Modified Sensory Processing in the Barrel Cortex of the Adult Mouse After Chronic Whisker Stimulation

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Quairiaux C, Armstrong-James M, Welker E. Modified sensory processing in the barrel cortex of the adult mouse after chronic whisker stimulation. J Neurophysiol 97: 2130–2147, 2007. First published November 22, 2006; doi:10.1152/jn.00338.2006. Chronic stimulation of a mystacial whisker follicle for 24 h induces structural and functional changes in layer IV of the corresponding barrel, with an insertion of new inhibitory synapses on spines and a depression of neuronal responses to the stimulated whisker. Under urethane anesthesia, we analyzed how sensory responses of single units are affected in layer IV and layers II & III of the stimulated barrel column as well as in adjacent columns. In the stimulated column, spatiotemporal characteristics of the activation evoked by the stimulated whisker are not altered, although spontaneous activity and response magnitude to the stimulated whisker are decreased. The sensitivity of neurons for the deflection of this whisker is not altered but the dynamic range of the response is reduced as tested by varying the amplitude and repetition rate of the deflection. Responses to deflection of nonstimulated whiskers remain unaltered with the exception of in-row whisker responses that are depressed in the column corresponding to the stimulated whisker. In adjacent nonstimulated columns, neuronal activity remains unaltered except for a diminished response of units in layer II/III to deflection of the stimulated whisker. From these results we propose that an increased inhibition within the stimulated barrel reduced the magnitude of its excitatory output and accordingly the flow of excitation toward layers II & III and the subsequent spread into adjacent columns. In addition, the period of uncorrelated activity between pathways from the stimulated and nonstimulated whiskers weakens synaptic inputs from in-row whiskers in the stimulated barrel column.

INTRODUCTION

The whisker-to-barrel pathway of rodents has become a model system to investigate cortical plasticity. Mystacial whisker follicles map in a highly somatotopic manner onto segregated clusters of neurons termed barrels in layer IV of the somatosensory cortex, easily identifiable both structurally and functionally (Armstrong-James and Fox 1987; Simons 1978; Welker 1976; Woolsey and Van der Loos 1970). Neurons within a barrel primarily process the information from the anatomically related whisker, i.e., the principal whisker (PW), but possess receptive fields spanning several whiskers (Armstrong-James and Fox 1987; Welker et al. 1993). In both rats (Armstrong-James et al. 1994; Diamond et al. 1993; Glazewski et al. 1998; Wallace and Fox 1999b) and mice (Glazewski et al. 2000; Knott et al. 2002; Yang et al. 2002), those receptive fields were shown to exhibit activity-dependent plasticity during the entire life on various alterations in the pattern of whisker experience; however, the exact mechanisms of cortical plasticity are not well known.

It has been shown that a prolonged period of chronic whisker stimulation in a normally behaving adult mouse induces functional and morphological changes in the corresponding barrel (Knott et al. 2002; Rocamora et al. 1996; Welker et al. 1989, 1992). An electron microscopical analysis demonstrated that a 24-h period of chronic whisker stimulation causes a fourfold increase in the density of spineous GABAergic synapses in the stimulated barrel. Physiological recordings revealed that the firing of barrel neurons evoked by the stimulated whisker is depressed during the second epoch of the response, i.e., from 12 to 25 ms postwhisker deflection, whereas the earliest epoch from 3 to 12 ms is unaffected (Knott et al. 2002). Previous electrophysiological studies in rats showed that after an initial period of thalamocortical (TC) activation, sensory processing in layer IV is predominantly driven by cortico-cortical rather than by TC excitatory connections (Armstrong-James and Callahan 1991; Pinto et al. 2000; Simons and Carvell 1989). In analogy, the diminished firing during the second epoch of the response observed in mice should be attributable to experience-dependent alterations in barrel cortex (Welker et al. 1993). Taken together, these structural and functional observations suggest that a habituation evolves for the chronically stimulated whisker in the corresponding barrel and that this habituation could be mediated through the increase in the density of GABAergic synapses and consequently an enhanced evoked inhibition.

The purpose of the present work was to further characterize the functional consequences of the period of modified experience on the processing of sensory signals throughout the barrel cortex. In the barrel column corresponding to the stimulated whisker, is the depression of responses specific for the stimulated input or is the integration of responses to nonstimulated surround whiskers (SWs) also modified? Is the sensitivity of neurons to deflections of the stimulated whisker as well as their dynamic response properties altered? Is further intracortical processing of responses to the stimulated whisker modified, i.e., the transmission of the sensory signals from layer IV to layers II & III within the home barrel column as well as the transcolumnar propagation? To address these questions, we examined the effects of a 24-h period of chronic stimulation using current-source density (CSD) analyses and quantitative measurements of the firing of single units. Recordings at the

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end of a 24-h chronic whisker stimulation period were made in the barrel column corresponding to the stimulated whisker as well as in adjacent barrel columns, whose related principal whiskers had not been stimulated. Control recordings were made from nonstimulated mice, whereas long-term effects were analyzed in a group of mice recorded 4 days after the cessation of the stimulation.

Our results suggest that an experience-dependent mechanism modulates the magnitude of the responses generated by the direct TC afferents from the stimulated pathway (i.e., PW responses) and that this in turn leads to a weaker propagation of excitation within the corresponding column and through the transcolumnar pathways in layers II & III of the barrel cortex. Responses to nonstimulated whiskers were unaltered in their corresponding columns. In the column corresponding to the stimulated whisker, responses to SWs were not altered, with the exception of those evoked by surround in-row whiskers that were transiently depressed. We propose that following a model of Hebbian-like synaptic depression (Armstrong-James et al. 1994; Cruikshank and Weinberger 1996), the transcolumnar synaptic inputs from in-row SWs are weakened in the stimulated barrel column.

METHODS

Recordings were made from a total of 160 adult female mice of the NOR strain derived from ICR stock (weight 25–35 g, between postnatal days 60 and 90; Van der Loos et al. 1986). All animal handling procedures used for this study were previously approved by the Office Vétérinaire Cantonal (Lausanne, Switzerland) in accordance with Swiss Federal Laws.

Chronic whisker stimulation

Sixty-seven mice were subjected to a 24-h period of chronic stimulation of their left C2 whisker immediately before electrophysiological experiments (24-h STIM). Seventy-five nonstimulated mice were used as control (NO STIM). Before the chronic stimulation procedure, mice were anesthetized with Nembutal [sodium pentobarbital, 60 mg/kg, administered intraperitoneally (ip)]; a small ferrous metal rod (ion/nickel, length 1.5 mm, diameter 0.2 mm, 0.6 mg) was then glued onto the C2 whisker of their left whiskerpad 3 mm away from the skin surface. After recovery from the anesthesia, animals were placed for a 24-h period in the Lausanne whisker stimulator (Melzer et al. 1985) to expose a large part of the somatosensory cortex including the postero medial barrel subfield region (PMBSF; Woolsey and Van der Loos 1970). The exposed region was covered with 1% agar dissolved in 0.9% saline. The depth of the anesthesia was continuously controlled using several criteria: absence of whisking or eye blink reflex and burst rate of layer V cortical neurons between two and four/s (Armstrong-James et al. 1985; Fox and Armstrong-James 1986). Supplementary urethane doses (10% of the original dose) were given when necessary.

Electrophysiological recordings

Extracellular neuronal activity was recorded using carbon-fiber microelectrodes of low impedance (1–5 MΩ), the tip of which had been sharpened by spark-etching (Armstrong-James and Millar 1979). The microelectrode was lowered in the cortex normal to the pial surface using a mechanical microdrive (Model S-11, 10-µm precision; Narashige Instruments, Tokyo, Japan) and a reference electrode was placed on the scalp. The differential voltage signal was recorded using a Neurolog modules arrangement (Neurolog System, Digitimer, Welywn Garden City, UK). This signal was first amplified (2K) then filtered for extracellular spike activity and local field potentials (LFPs) through parallel band-pass filters of 0.8–5 kHz and 0–45 Hz, respectively. Single units were isolated by means of a waveform time window discriminator that provided digital output of the discriminated spike (DIS-1; BAK Electronics, Mt. Airy, MD). Digitized spike times and analog LFP waveforms were processed using the CED-Power 1401 interface [Cambridge Electronic Design (CED), Cambridge, UK].

SINGLE-UNIT ANALYSES. Neurons were recorded during the cortical active periods of slow-wave sleep. Spike times were recorded during a time period of 1,000 ms starting 250 ms before whisker deflection. They were collected into 1-ms bins in two separate channels, retrieving either all spike times to build peristimulus time histograms (PSTHs) or the time of only the first spike after the onset of the whisker deflection to build latency histograms (LHs). The response magnitude (RM) of a single unit was defined as the mean number of spikes evoked per deflection, corrected for the spontaneous activity. It was calculated using the PSTH data by counting the number of spikes recorded during the 3- to 100-ms interval after the onset of whisker deflection, from which we subtracted the number of spikes that occurred during a similar time interval before the whisker deflection. All neurons recorded during this study had significant responses to the deflection of the PW (RM ≥0.1). Epoch analyses of responses were carried out by dividing PSTHs in five consecutive poststimulus epochs that were chosen based on the temporal profile of the PSTHs of layer IV neuronal response to PW deflection (see RESULTS for determination of these epochs). Based on the LH, we calculated the mode (modal latencies; Armstrong-James and Fox 1987), the median, and the interquartile (IQR) range q75–q25 of the distribution of the spike latencies (median latencies and IQR range; Welker et al. 1993). Analyses were carried out using homemade scripts written in Spike2 software language (CED).

RECORDING SITES AND HISTOLOGY. Per animal, one to three electrode penetrations were made in barrel columns C2, C1, and/or D2. The penetrations were tangentially separated by a minimal distance of 100 µm. During a penetration, three to eight neurons were recorded separated from each other by a minimal distance of 50 µm along the depth. Radial position within a penetration was monitored from the micrometer setting of the stereotactic manipulator and used to allocate units to cortical layers as previously determined for mice of the NOR strain (Welker et al. 1993): layers II & III = 150–350 µm from the pial surface, layer IV = 350–480 µm. At the end of the recording session, electrolytic microlesions (diameter 25 µm) were made in layer IV of each of the penetrations achieved. For this, the microelec-
trodex was lowered at 400 μm from the pial surface and a 1.6-μA negative current was passed through the electrode for 7 s.

Animals then received an ip injection of 0.1 ml sodium pentobarbital (5 mg) and were transcardially perfused with 10% formalin in 0.9% NaCl. The brains were removed from the skull and postfixed for 1 to 3 wk. After 24 h of cryoprotection in 30% sucrose, the right hemispheres were oriented using a guillotine (Rice and Anders 1977) and sectioned tangentially to the pial surface overlying the barrel cortex on a cryotome (section thickness 40 μm). Sections were then stained for Nissl substance with cresyl-violet to visualize the position of the microlesions with respect to the barrel outlines.

LOCAL FIELD POTENTIAL AND CSD ANALYSES. To calculate CSDs, PW-evoked LFP waveforms were recorded in the C2 barrel column with one microelectrode successively placed at incremental intervals (Δz = 50 μm) from the pial surface to 1,000 μm beneath, encompassing the entire thickness of the cortex. At each depth, the LFP waveform was averaged for a minimum of 50 whisker deflections processed with the standard protocol (1.43° upward, 0.5/s; see Whisker deflection). LFP waveforms were used to calculate the second spatial derivative of potentials along the depth, referred to as the one-dimensional CSD, and yields spatiotemporal profiles of extracellular current sinks and sources. Those CSD traces were calculated in one dimension (z), with the assumption that currents in the x and y directions were negligible and that intracortical conductivity was constant, using the following formula: CSD = −[V(z + Δz, t) − 2V(z, t) + V(z − Δz, t)]/Δz², where V(z, t) is the measured voltage at a subdial depth z (t = time; Δz = 50 μm) (Mitzdorf 1985; Nicholson and Freeman 1975). CSD calculations were carried out using homemade scripts written in Matlab software (The MathWorks, Natick, MA).

Whisker deflection

The whisker deflection consisted of an upward movement of 3-ms duration. To apply this deflection, the whisker (trimmed to a length ~15 mm) was inserted into a thin (inner diameter 0.5 mm) borosilicate-glass tube attached to a piezoelectric bimorph slab. The upward movement of the probe was evoked by square-wave voltage pulses delivered by a stimulator with high-voltage output (stimulator DS59A; Digitimer) gated by the CED–Power 1401 interface. Field potentials were carefully observed to ensure that deflections were applied only during appropriate stages of slow-wave sleep (Armstrong-James and Fox 1988). The following deflection amplitudes and repetition rates were used:

Standard protocol, which corresponds to the one used in previous studies in our laboratories (Armstrong-James and Fox 1987; Welker et al. 1993). The amplitude of the upward deflection was set to engage an angular displacement of the whisker of 1.43° from the horizontal plane. Evoked responses of barrel column neurons were all recorded toward their PW and six to eight immediate surround whiskers, one at a time. Each individual whisker was deflected 50 times, at a repetition rate of 0.5/s.

Varying amplitude protocol, applied to record the responses of layer IV neurons on deflections of different amplitudes (0.06, 0.18, 0.30, 0.68, 1.02, 1.43, and 2.1°) at a repetition rate of 0.5/s. Note that using our stimulator, both the amplitude and the velocity of the deflection were concomitantly decreased with decreasing voltage pulses, as illustrated in Armstrong-James and Fox (1987). Here we did not measure the actual variations of the velocity.

Varying repetition rate protocol, in which the PW was deflected at various rates (0.5, 1, 2, 4, 8, and 16/s) at an angular displacement of 1.43° upward. Trains of 10 deflections were used for each repetition rate and applied with a 2-s deflection-free interval. Five runs of pseudorandomly interleaved trains were performed, resulting in a total of 50 deflections for each repetition rate.

Statistics

Single-unit data were grouped by cortical layers (II/III and IV), barrel columns (C2, C1, and D2), and experimental groups (NO STIM, 24h STIM, 4-days-after-STIM). Within each class and for each whisker deflected, distributions of the different response variables (RM, modal and median latencies, epoch’s activity) were tested for normality (Shapiro–Wilk test). Normality was rejected in half of the cases; data were therefore normalized with a transform-ranking method before entering a multivariate hierarchical model [general linear model (GLM) procedure]. For each response variable, the hypothesis of no overall group effect on the cluster of dependent variables was tested with a MANOVA (applying Wilk’s lambda test criteria). Where the null hypothesis was rejected, multiple comparisons of each variable between groups could then be performed with minimized risks of false positive (Type II error) using Tukey’s Studentized range HSD (honestly significant difference) tests, which took the interanimal variability as a potential error source. For comparisons of the epoch’s activity between groups, a procedure of repeated-measures ANOVA with contrast variables was used. For the series of recordings in which the PW was deflected at different amplitudes or rates, mean values of response variables were compared within groups using a MANOVA and Tukey’s tests. We applied a GLM model of ANOVA with covariable to test whether the cortical responses in the two groups of mice (24h STIM and NO STIM) were modified in a similar manner by altering the deflection variables. All statistics were carried out using the SAS package for windows (SAS Institute, Cary, NC).

RESULTS

Spatiotemporal distribution of evoked current flows

The sequential processing of sensory signals within a cortical column can be revealed by measuring the laminar LFPs evoked by whisker deflection and processing them through a one-dimensional CSD analysis that reveals the spatiotemporal pattern of synaptic activation (Mitzdorf 1985; Nicholson and Freeman 1975). We recorded the LFPs evoked by deflection of the C2 whisker according to the standard protocol (see methods) in the C2 barrel column of animals that were either nonstimulated (NO STIM; n = 7) or of those that had been stimulated 24 h for their C2 whisker (24h STIM; n = 7). Figure 1A shows a representative example of the laminar traces of the evoked LFPs in a nonstimulated mouse. The variations in LFP profiles across laminae indicate a heterogeneous pattern of current flow. During the first 40–50 ms of the cortical response, a biphasic extracellular field potential was evoked from 200- to 800-μm subpial depth that consisted of a short-latency negative-potential phase (5- to 7-ms onset in both groups) immediately followed by a positive phase. In all cases, the magnitude of voltage gradients was largest between lower layer III and upper layer V, with negative and positive peak amplitudes invariably present in layer IV. The initial negative phase terminated, respectively, 25.0 ± 4.1 and 24.2 ± 3.4 ms poststimulus in layer IV of NO STIM and 24h STIM groups (mean ± SD). In the most superficial (0–100 μm) and deepest layers (850–1,000 μm), field potentials reversed polarity, with positive–negative phases variable in duration across mice. During the next 50 ms, a biphasic positive–negative potential sequence of small magnitude was observed in both groups that was spatially restricted between lower layer III and upper layer V. Subsequently, the average field potentials gradually returned to baseline level. The total duration of the evoked LFP response was 90–95 ms.
The CSD analysis allows the localization of the current generators (Mitzdorf 1985; Nicholson and Freeman 1975). The CSD traces computed from the laminar LFP traces (Fig. 1A) point to a short-latency current sink (positive CSD phase) present in layers II & III to layer V by the PW deflection that was followed by a current source (negative CSD phase). These current events are more easily identifiable in Fig. 1B that shows two examples of color-coded contour plots, interpolated from the laminar CSD traces recorded in a nonstimulated (left) and a stimulated (right) mouse. Color coding shows current sources in blue, current sinks in red, and isopotentials in green.

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**Modifications in single-unit activity in the “stimulated barrel column”**

We quantitatively analyzed the effects of a 24-h period of chronic C2 whisker stimulation on the response properties of individual neurons in the C2 barrel column, as well as in neighboring barrel columns C1 and D2. In stimulated animals, the C2 barrel column will be referred to hereafter as the “stimulated barrel column” in contradistinction to neighboring “nonstimulated barrel columns.” In all, 1,042 neurons were recorded in 68 animals from the NO STIM (469 neurons), 60 from the 24h STIM (n = 438), and 18 from the 4-days-after-STIM (n = 135) groups. Data from 224 of these neurons were already partially presented in a previous study (Knott et al. 2002).

**TRANSIENT DECREASE IN SPONTANEOUS ACTIVITY.** In the C2 barrel column of nonstimulated mice, mean spontaneous activity was 1.7 ± 0.8 spikes/s for neurons in layer IV (mean ± SD; n = 102; Fig. 2) and 1.3 ± 0.8 spikes/s in layers II & III (n =
After 24 h of whisker stimulation, mean spontaneous firing of neurons was significantly reduced by 47% in layer IV (n = 77) and 40% in layers II & III (n = 70) of the stimulated barrel column compared with values in nonstimulated animals (P < 0.01, Kruskal–Wallis). In mice recorded 4 days after the chronic stimulation, spontaneous activity of neurons (n = 82 and 53 in layer IV and layers II & III, respectively) was similar to values in nonstimulated mice. This transient decrease in spontaneous activity was restricted to barrel column C2 because in barrel columns C1 and D2 it was identical in mice of the 24h STIM group and of the NO STIM group.

DECREASE IN PW RESPONSES. Figure 3A shows the distributions of the latency of PW responses as a function of cortical depth for all three groups of mice analyzed. Responses of single units in the three groups were evoked using the standard protocol of whisker deflection. In a comparison of the three distributions, it does not seem that chronic whisker stimulation affects the latency of the responses in any of the cortical layers. This was confirmed (Fig. 3B) by the statistical analysis of the median latency of the responses of neurons of layer IV (NO STIM, n = 102, latency = 11.4 ± 3.8 ms; 24h STIM, n = 77, latency = 10.9 ± 3.4 ms; 4-days-after-STIM, n = 82, latency = 10.7 ± 2.7 ms) and layers II & III (NO STIM, n = 73, latency = 14.7 ± 5.8 ms; 24h STIM, n = 70, latency = 14.8 ± 8.8 ms; 4-days-after-STIM, n = 53, latency = 13.9 ± 3.4 ms).

Analysis of the response magnitude (RM) of these sets of neurons showed striking effects of the chronic whisker stimulation. Figure 4A shows the frequency distributions of response magnitude of neurons in barrel column C2 to deflections of the C2 whisker. In layer IV of NO STIM mice the distribution was centered at 0.8–1.0 spike per deflection; <25% of neurons showed responses <0.6 spike per deflection. The RM distribution for the 24h STIM group became clearly asymmetrical, with 50% of the neurons showing responses <0.6 spike/deflection. This distribution is significantly different from that of the NO STIM group (P < 0.01; chi-square). The RM distribution of the 4-days-after-STIM group was significantly different from the NO STIM and 24h STIM groups. Compared with the distribution in the NO STIM group, the distribution in the 4-days-after-STIM group shows an increase in highly responsive neurons (>1.4 spikes/deflection). Figure 4B displays the mean RM of the neurons in layer IV in the three groups of mice and indicates the significant depression of RM of neurons in the C2 barrel after 24 h of chronic whisker stimulation (mean ± SD = 0.97 ± 0.53 in NO STIM vs. 0.71 ± 0.40 in 24h STIM; P < 0.01, MANOVA and Tukey’s HSD). However, the RM increased significantly (15%) at 4 days after stimulation compared with NO STIM (4-days-after-STIM: 1.14 ± 0.52; P = 0.03). A similar analysis was performed on the RM of neurons in layers II & III. Figure 4A
shows the frequency distribution of the RM of these neurons in the three groups of mice studied. After 24 h of whisker stimulation the RM distribution was significantly shifted to smaller values compared with the NO STIM ($P < 0.01$). Four days after the stimulation, the distribution was no longer significantly different compared with NO STIM.

Statistical analysis of the mean RM values showed significant depression of RM of neurons in layers II & III of the 24h STIM group (mean $\pm$ SE: $0.74 \pm 0.36$ in NO STIM; $0.47 \pm 0.36$ in 24h STIM; $P < 0.01$). After 4 days of recovery, the mean RM of neurons was not significantly different from neurons in nonstimulated mice ($4$-days-after-STIM: $0.67 \pm 0.32$).

Thus a 24-h period of chronic stimulation induced a decrease in RM toward the deflection of the stimulated whisker both in layer IV and in layers II & III. This depression of PW-evoked responses was transient because the mean RM of neurons recorded 4 days after the stimulation period was no longer smaller than that in nonstimulated animals. The data of the 4-days-after-STIM group show that in layers II & III, the RM returns to control level, whereas in layer IV we detected a small but significant increase in RM compared with the value of the NO STIM group.

Furthermore, this part of the analysis was complemented with a comparison of the PW response between neurons of layers II & III and layer IV. In the 24h STIM group the mean RM of neurons in layers II & III was decreased by 36% relative to the control group, whereas in layer IV the decrease was 26%. To test this difference for statistical significance, we calculated for each neuron of the 24h STIM group an index representing the ratio between its RM and the mean RM of neurons in the same layer of the NO STIM group ($\text{RM}_{\text{24h STIM}} / (\text{RM}_{\text{mean NO STIM}})$). This comparison revealed that the mean value of this index for neurons in layers II & III was significantly smaller than that for neurons in layer IV, indicating that the effect of stimulation on RM depression was stronger in layers II & III than in layer IV ($mean \pm SD: 0.40 \pm 0.12$ in layer IV and $0.32 \pm 0.12$ in layers II & III).

**QUANTITATIVE ANALYSIS OF PW RESPONSES IN NONSTIMULATED BARREL COLUMNS.** To test whether the effect of the C2 stimulation is restricted to PW responses inside the C2 barrel...
column, we also analyzed neuronal responses in neighboring barrel columns C1 and D2 for deflection of the corresponding whisker using the standard protocol (C1 column: layer IV NO STIM, n = 54; 24h STIM, n = 49; layers II & III NO STIM, n = 53; 24h STIM, n = 50; D2 column: layer IV NO STIM, n = 65; 24h STIM, n = 58; layers II & III NO STIM, n = 48; 24h STIM, n = 68). No statistically significant differences were revealed at P < 0.05 in mean RM between stimulated and nonstimulated animals in either layer IV or layers II & III (Fig. 4), indicating that the decrease in PW-evoked response was restricted to the barrel column corresponding to the stimulated whisker. C2 whisker stimulation does not affect PW responses in neighboring barrel columns.

EPOCH ANALYSIS OF PW RESPONSES. To determine whether the decrease in the magnitude of PW responses in the stimulated column reflected a general suppression of evoked firing or affected only parts of the response, we performed a quantitative poststimulus epoch analysis of the responses to C2 deflection according to the standard protocol. This analysis included the responses of all neurons in layer IV and layers II & III of the C2 barrel column in the three groups of mice (layer IV: NO STIM, n = 102; 24h STIM, n = 77; 4-days-after-STIM, n = 82; layers II & III: NO STIM, n = 73; 24h STIM, n = 70; 4-days-after-STIM, n = 53; same neurons as in Fig. 4).
ANOVAs with repeated measures). During T3 neuronal activity remains at the spontaneous level in all groups. The characteristic rebound in neuronal activity evoked by deflection of the PW during T4 was strongly reduced in 24h STIM mice relative to NO STIM mice \( (P < 0.01) \). Four days after the stimulation period, however, evoked spiking was significantly enhanced \( (P = 0.02) \) during this late poststimulus epoch compared with the activity of neurons of the NO STIM animals.

**Layer II/III.** The same epochs were used for analysis of the responses of cells in layers II & III. Evoked activity began at 6 ms poststimulus (Fig. 5A). The peak of spiking probability was reached 2–3 ms later than in layer IV, at 13–14 ms poststimulus. Spiking probability then gradually decreased to reach a plateau of low probability of spiking above the spontaneous level around 25 ms poststimulus, until 100 ms poststimulus. There is no difference in duration and temporal profile of the evoked population spiking in the three groups.

As illustrated in Fig. 5B, chronic stimulation did not alter mean spike rate during the first epoch \( (P = 0.7) \) but significantly decreased evoked firing during the following time epochs T2, T3, and T4 \( (P < 0.01) \). After the initial epoch T1, during which layer IV neurons reached their maximal firing, layers II & III firing strongly increased until a peak at 13–14 ms in mice of the NO STIM group, whereas in the 24h STIM group mean spiking probability did not exceed the level attained at 11 ms poststimulus and decreased rapidly after 13 ms poststimulus. Accordingly, the mean spiking rate was higher during T2 compared with that during T1 in the NO STIM group \( (P < 0.01) \), whereas it was not in 24h STIM animals. Mean spike rates per epoch were not significantly different in the 4-days-after-STIM and the NO STIM groups.

**DECREASED DYNAMIC RANGE FOR DEFLECTION-AMPLITUDE–DEPENDENT RESPONSES IN THE STIMULATED BARREL.** To test whether the decrease in response magnitude of layer IV neurons is paralleled by a change in sensitivity of cortical neurons to whisker deflection, we applied the varying amplitude deflection protocol (see METHODS) and quantified the responses of neurons in layer IV of four mice of the 24h STIM group \( (n = 31) \) and five mice of the NO STIM group \( (n = 35) \). As illustrated in the example of Fig. 6A, LFPs and single-unit spikes exhibited similar input–output relationships: the magnitude of field potentials and of neuronal firing increased with incremented deflection amplitude. Concomitantly, both the onset of evoked LFP waveforms and the mode of single-unit spiking distributions were delayed with decremented amplitude. The GLM procedure of ANOVA with covariable showed that mean modal latency decreased with deflection amplitude (Fig. 6B), whereas the mean RM similarly increased with deflection amplitude (Fig. 6C) in both the 24h STIM and the NO STIM groups at \( P < 0.05 \).

All neurons in both groups of mice showed a strong correlation between deflection amplitude and RM \( (P < 0.0001, \) Spearman correlation test). The maximal values of RM were reached at a deflection amplitude of 1.43° in both groups of mice. The lowest deflection amplitude assessed still evoked significant responses above spontaneous activity in two thirds of the neurons recorded in both groups \( (24/35 \text{ in NO STIM}, 25/31 \text{ in 24h STIM animals}) \), whereas all cells were significantly responsive after two increments in deflection amplitude. Mean RM was smaller in stimulated animals than that in nonstimulated animals at all deflection amplitudes. These differences reach a significant level for responses to PW deflections \( >0.68° \) \( (P < 0.05; \) MANOVA and Tukey’s test). Thus although chronic stimulation did not alter the sensitivity of layer IV neurons to whisker deflections of small amplitude, it reduced the dynamic range of neuronal response for deflection amplitude.

**ADAPTATION TO INCREASED REPETITION RATE OF WHISKER DEFLECTION.** We further explored the effect of chronic whisker stimulation on response properties of cortical neurons by varying the repetition rate of the whisker deflection. The following repetition rates were used: 0.5, 1, 2, 4, 8, and 16 deflections/s while recording neurons in the C2 barrel column from five nonstimulated \( (\text{layer IV, } n = 19; \text{layers II & III, } n = 20) \) and four stimulated mice \( (\text{layer IV, } n = 16; \text{layers II & III, } n = 18) \). At 0.5 deflection/s neuronal firing does not vary during a train of deflections, whereas at higher repetition rates the response declines rapidly at subsequent deflections, reaching a steady level of firing after the third or fourth deflection (Fig. 7A). The number of deflections at which this steady level is reached is not different between control and 24h STIM.

For each single unit we determined the RM as the average evoked firing per stimulus during five trains of 10 deflections, taking into account the response to all deflections within a train. Figure 7B gives the mean values of RM per repetition rate tested for the two groups of mice per layer analyzed. The RM of all neurons in layer IV and layers II & III decreased with incremental repetition rate between 0.5 and 8 deflections/s in both NO STIM and 24h STIM groups \( (P < 0.0001, \) Spearman correlation test).

**FIG. 6.** Neuronal response in layer IV of the C2 barrel as a function of whisker deflection amplitude. A: examples of LFPs and PSTHs in layer IV of a nonstimulated mouse using 3 different amplitudes of PW deflection. Both parameters show an increase in magnitude with incremented deflection amplitude. B: mean modal latencies (±SD) of single unit’s responses on incremental deflection amplitudes in nonstimulated and stimulated animals. C: mean RM (±SE) of single-unit responses increased with deflection amplitude. Asterisks indicate differences between RM of nonstimulated and stimulated animals at \( P < 0.01 \) (GLM procedure; MANOVA and Tukey’s HSD). Amplitude of whisker deflection at 1 to 7: 0.06, 0.18, 0.30, 0.68, 1.02, 1.43, and 2.10°, respectively.
In layer IV, chronic stimulation significantly reduced mean RM of the neuronal population \((P < 0.05; \text{MANOVA})\). The GLM procedure of ANOVA with covariable confirmed this effect of chronic whisker stimulation and showed that the mean RM is dramatically affected by repetition rate in a similar manner in both stimulated and control mice. In both groups of mice, neurons adapt to incremental repetition rate in the same manner, graphically expressed by the two, almost parallel lines in Fig. 7B. In this respect for both groups, we observed a similar twofold reduction of RM for responses recorded at 8 deflections/s compared with those at 0.5/s.

In layers II & III, RM was depressed after chronic stimulation for responses at 0.5 and 4/s at \(P < 0.05\) (MANOVA and Tukey’s test); however, the responses to 8 deflections/s were not significantly different in both groups \((P = 0.2709)\). Between 0.5 and 8 deflections/s, we observed a 4.4-fold reduction of RM in nonstimulated animals and a threefold reduction in stimulated animals. This difference in the effect of the repetition rate between the NO STIM and the 24h STIM groups was significant, as revealed by the GLM procedure of ANOVA with covariable \((P = 0.0025)\). In agreement, the two regression lines between repetition rate and RM (Fig. 7C) for the neurons in this layer are not in parallel and the mean slope is significantly smaller in 24h STIM compared with that in the NO STIM group \((\text{mean } b_{y,x} \pm \text{SD}: 0.27 \pm 0.1 \text{ vs. } 0.12 \pm 0.7; P = 0.0004, \text{two-tailed } t\text{-test, using unitary values of increment for the different repetition rates})\). This may be explained by the fact that the first deflection within a train evokes a very small response of layers II & III neurons in chronically stimulated animals.

Altered surround receptive field in the “stimulated barrel column”

For all neurons recorded in layer IV and layers II & III of the C2 barrel column, the PW deflection invariably evoked a stronger response than did any SW (number of neurons analyzed given in third paragraph of RESULTS). Figure 8 presents the mean RM of those neurons on the deflection of the PW and all SWs using the standard deflection protocol. In layer IV and layers II & III, \(<10\% of neurons responded to the PW only, the majority responding to five SWs. In layer IV, 5\% of neurons responded to all eight SWs tested, whereas in layers II & III this characterizes \(10\% of the neurons. The number of SWs eliciting significant responses per neuron was not altered by chronic stimulation, suggesting that the size of the surround receptive field was not diminished. Also the median latencies to SW deflection were not altered by the chronic stimulation.

In agreement with a previous study in the same mouse strain (Welker et al. 1993), neuronal responses vary as a function of the position of the deflected SW on the whiskerpad relative to the PW; usually the SW positioned within the same row as the
PW elicited the strongest and fastest response. In layer IV and layers II & III of barrel column C2, the deflection of in-row whiskers C1 and C3 evoked faster responses than the deflection of any other SWs ($P < 0.05$; Kruskal–Wallis). Whisker C1 evoked the strongest response followed by whisker C3 ($P < 0.05$). With respect to responses evoked by the deflection of whisks in rows B and D, the caudal whiskers (Arc-1) evoked stronger responses than the rostral whiskers (Arc-3); however, these differences did not reach significant levels. Because of the anisotropy between responses to SWs, these were grouped as follows: responses to caudal whisker C1 and rostral whisker C3 were kept separated, whereas responses to whiskers from Arc-1 (B1, D1) were pooled, along with responses to whiskers from Arc-2 (B2, D2) and Arc-3 (B3, C3).

Multivariate analyses revealed a significant effect of chronic stimulation on RM to SW deflection in both layer IV and layers II & III at $P < 0.05$ (MANOVA). For layer IV neurons, multiple comparisons (Tukey’s test) identified that the RM to the deflection of the caudal SW C1 was significantly reduced (42%) in 24h STIM compared with the NO STIM group (mean $\pm$ SD: 0.29 $\pm$ 0.25 in NO STIM vs. 0.17 $\pm$ 0.23 in 24h STIM; $P < 0.01$; Fig. 8A). The response magnitude to rostral SW C3 was also reduced after stimulation; however, this decrease did not reach a significant level. Responses evoked by SWs on Arc-1 to Arc-3 were not affected. Four days after the stimulation was stopped, a tendency toward lower values of mean RM was still observed to the deflection of the C1 and C3 whiskers compared with nonstimulated mice, but these differences were not statistically significant. Figure 8B shows the mean population PSTHs for the C1 whisker for the three groups of mice. Evoked activity above spontaneous firing starts at 9–10 ms poststimulus, the peak of activity was reached around 17–18 ms poststimulus, then firing gradually decreased until a long-lasting plateau of low firing took place. The temporal response profile remained unaltered after the 24-h period of chronic stimulation and modal latency of neurons to the deflection of SW C1 was not altered by chronic stimulation ($20.7 \pm 17.5$ ms in NO STIM, $19.3 \pm 18.2$ ms in 24h STIM, $24.0 \pm 14.4$ ms in 4-days-after-STIM; mean $\pm$ SD). However, evoked spiking probability of C1 responses was significantly lowered during almost the entire duration of the response, i.e., from T2 to T4, in the 24h STIM group of animals compared with the NO STIM and the 4-days-after-STIM groups.

In layers II & III, chronic sensory stimulation led to effects similar to those in layer IV: besides a decrease in PW-evoked mean RM, we observed a 52% decrease in mean RM to deflection of the C1 whisker (0.32 $\pm$ 0.30 in NO STIM vs. 0.15 $\pm$ 0.23 in 24h STIM; $P < 0.01$; Fig. 8A). Mean RMs to deflection of all the other SWs were not significantly altered by the chronic stimulation. Asterisks indicate epochs during which mean firing rate is significantly lower in the 24h STIM group compared with the NO STIM group at $P < 0.01$ (Kruskal–Wallis).

**Fig. 8.** Modifications in principal vs. surround whisker (SW) RM in barrel column C2. A: mean RM ($\pm$ SE) for layer IV and layers II & III neurons on PW and SW deflections. Responses to SWs that did not belong to row C were pooled (see left diagram); Arc-1 (B1 + D1), Arc-2 (B2 + D2), and Arc-3 (B3 + D3). Asterisks indicate values that differ significantly between groups at $P < 0.05$ (GLM procedure; MANOVA and Tukey’s HSD). After chronic stimulation of whisker C2, the responses to C1 deflection were decreased in layer IV and layers II & III. Responses to deflection of other SWs were not significantly altered by the chronic stimulation. B: mean population PSTHs (2-ms bins) toward C1 whisker deflections. Asterisks indicate epochs during which mean firing rate is significantly lower in the 24h STIM group compared with the NO STIM group at $P < 0.01$ (Kruskal–Wallis).

**Reduction of evoked responses in adjacent barrel columns**

Neurons recorded in the C1 and D2 barrel columns that were used for the quantitative analysis of PW responses described above were also tested for deflection of their SWs using the standard protocol. The MANOVA did not detect a global effect of the group on RM either in the C1 or in the D2 barrel columns. However, this model could have underestimated the potential effect of the stimulation period on RM of neurons to individual whiskers because it was designed to be highly robust, minimizing type II error (false positive). Therefore despite the negative MANOVA overall test, we decided to compare separately the RMs of neurons for each individual whisker deflection between the two groups of animals using nonparametric statistics. Four or seven SWs were tested in D2 and C1 columns, respectively. Therefore the level of significance of the nonparametric tests was lowered to $P = 0.005$ to test whether stimulation alters RM (standard Bonferroni correction for multiple comparisons). In layer IV of barrels C1 and D2,
RM values were not altered after chronic stimulation on deflection of any SW (all $P \gg 0.05$; Fig. 9, A and B). In layers II & III, however, mean RM to the deflection of the C2 whisker decreased with high $P$ values in barrel columns C1 ($P = 0.0007$) and D2 ($P = 0.0038$), whereas $P$ values for all other SWs were $>0.05$. The specific decrease on the deflection of the C2 whisker and the deliberate robustness of our multivariate model could explain why the MANOVA test did not reveal an overall group effect.

**DISCUSSION**

**Unaltered spatiotemporal characteristics of barrel column activation**

The CSD analysis showed a stereotype sequence of activation throughout the barrel column after deflection of the corresponding whisker. This activation pattern starts with a current sink centered in layer IV at 5–7 ms after the deflection and lasts for roughly 20 ms. Both the onset and the spatial location of the subsequent events that we report here are almost identical to the results of CSD analyses performed in the rat barrel cortex (Castro-Alamancos and Connors 1996; Di et al. 1990). Current sinks and sources originate mainly from local changes in neuronal membrane conductance produced by synaptic activation and less by those created by propagated APs (Leung 1990; Mitzdorf 1985). The early layer IV sink presumably reflects the excitatory drive of the glutamatergic TC projections that arborize predominantly in this layer (Agmon and Connors 1991; Agmon and Odowd 1992; White 1978). This assumption is supported by the dependency of the negative going phases of LFPs evoked in layer IV on $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and $N$-methyl-$d$-aspartate (NMDA) glutamate receptors as shown in rat cortical slices (Wirth and Luscher 2004). After 1–3 ms the sink in layer IV invades layers II & III and is followed by a sink in the infragranular layers. This sequence is in agreement with observations in rats demonstrating excitatory outputs of layer IV to layers II & III (Feldmeyer et al. 2000; Lübke et al. 2000; Petersen and Sakmann 2001) and of layer IV and layers II & III to infragranular layers (Gottlieb and Keller 1997; Harris and Woolsey 1983; Zhang and Deschenes 1998). It is in agreement with the postulated “VB to layer IV to layers II & III to layers V and VI” pathway in rodents (Armstrong-James and Fox 1987; Armstrong-James et al. 1992; Welker et al. 1993). The current source that follows the early sink in layer IV may be driven by feedforward and feedback inhibition from layer IV inhibitory interneurons (Porter et al. 2001). This current source temporally matches a positive LFP phase and goes with a weak spiking period. Wirth and Lusher (2004) showed that the positive LFP phase that interrupts the negative LFP phase evoked by the activation of layer IV is dependent on $\gamma$-aminobutyric acid type A (GABA$_A$) receptors. Castro-Alamancos and Connors (1996) showed that the spatiotemporal sequence of barrel cortex activation as determined by CSD analysis in rats can be altered by repetitive thalamic stimulation. However, our study reveals that a chronic stimulation period does not modify the laminar locations, onset latencies, and duration of the current sinks and sources evoked by the stimulated whisker.

**Altered activity of single units after chronic stimulation**

**DECREASED SPONTANEOUS FIRING IN THE STIMULATED BARREL COLUMN.** Spontaneous activity of single units decreases in layer IV and layers II & III of the stimulated but not of the adjacent nonstimulated barrel columns (Fig. 2). This may be explained by modifications in the intrinsic properties of neurons within the stimulated barrel column, but could also be the consequence of modifications in the chemical environment of the neurons. For instance, an elevated concentration of extracellular GABA induced by the 24-h stimulation period could persist in the stimulated column during the recording session (Welker et al. 1989; see following text). This could modulate neuronal firing by enhancing tonic inhibitory currents by high-affinity extrasynaptic GABA$_A$ receptors (Banks and Pearce 2000; Fritschy et al. 1992; Rossi and Hamann 1998; Semyanov et al. 2003) and induce a decrease in the probability of spontaneous spikes without preventing spiking driven by evoked activity. Alternatively, the decreased spontaneous firing could be the consequence of a persistent reduction in the concentration of glutamate in the extracellular medium.
ATTENUATION OF RESPONSES TO THE PW IN LAYER IV OF THE “STIMULATED” BARREL COLUMN. Layer IV is the cortical entry of peripheral signals. In rodents, each barrel processes primarily sensory signals ascending from the corresponding whisker (Armstrong-James and Fox 1987; Simons 1978; Welker et al. 1992), reflecting the segregation of TC-axonal branches from individual barreloids within the corresponding barrels (Arnold et al. 2001; Bernardo and Woolsey 1987; Frost and Caviness 1980; Jensen and Killackey 1987; Killackey 1973). In mice and rats barrels contain glutamatergic neurons, mainly spiny stellate cells, and GABAergic inhibitory aspiny neurons (Harris and Woolsey 1983; Porter et al. 2001; White 1978; White and Rock 1980). Both cell types receive glutamatergic TC synapses of high efficacy, which should ensure a robust impact of TC activation in layer IV (Agmon and Odowd 1992; Benshalom and White 1986; Gabernet et al. 2005; Gil et al. 1999). After initial monosynaptic TC activation of layer IV through AMPA and NMDA receptors, subsequent evoked neuronal spiking is mediated by excitatory interactions of cortical origin that depend almost entirely on NMDA receptors, as demonstrated in the rat and in the mouse (Armstrong-James et al. 1993; Fiedervish et al. 1998; Gil and Amitai 1996). In both species, spiny stellate neurons form a densely interconnected excitatory network within the barrel (Beierlein et al. 2003; Feldmeyer et al. 1999; Gil et al. 1999; Lübke et al. 2000) that mediates a selective amplification of thalamic signals before its propagation throughout the cortical column by axonal collaterals. Concomitantly, inhibitory interneurons generate strong disynaptic feedforward inhibition: on TC activation, an excitatory postsynaptic potential (EPSP) is evoked at the soma of virtually all barrel neurons and is followed within 1–3 ms by an inhibitory postsynaptic potential (IPSP) that curtails the EPSP as soon as 10–11 ms after its onset, as shown both in rats (Carvell and Simons 1988; Higley and Contreras 2003; Moore and Nelson 1998; Sun et al. 2006) and in mice (Agmon and Connors 1992; Gabernet et al. 2005; Porter et al. 2001). Evoked inhibition is often followed by a second EPSP at variable latencies (50–200 ms poststimulus). The sequence of those subthreshold events is consistent with the suprathreshold profile generated by the PW that we observed in layer IV population PSTHs (Fig. 5A): a strong feedforward inhibition could explain the decrease in evoked AP firing after it has peaked around 11 ms, whereas a second wave of EPSP after release from inhibition could match the late period of low evoked firing (T4). This biphasic profile remains unaltered after chronic stimulation.

In a previous work we demonstrated that the magnitude of PW-evoked single-unit spiking decreases in the stimulated barrel relative to spiking in the same barrel of nonstimulated mice (Knott et al. 2002); here supplementary neurons were added to the data and we confirm this observation: RM significantly decreases by 25%. In addition, we show that the depression of PW-evoked response is restricted to the “stimulated” barrel because responses in adjacent “nonstimulated” barrels C1 and D2 to their corresponding PW are not altered. These observations correlate well with the similarly localized decrease in deoxyglucose uptake described by Welker et al. (1992). The depression of neuronal firing may be attributable to homeostatic downregulations of the cortical network to stabilize its own level of activity in response to a period of chronic increase in sensory drive. Even though far removed from a natural setting, one can also imagine that the depression of cortical responses to the stimulated whisker represents a habituation-like phenomenon to a continuous and behaviorally irrelevant stimulus.

To investigate the functional consequences of the decreased response we modified in a set of experiments the amplitude and the repetition rate of the whisker deflection during the recoding session. As previously reported in rats (Armstrong-James and Fox 1987; Peterson et al. 1998; Pinto et al. 2000; Wilent and Contreras 2004), here we show in the mouse that the magnitude of layer IV neuronal response is dependent on the amplitude of the PW deflection (Fig. 6). With incremented deflection amplitude, latency decreases while firing robustly increases, which presumably reflects stronger and more synchronized TC inputs to layer IV units (Pinto et al. 2000). After chronic stimulation the neuronal responses were weaker at all deflection amplitudes, although at the smallest amplitude this difference does not reach significance. At this amplitude RM still exceeds spontaneous level in both groups of animals. This amplitude is near-threshold; it induces an upward deflection of only 0.06°, close to the threshold estimated in rats by Peterson et al. (1998), and mean RM of neurons is weak, barely exceeding 0.1 spike per deflection, one third of neurons being unresponsive in both groups of mice. Thus RM varies from virtually 0 at the smallest amplitude to an average of 1.18 spikes in nonstimulated and 0.85 spike in stimulated animals at the largest amplitude. Thus the dynamic range of response for deflection amplitude is reduced after chronic whisker stimulation.

We further analyzed the cortical responses after varying the repetition rate of the whisker deflection. In rats, layer IV neurons exhibit adaptation in their response to increasing rates of repetitive deflections (Ahissar et al. 2000; Garabedian et al. 2003; Khatri et al. 2004; Melzer et al. 2006; Simons 1978). Although the absolute value of the response magnitude varies
between experimental paradigms used (method of stimulation, type of anesthesia, determination of RM), we showed here that layer IV neurons in the mouse adapt their response magnitude in a manner similar to that in the rat. It is thought that adaptation in layer IV results from short-term depression at TC synapses to both excitatory and inhibitory neurons (Chung et al. 2002; Hartings et al. 2003) and at cortico-cortical excitatory and inhibitory synapses (Finnerty et al. 1999; Gabernet et al. 2005; Gil et al. 1997; Higley and Contreras 2006; Sosnik et al. 2001; Webber and Stanley 2006). We observed here that in layer IV the adaptation of the neuronal response is not affected after chronic stimulation, suggesting that short-term dynamics at TC and cortico-cortical synapses within layer IV is presumably unaltered. However, neurons in layers II & III show a significant modification in the degree of adaptation of their responses to incremented deflection rates. This difference between cortical layers is most likely explained by the fact that the RM of neurons in layers II & III is more depressed than that of layer IV neurons after the chronic whisker stimulation period (see following text).

To conclude, chronic stimulation leads to a modulation of the magnitude of the response that does not alter the sensitivity of neurons for the deflection of the stimulated whisker. The reduced dynamic ranges of amplitude- and repetition-rate-dependent responses, however, may affect the retrieving and encoding of crucial stimulus clues (Moore 2004; Petersen 2002).

The epoch analysis further elucidated the possible origin of RM depression of layer IV neurons after chronic whisker stimulation. Although onset of the layer IV response is not modified (T1; 3–10 ms after deflection onset), the analysis revealed a significant RM depression during the second epoch (T2; 12–25 ms poststimulus; see Fig. 5). In urethane-anesthetized rats, the period of strongly synchronized spiking of barreloid neurons peaks 5–6 ms after the stimulus and lasts between 4 and 10 ms poststimulus (Armstrong-James and Callahan 1991; Diamond et al. 1992; Glazewski et al. 1998; Pinto et al. 2000). The strength of the TC volley is then strongly reduced, presumably because of the strong inhibition generated by the reticular nucleus (Brecht and Sakmann 2002b). Taking this to the mouse, the unaltered T1 response indicates a TC activation that is not modified after chronic stimulation. Quantification of evoked firing in barreloid neurons of stimulated and nonstimulated mice will be useful to confirm this assumption.

Other studies on the barrel cortex showed that short-term experience-dependent changes of cortical receptive field are mediated by synaptic plasticity occurring predominantly at intracortical synapses (Armstrong-James et al. 1994; Feldman and Brecht 2005; Glazewski et al. 1998; Rema et al. 1998; Wallace and Fox 1999a). Only after long periods of modified sensory experience such as 30 days of whisker-pairing (Armstrong-James et al. 1994; Diamond et al. 1993) does TC plasticity contribute to an altered cortical response. However, this concept—based on quantitative analysis of response properties of layer IV neurons in anesthetized rats—was recently challenged by an in vivo recording study that indicates that TC activation may be altered within 4 h of sensory deprivation (Sellien and Ebner 2006).

The depression of PW-evoked firing during T2 in the “stimulated” barrel is likely a functional consequence of the increased density of inhibitory synapses mentioned above. The activation of inhibitory neurons by the TC volley and subsequently by excitatory layer IV neurons could engage stronger feedforward and feedback inhibition by GABA_A and GABA_B postsynaptic receptors (Barnard et al. 1998; Bowery et al. 2002). Additionally, GABA release from inhibitory synapses can escape from the synaptic cleft and activate GABA_B presynaptic receptors on neighboring excitatory synapses, depressing the glutamate release (Gil et al. 1997; Isaacson et al. 1993; Porter and Nieves 2004). Presynaptic effects of GABA may be of importance in the context of the experience-dependent depression of neuronal spiking observed in the present study because, being prominent on spines, the newly formed inhibitory synapses will be in close proximity with excitatory synapses.

The form of activity-dependent cortical plasticity reported here and graphically summarized in Fig. 10 can be considered...
a form of disfacilitation arising from either a reduction in excitatory drive (Higley and Contreras 2005) or a potentiation of postsynaptic inhibition. In our study, we were unable to discriminate between the two because this would require intracellular recordings. In barrel cortex, the precise timing of postsynaptic spikes in relationship to presynaptic EPSPs determines whether use-dependent potentiation or depression occurs (Markram et al. 1997). Markram and colleagues observed that LTP occurred when EPSPs preceded single evoked APs, whereas the reverse, long-term depression (LTD) of EPSPs occurred when APs preceded EPSPs. Feldman (2000) demonstrated in rat barrel cortex that for LTP to be produced the EPSP has to occur within a very narrow time window before the AP. On the other hand, LTD occurred when APs preceded EPSPs within a much broader temporal window; about fivefold the LTP window. In the present study, chronic stimuli were imposed unrelated in time to any exploratory sensory information and evoked activity. Thus incoming sensory EPSPs were randomly related to our imposed regular stimulus-driven spikes and therefore more likely to fall in the longer LTD than the LTP window. This is one probable origin for the “habituation” of sensory transmission found in our study.

ATTENUATION OF RESPONSE TO THE PW IN LAYER II/III OF THE “STIMULATED” BARREL COLUMN. In rodents, electrophysiological studies (Agmon and Commins 1992; Armstrong-James et al. 1992; Moore and Nelson 1998; Wilent and Contreras 2004) combined with optical imaging studies (Laaris and Keller 2002; Petersen and Sakmann 2001) showed that after their activation by TC afferents, layer IV neurons relay neuronal signals to layers II & III in a columnar fashion. Axonal projections of layer IV excitatory neurons in layers II & III are confined to the width of their home-barrel column. They contact pyramidal neurons, whose dendritic arborizations area is similarly restricted to the border of the column, thus providing an anatomical substrate for a columnar propagation of excitation (Feldmeyer et al. 2002; Harris and Woolsey 1983; Lübke et al. 2000; Petersen et al. 2003b).

After chronic stimulation, RM to the PW in layers II & III of the “stimulated” barrel column decreases, whereas RM in adjacent barrel columns to their own PW are not altered (Fig. 4). Given the depression of evoked firing in the “stimulated” barrel, the decreased response in layers II & III is most likely the result of a reduced vertical flow of excitation from layer IV neurons to layers II & III. However, the reduction of RM is even stronger in layers II & III than in layer IV (38 vs. 25%). This could reflect the fact that a significant portion of evoked firing in layer IV is generated by the VC volley during T1, which was not altered after chronic stimulation (Fig. 5). Furthermore, feedforward inhibition transmitted to layers II & III from layer IV inhibitory neurons could also have increased. Porter et al. (2001) found in the mouse that layer IV inhibitory interneurons strongly project toward layers II & III, suggesting that after thalamocortical input, a vertical flow of inhibition follows excitation along its intracortical course toward upper layers. The hypothesis of an experience-dependent increase in inhibition in layers II & III is supported by the observation that after 4 days of whisker stimulation GAD immunoreactivity increases not only in layer IV but also in layers II & III (Welker et al. 1989). Nonetheless, we do not know whether structural changes occur in the inhibitory synaptic network in layers II & III after chronic stimulation. Alternatively, the strength of layer IV to layers II & III synapses could have been weakened through a LTD-like process in the stimulated barrel column (Feldman 2000; Finnerty et al. 1999). Finally, it was suggested that the layer IV to layers II & III connection could act as an activity-dependent gate, requiring a relatively high level of layer IV input excitation to propagate activity into layers II & III (Feldman et al. 1998; Feldmeyer et al. 2002; Silver et al. 2003). Thus a reduction in output from layer IV neurons may lead to a disproportional decrease in evoked responses of layers II & III neurons.

In both nonstimulated and stimulated animals, the RM of layers II & III neurons decreases with incremental repetition rate between 0.5 and 8 deflections/s. This adaptation is stronger than that in layer IV, indicating additional short-term depression in layers II & III. At 8/s, the RM of layers II & III neurons is not significantly altered by chronic stimulation, which could be the result of a repetition rate–dependent reduction in the amplitude of TC-feedforward inhibition as demonstrated in the rat barrel cortex by Gabernet et al. (2005). Finally, statistical analyses reveal that the effect of repetition rate on RM in layers II & III is more pronounced in nonstimulated than in stimulated animals. This reduction of adaptation may reflect the fact that stimulus-evoked responses, even to the first deflection, are already smaller in stimulated animals.

ATTENUATION OF RESPONSE TO THE STIMULATED WHISKER IN ADJACENT “NONSTIMULATED” BARREL COLUMNS. Response magnitude to the chronically stimulated C2 whisker decreases in layers II & III of “nonstimulated” columns but not in layer IV of the columns neighboring the C2 barrel column (Fig. 9). We propose this to be the consequence of a reduced level of transcolumnar excitation according to the classical model of activation of the barrel cortex (Armstrong-James et al. 1991), in which PW-evoked excitation first invades the corresponding barrel, then engages the entire column and spreads tangentially into neighboring barrel columns through intracortical connections, synthesizing surround receptive fields (SRFs) of cortical neurons. There is considerable physiological evidence in the rat barrel cortex that SRFs are constructed intracortically (Armstrong-James and Callahan 1991; Armstrong-James et al. 1992; Fox 1994; Fox et al. 2003). The long-range horizontal projections of layers II & III axons support the existence of monosynaptic intracortical inputs for SW responses (Gottlieb and Keller 1997; Lübke et al. 2003; Petersen et al. 2003b). Additionally, a small proportion of layer IV axon collaterals to supragranular layers fans out to adjacent columns (Lübke et al. 2000) and collaterals of the projections between supragranular and infragranular layers often terminate in adjacent columns (Bernardo et al. 1990; Chagnac-Amitai et al. 1990). Because of the paucity of direct interbarrel connections and the observations that layers II & III transcortical projections mainly avoid barrels (Brecht et al. 2003; Lübke et al. 2000; Petersen et al. 2003b), it has been hypothesized that SW responses of layer IV barrel cells could originate from TC cells in the corresponding barreloid transmitting their SRF to the cortex (Brecht and Sakmann 2002b; Kwegyir-Afful et al. 2005; Petersen and Sakmann 2001; Simons and Carvell 1989) or from divergent TC projections entering neighboring barrels (Arnold et al. 2001; Gheorghita et al. 2006; Keller and Carlson 1999; Land et
al. 1995). However, direct barrel-to-barrel connections were previously demonstrated at a functional level (Schubert et al. 2003; Staiger et al. 2004). Other likely routes for the intracortical relay of SW responses in layer IV are the collaterals of layer VI neurons (Gottlieb and Keller 1997; Staiger et al. 1996; Zhang and Deschenes 1997) and the apical dendrites of neurons in layer IV that sample the activity of horizontal projections in layers II & III (Brecht and Sakmann 2002a; Lübke et al. 2000; Simons and Woolsey 1984; Valverde 1986). Finally, it remains possible that subcortical and cortical pathways cooperate to generate the SRF of layer IV neurons in mouse barrel cortex. The unaltered SW responses to the chronically stimulated whisker in layer IV suggest that SW activity in this layer results from a different source or follows different rules of activity-dependent plasticity than SW activity in layers II & III. Related to this second hypothesis, it was shown that in the adult rat, TC transmission is less sensitive than intracortical transmission to plasticity experiments (Feldman and Brecht 2005; Wallace and Fox 1999a).

Alteration within the surround receptive field of neurons in the stimulated barrel column

Within the C2 barrel column, responses to the C1 SW are also significantly depressed (Fig. 8). This is not the result of a global dampening of neuronal responsiveness in the stimulated barrel column because responses to SWs that are not in row C (Arc-1, Arc-2, and Arc-3 whiskers) are not altered. Furthermore, responses to the C1 whisker are not altered in the barrel column C1, suggesting a mechanism that takes place within the synaptic network of the stimulated C2 barrel column itself or at the synaptic inputs from the C1 whisker to the C2 barrel column.

During chronic stimulation, activity in the stimulated pathway is uncorrelated with the activity in the pathways from intact whiskers. This chronic “uncoupling” may drive depression at synaptic inputs in the stimulated barrel column from SWs following Hebbian-like rules of plasticity (Cruikshank and Weinberger 1996; Hebb 1949; Martin et al. 2000). Such modifications of effective intracortical and/or thalamocortical connectivity following Hebbian-like rules were proposed to account for functional plasticity induced by selective whisker deprivation (Armstrong-James et al. 1994; Fox 1994; Wang et al. 1995). Among the SWs, in-row whiskers evoke the strongest and fastest responses in barrel column C2. Preferred responses to in-row SWs were previously reported in both rats and mice and presumably rely on the preferred propagation of evoked excitation along the rows of barrel columns (Armstrong-James et al. 1992; Brecht et al. 2003; Petersen et al. 2003b; Simons 1978; Welker et al. 1993). The activity evoked at the specific synaptic inputs from the in-row whiskers, whether originating from interbarrel projections or direct TC projections, could have reached a threshold for plasticity during the prolonged period of stimulation, whereas the level of activity generated by other SWs does not surpass such a threshold.

Alternatively, it is possible that our measurements of neuronal activity were not sufficiently sensitive to detect changes at the lower levels of neuronal firing evoked by SWs other than C1 and that unspecific depression alters all responses in the stimulated barrel column. After chronic stimulation, however, we do not observe a tendency toward lower level of RM to SWs from Arc-1 to Arc-3, and the number of SWs eliciting a response per neuron is not altered, which would have been unlikely if responses to weak inputs from SWs had been reduced.

Transient and long-term effects of chronic stimulation

Four days after the cessation of the stimulation, spontaneous activity returns to control levels in both layer IV and in layers II & III of the stimulated barrel column. Depression of neuronal activity evoked by both the stimulated whisker and the caudal in-row whisker is no longer present. However, RM of neurons to the stimulated whisker is slightly but significantly increased in layer IV of the corresponding barrel column. The epoch analysis reveals a significant increase of the late component of AP spiking (i.e., during T4). This late component of layer IV spiking has often been referred to as a postinhibitory rebound (PIR), an intrinsic property of neurons in many parts of the brain (Fanselow and Nicolelis 1999; Simons et al. 1992). It was previously shown that the recruitment of local inhibition in cortical neurons is followed by a rebound depolarization that can be strong enough to increase spiking probability and that the rebound amplitude is correlated to the degree of the preceding hyperpolarization (Dean et al. 1989; Fan et al. 2000; Grenier et al. 1998; Huguenard 1996; Stafstrom et al. 1984; Steriade and Timofeev 1997). Morphologically, 4 days after the cessation of the stimulation, the total synaptic density in the stimulated barrel regains the level measured in nonstimulated animals; however, the density of GABAergic synapses remains equivalent as in animals analyzed immediately after the stimulation, i.e., greater than that in nonstimulated animals (Knott et al. 2002). It is tempting to speculate that the increase in rebound activity may be related to the maintenance of GABAergic synapses on spines: a modified equilibrium between excitatory and inhibitory synapses amplifies the level of hyperpolarization that follows the activation of layer IV neurons and in turn potentiates the PIR. Interestingly, the dynamic of the PIR in mice establishes a periodicity that matches the whisking frequency range (5–15 Hz; Welker 1964), which may allow an ideal temporal frame for the extraction of spatiotemporal clues during exploratory behaviors. Spikes that are not stimulus locked should be specifically suppressed by the long-lasting inhibition that is thought to precede the second component of AP spiking. The increased magnitude of the second component of evoked firing may represent a long-term effect of a prolonged period of sensory experience during which trains of passive whisker stimulus were separated by about 60 ms. Thus although the modifications of neuronal responses observed immediately after the stimulation period are transient, chronic stimulation leaves a persistent mnesic trace in the cortical circuitry. Further investigations should address the duration of these functional alterations. Finally, it raises interesting questions as to whether, and how, these alterations should influence the experience-dependent adaptations of the cortical network to a second period of chronic stimulation.

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