/3.

^c PSTH bin containing the most spikes.

^d Derived from cross-correlograms for simultaneously recorded L4-L2 and L4-L3 cell pairs.