Experience-Dependent Rewiring of Specific Inhibitory Connections in Adult Neocortex

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Abstract

Although neocortical connectivity is remarkably stereotyped, the abundance of some wiring motifs varies greatly between cortical areas. To examine if regional wiring differences represent functional adaptations, we have used optogenetic raster stimulation to map the laminar distribution of GABAergic interneurons providing inhibition to pyramidal cells in layer 2/3 (L2/3) of adult mouse barrel cortex during sensory deprivation and recovery. Whisker trimming caused large, motif-specific changes in inhibitory synaptic connectivity: ascending inhibition from deep layers 4 and 5 was attenuated to 20–45% of baseline, whereas inhibition from superficial layers remained stable (L2/3) or increased moderately (L1). The principal mechanism of deprivation-induced plasticity was motif-specific changes in inhibitory-to-excitatory connection probabilities; the strengths of extant connections were left unaltered. Whisker regrowth restored the original balance of inhibition from deep and superficial layers. Targeted, reversible modifications of specific inhibitory wiring motifs thus contribute to the adaptive remodeling of cortical circuits.

Introduction

Neocortex has a similar multilayered histology throughout [1,2], and different cortical areas are able to adapt, depending on their inputs, to the normal function of other regions [3]. This versatility may reflect the existence of a “canonical” information-processing architecture, underpinned by stereotyped patterns of excitatory connectivity [2,4].

The organization of inhibitory neocortical circuits also obeys principles of some generality. A recent survey of inhibitory-to-excitatory wiring patterns in primary motor (M1), somatosensory (S1), and visual cortex (V1) of the mouse uncovered 25 interlaminar connection motifs common to all three regions [5]. Whereas most of these motifs were found at comparable frequencies in all cortical areas, the abundance of four motifs varied widely: ascending inhibition from layer 5B (L5B) to L2/3 and L4, as well as from L6 to L5B, was prominent in V1 and S1, but not in M1; descending inhibition from L4 to L5A featured notably in S1. These motifs may therefore represent adaptations of a common blueprint to region-specific information-processing demands.

This interpretation raises several questions. Is the presence of a specific wiring motif linked to the particular type of input a cortical area receives? In other words, does the motif change when the type of input changes? If so, is plasticity limited to a critical developmental period, or does the capacity to adapt persist into adulthood? And how motif-specific is the change? Are variable wiring motifs inserted or removed on demand, akin to plug-in devices that add new functionalities, or are circuits reconfigured more broadly?

To answer these questions, we have analyzed and compared the laminar organization of inhibitory inputs to pyramidal neurons in L2/3 of adult mouse barrel cortex (S1) under physiological conditions, during sensory deprivation (whisker trimming), and after recovery (whisker regrowth). In agreement with recent observations in visual cortex [6–9], we found that sensory deprivation of adult barrel cortex induced changes in inhibitory circuits. Importantly, the nature of these changes was not only an overall reduction in cortical inhibition, as had been inferred from the decrease of inhibitory neuron spine and bouton numbers observed earlier. Instead, inhibitory connections from particular cortical layers underwent large, reversible, motif-specific, and sometimes antagonistic adjustments. Individual inhibitory network motifs are thus altered selectively and independently to adapt a cortical area to functional change.

Results

Experiments were performed on acute neocortical slices of a mouse knock-in line expressing the optogenetic actuator [10–12] chanelrhodopsin-2 (ChR2, GenBank accession number AF461397; [13–15]) in inhibitory interneurons. ChR2 was present...
Author Summary

Many natural and engineered networks contain recurring patterns of local connectivity. Although these so-called network motifs are thought to have functional significance, direct tests of the idea that network topology reflects function remain scarce. We have performed such a test in the area of mammalian neocortex that is devoted to the sensory representation of touch. To this end, we equipped inhibitory interneurons in the mouse with light-activated ion channels that allowed us to stimulate interneuron activity optically and record light-evoked inhibitory currents in their postsynaptic partners, thereby revealing maps of connectivity. We find that excitatory pyramidal cells in layer 2/3 of primary somatosensory cortex receive inhibition from GABAergic interneurons located in different cortical layers, with a characteristic balance of inhibitory connections from deep and superficial layers. Trimming the whiskers to remove sensory input in adult animals alters this balance—inhibitory connections from deep cortical layers are depleted, while inhibitory connections from superficial layers are augmented. These changes revert when the whiskers regrow, restoring the original balance between wiring motifs. This see-saw relationship between deep and superficial inhibition demonstrates that mature cortical circuits adapt to functional change by selectively altering specific network motifs.
plasticity was antagonistic changes occurring simultaneously to different inhibitory connections in the same barrel-related column: while inhibition from L5B weakened 5-fold in deprived columns, inhibition from L1 nearly doubled (Figure 3B,C).

After whiskers had been allowed to regrow for 3 mo, the inhibitory charge flow from all cortical layers returned to baseline values (Figure 3). Full whisker regrowth restored the original balance between L5B- and L1-derived inhibition, by strengthening the former and weakening the latter (Figure 3B,C). The antagonistic relationship between L1- and L5-derived inhibition held even during a transient stage of overcompensation when whisker regrowth was partial; at this stage, the aggregate strengths of inhibitory inputs from the two layers overshot their targets in opposite directions (Figure 3B,C).

Sensory Deprivation Leaves the Horizontal Organization of Inhibitory Circuits Unperturbed

Sparing a single central whisker from deprivation can cause excitatory circuits to expand, so that signals from the spared whisker now activate surrounding deprived barrel-related columns [28,29]. We examined if inhibitory circuits in columns representing intact whiskers similarly expanded into or retracted from deprived cortical areas. If this were the case, the inhibitory input distributions of whisker-related column rows B and D, which in our deprivation protocol neighbor the spared row C, would be expected to become asymmetric. No evidence for this type of territorial reorganization was found: neither the horizontal reach of inhibitory connections into deprived versus nondeprived barrel-related columns (Figure 4A), nor the number of inputs from these columns (Figure 4B,C), differed.

We also analyzed the horizontal inhibitory input distribution of L2/3 pyramidal neurons residing in the spared barrel-related columns of row C. Trimming the principal whiskers associated with adjacent barrel-related columns altered neither the horizontal spread (Figure 4D) nor the number of locations in deprived columns (Figure 4E,F) that gave rise to IPSCs in spared columns: the profile of horizontal inhibitory connections from deprived to spared columns was the same as that between deprived columns (Figure 4D). Our regime of sensory deprivation thus selectively altered the vertical (laminar) but not the horizontal (columnar) organization of inhibitory circuits.

Remarkably, deprivation-induced changes in vertical inhibitory connectivity also affected the spared barrel-related columns of row C. As in deprived columns, the number of home column inputs decreased significantly (Figure 4E), but the detailed pattern of laminar reorganization differed subtly. spared and deprived whisker columns suffered an equally sharp drop of inhibitory charge flow from the thalamorecipient layers 4 and 5A (Figure 5A). However, some inhibition from L5B was preserved in spared columns (Figure 5A), and the antagonistic increase of L1-derived inhibition was lacking (Figure 5B,C).

Motif-Specific Changes in Connection Probability

Several mechanisms could generate these adaptations, singly or in combination. Elaboration or retraction of inhibitory terminals could alter the number of pyramidal cells contacted by one interneuron (a change in connection probability) or the number of synapses between one interneuron and one pyramidal cell (a change in connection strength). Differences in connection strength could also arise if synaptic release probability or quantal size were modulated. A formal, though remote, possibility is that the number of interneurons themselves might change during deprivation.

In adult mice with intact or fully regrown whiskers, identically sized majorities of pyramidal cells in L2/3 were targeted by L5B
interneurons (19/23, or 82.6%, of cells in control conditions; 14/17, or 82.4%, of cells after 3 mo of whisker regrowth). By contrast, in deprived barrel-related columns approximately one half (11/23) of L2/3 pyramidal cells lacked any detectable input from layer 5B (Figure 6). Where connections from L5B remained after deprivation, their numbers were severely, selectively, and reversibly depleted (Figure 6 and Figure S2). The average number of connected locations in L5B dropped from 8.0±9.9 in columns representing intact whiskers to 1.7±2.2 in deprived columns; input numbers returned to 8.5±9.5 and 5.9±6.6, respectively, during and after whisker regrowth (means ± 1 SD; p = 0.002; ANOVA).

Most of the few surviving sources of inhibition attributed to L5B arose from stimulation sites that straddled the border to L5A (Figure 3A and Figure S2), raising the possibility that the depletion of inhibitory connections from L5B was virtually complete. In columns representing intact whiskers, in contrast, presynaptically connected interneurons populated the full depth of L5B (Figure 3A). IPSCs could be elicited from 13.4%±16.0% of L5B stimulation spots in control cortex and from 15.3%±17.3% in previously deprived columns after whisker regrowth, but only from 3.3%±4.4% of all L5B locations in deafferented columns (means ± 1 SD; p = 0.023; ANOVA).

Inhibition from layers 4 and 5A underwent qualitatively similar but less extensive changes. Absolute input numbers, as well as the fractions of connected locations, fell during sensory deprivation but rebounded fully when sensory input was restored (Figure 6). Connections from L1 followed a trend opposite to that of connections from deep layers. The average number of inhibitory inputs from L1 rose from 7.2±3.3 in control barrel cortex to 9.2±3.1 in deprived whisker columns, and returned to 5.5±3.1 and 8.5±3.5 during and after whisker regrowth, respectively (means ± 1 SD; p = 0.001; ANOVA; Figure 6). These profound

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**Figure 2. Light sensitivity of ChR2-expressing interneurons.** (A) Classification of interneurons into fast-spiking (fs; left) and two types of non-fast-spiking (non-fs) cells, termed nonaccommodating or regular-spiking (center) and accommodating interneurons (right). Traces from top to bottom were recorded during 1-s current pulses of −50 pA, +25 pA, +150 pA, and +300 pA (B). Top: Example of a spike train evoked by optical pulses (1.8 mW, 20 ms, gray bars) at a stimulation frequency of 0.2 Hz in a L5B interneuron. (Center and Bottom) Probability of evoking ≥1 spike per optical pulse during repeated optical stimulation (n = 3–5 trains of 10 light pulses each) of L1 (center) and L5B interneurons (bottom). Light pulses were presented at 0.2 Hz and carried optical powers of 0.5 mW (small solid circles) or 1.8 mW (large open circles); small solid within large open circles indicate identical spiking probabilities at both power levels. Interneuron responses were classified as fast-spiking (fs) or non-fast-spiking (non-fs) and recorded in control (left) or deprived conditions (right). No significant differences exist between any of these groups (p > 0.05, t test). See Materials and Methods for criteria used to distinguish fs from non-fs cells. Yellow, fs interneurons; blue, nonaccommodating non-fs interneurons; red, accommodating non-fs interneurons. Only non-fs interneurons of the accommodating type were encountered in L1. (C) Same display as (B), but at 5 Hz stimulation frequency. No significant differences exist between any of the groups shown (p > 0.05, t test). (D) Top: Examples of single spikes (left), spike doublets (center), and spike triplets (right) evoked by a single optical pulse in a train (1.8 mW, 20 ms, 0.2 Hz, gray bars). (Center and Bottom) Average number of spikes evoked per optical pulse in L1 (center) and L5B interneurons. Interneuron responses were classified as fast-spiking (fs) or non-fast-spiking (non-fs) and recorded in control (left) or deprived conditions (right). No significant differences exist between any of these groups (p > 0.05, t test). See Materials and Methods for criteria used to distinguish fs from non-fs cells. Yellow, fs interneurons; blue, nonaccommodating non-fs interneurons; red, accommodating non-fs interneurons. Only non-fs interneurons of the accommodating type were encountered in L1. (D) Same display as (B), but at 5 Hz stimulation frequency. No significant differences exist between any of the groups shown (p > 0.05, t test). (E) Spiking probabilities of L1 and L5B interneurons as functions of the distance of the stimulation spot from the soma, using the same pseudorandom 14×20-spot stimulation sequence as in mapping experiments (see Figure 1). The graphs represent data from 10 L1 interneurons, 5 non-fs L5B interneurons, and 5 fs L5B interneurons in control conditions, and from 13 L1 interneurons, 7 non-fs L5B interneurons, and 4 fs L5B interneurons in deprived conditions. (F) Same data as in (E), but displaying the average number of spikes evoked per 14×20-spot stimulation sequence for interneurons in L1 (left) and L5B (right). (G) Probability of evoking an IPSC during repeated optical stimulation (n = 8–10 trials) of the same presynaptic location in the indicated layers (n = 23 slices). Black columns, control condition; gray columns, deprived condition; error bars represent 1 SD; asterisks indicate significant differences (p < 0.05, t test). The red line represents the most stringent criterion for detecting a synaptic input (≥3 IPSCs in eight trials; see Materials and Methods). In all cases, the reliability of transmission exceeds the criterion for detection by a wide margin.

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**Figure 3. Sensory deprivation causes motif-specific changes in laminar inhibitory connectivity.** (A) Maps of inhibitory inputs to L2/3 pyramidal neurons in columns representing intact (left, n = 23), trimmed (center left, n = 23), or previously deprived whiskers after regrowth for 1 mo (center right, n = 19) or 3 mo (right, n = 17). The maps are scaled to the size of a standard barrel (yellow outline) and overlaid to depict the distribution of inhibitory input sources. The intensity of gray shading at each location indicates the cumulative inhibitory charge transfer. This normalized index measures the frequency with which IPSCs are elicited from corresponding locations in different slices, weighted by the average charge transfer per IPSC. (B) Normalized inhibitory charge flow from the indicated source layers (rows) to L2/3 pyramidal neurons in columns representing intact (left, n = 23), trimmed (center left, n = 23), or previously deprived whiskers after regrowth for 1 mo (center right, n = 19) or 3 mo (right, n = 17). Values are represented numerically (± 1 SD) and in normalized gray scale. Red outlines mark significant differences associated with whisker trimming (p < 0.05; ANOVA; blue outlines indicate groups whose means differ from the whisker-trimmed state (Bonferroni-corrected t test). (C) Same display as (B), but illustrating absolute laminar inhibitory charge flow in pC (mean ± 1 SD). An additional significant difference exists in L1 between the 1-mo-regrowth condition (red asterisk) and columns representing intact and fully regrown whiskers (blue asterisks) (p < 0.05; Bonferroni-corrected t test).

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and antagonistic changes to four translaminar wiring motifs occurred against a backdrop of stable inhibitory-to-excitatory connectivity in the home layer. The number of inhibitory inputs from L2/3 fluctuated only marginally between a minimum of 23.2 ± 9.7 after 1 mo of whisker regrowth and a maximum of 27.1 ± 8.8 after 3 mo of regrowth (Figure 6).

In contrast to the antagonistic relationship between inhibitory inputs from superficial and deep cortical layers in deprived
barrel-related columns (Figure 6 and Figure 7), transcalamin inhibitory connections in the spared whisker-related row C of deprived cortex became uniformly sparse. In deep cortical layers, the number of connected locations dropped significantly: from 17.2±8.5 to 6.2±3.6 in L4, from 5.0±4.5 to 1.8±2.8 in L5A, and from 5.1±6.5 to 2.6±5.0 (means ± 1 SD; p<0.05; t test; Figure 7). The percentages of stimulation spots from which IPSCs could be elicited decreased from 34.7% to 11.4% in L4, from 23.9% to 8.2% in L5A, and from 10.2% to 4.8% in L5B (p<0.05, t test). Even in L1 the number of connected locations fell slightly (Figure 7), in keeping with the lack of a deprivation-induced surge in inhibitory charge flow from that layer (Figure 5B,C).

Our experimental manipulations altered neither the optical excitability of ChR2-expressing interneurons (Figure 2B-G) nor the reliability of optically evoked synaptic transmission (Figure 2H). The rearranged inhibitory input maps of L2/3 pyramidal cells in deprived cortex must therefore reflect changes in the number or subclass distribution of presynaptic interneurons, or changes in connection probabilities between these neurons and their postsynaptic targets. Immunohistochemistry ruled out the first mechanistic: neither the densities of ChR2-expressing interneurons in the plastic layers 1, 4, and 5, nor the distributions of the major subpopulations of parvalbumin- and somatostatin-positive cells, changed (Figure 8A,B). The measured variations in the number and locations of sites where IPSCs could be stimulated are thus indicative of changes in connection probabilities.

To search for structural correlates of these functional changes, we analyzed the wiring motif undergoing the largest deprivation-induced change: ascending inhibition from L5B (Figure 3 and Figure 6). Forty-nine interneurons in L5B were filled with neurobiotin; of these, 11 cells showed high-contrast axonal staining in upper cortical layers (Figure 8C,D). All of these 11 cells were non-fast-spiking interneurons of the accommodating (n=6) or regular-spiking (ionaccommodating) type (n=5), consistent with the notion that non-fast-spiking Martinotti cells are the principal conduits of L5-to-L2/3 inhibition [30,31]. The neurobiotin-filled axons of L5B interneurons extending into L2/3 carried fewer varicosities per unit length in deprived cortex than did their counterparts in control conditions, as would be expected if presynaptic terminals were eliminated following sensory deprivation (Figure 8D–F). There were no statistically significant differences between the volumes, surface areas, and maximal diameters of varicosities in deprived and control conditions; qualitatively, varicosities in deprived cortex even appeared somewhat larger than in intact cortex (Figure 8G).

The fractional loss of inhibitory varicosities after whisker trimming was, however, smaller than the fractional reduction in inhibitory charge flow from L5B (compare Figure 3B, Figure 6, and Figure 8F). This apparent mismatch may be accounted for in several ways. First, it is conceivable that varicosities remain visible after deprivation but the associated synapses have fallen silent. Second, connections with pyramidal cells may represent only a fraction of all synapses formed by L5B pyramidal neurons in barrel-related columns representing intact (left, n=13, column C), spared (center, n=12, column C), or deprived whiskers (right, n=23, columns A, B, D, and E; same data as in Figure 3B). Values are represented numerically (±1 SD) and in normalized gray scale. Red outlines mark significant differences between the spared column in deprived cortex and either of the other two (blue) conditions (p<0.05, t test). (C) Same display as (B), but illustrating absolute laminar inhibitory charge flow in pC (mean ± 1 SD). doi:10.1371/journal.pbio.1001798.g005

Figure 5. Sensory deprivation causes motif-specific changes in laminar inhibitory connectivity also in spared barrel-related columns. (A) Maps of inhibitory inputs to L2/3 pyramidal neurons in columns representing intact (left, n=13, C column), spared (center, n=12), or deprived whiskers (right, n=23; same data as in Figure 3B). The maps are scaled to the size of a standard barrel (yellow outline) and overlaid to depict the distribution of inhibitory input sources. The intensity of gray shading at each location indicates the cumulative inhibitory charge transfer. This normalized index measures the frequency with which IPSCs are elicited from corresponding locations in different slices, weighted by the average charge transfer per IPSC. (B) Normalized laminar charge flow from the indicated source layers (rows) to L2/3 pyramidal neurons in barrel-related columns representing intact (left, n=13, column C), spared (center, n=12, column C), or deprived whiskers (right, n=23, columns A, B, D, and E; same data as in Figure 3B). Values are represented numerically (±1 SD) and in normalized gray scale. Red outlines mark significant differences between the spared column in deprived cortex and either of the other two (blue) conditions (p<0.05, t test). (C) Same display as (B), but illustrating absolute laminar inhibitory charge flow in pC (mean ± 1 SD). doi:10.1371/journal.pbio.1001798.g005

Motif-Specific Changes in Connection Strength

Interneurons are thought to innervate each of their postsynaptic targets via multiple boutons (typically ~15) [30,32,33]. Whisker deprivation might cause some of these boutons to be eliminated at random. The loss of a measurable connection would then simply result from the stochastic depletion of all synapses between two locations. If boutons were indeed silenced or pruned in this shotgun manner, surviving connections would be expected to suffer partial bouton losses and, therefore, be weaker than those in...
significant differences exist between control and regrowth conditions. Opposite adjustments that canceled one another in the average. Different subsets of inhibitory synapses underwent large but remained unchanged (Figure S3). It is therefore implausible that coefficients of variation of the individual IPSC amplitudes also virtually unchanged (Figure 9 and Figure 10). Importantly, the average strength of extant connections from deep layers, measured as the mean integrated current per IPSC, deprivation left the strengths of extant connections from deep layers, measured as the mean integrated current per IPSC, virtually unchanged (Figure 7 and Figure 10). This constellation of changes indicates that the average L1-derived synapse gained in strength. In L1 of deprived columns, in contrast, both the average charge transfer per IPSC and the number of connected locations rose insignificantly. The statistically significant increase in laminar charge flow from L1 after whisker trimming (Figure 3) thus remains an unresolved consequence of combined increases in monosynaptic connection probability and monosynaptic connection strength.

A history of deprivation had profound aftereffects on the strengths of inhibitory inputs originating in superficial layers 1 and 2/3. The inhibitory charge flow per IPSC from these layers increased marginally upon sensory deprivation, echoing similar changes during the critical period [35], but plummeted during subsequent whisker regrowth (Figure 9 and Figure S4). In contrast to the rapid and complete resurgence of inhibitory input numbers upon sensory restoration (Figure 6), the recovery of connection strengths was delayed and partial, even after 3 mo of whisker regrowth (Figure 9 and Figure S4). Although the causes and significance of this hysteretic effect are currently unknown, the phenomenon provides clear further evidence that sensory plasticity operates by tuning probabilities and strengths of synaptic connections independently of each other.

**Discussion**

The adaptations documented here lay bare four remarkable features of experience-dependent plasticity of inhibitory connections. First, extensive changes take place in adult neocortex, long after the critical period for refining neuronal connections has closed [29]. Second, different wiring motifs are altered selectively and independently of one another. The most compelling illustration of this principle is the see-saw relationship between L1- and L5B-derived inhibition in deprived cortex: upon whisker trimming and regrowth, connections to a common postsynaptic target, the L2/3 pyramidal neuron, undergo simultaneous but opposite functional changes (Figure 3). Third, adjustments of inhibitory connection probabilities are fully reversible upon sensory restoration, even in cases where entire connections appear to have been lost during deprivation (Figure 3 and Figure 6). The removal of a measurable connection may thus not entail the physical retraction of axonal and/or dendritic branches, as is the case in critical period plasticity [36,37], but rather the shutdown of transmission between synaptic partners that remain in close apposition [7,38]. Fourth, probability and strength of a connection are independent dimensions for functional adjustment (Figure 6, Figure 7, Figure 9, and Figure 10).

Although the experimental settings and analytical approaches differ, it is instructive to compare our present findings with those of control conditions. Surprisingly, this was not the case: sensory deprivation left the strengths of extant connections from deep layers, measured as the mean integrated current per IPSC, virtually unchanged (Figure 9 and Figure 10). Importantly, the coefficients of variation of the individual IPSC amplitudes also remained unchanged (Figure S3). It is therefore implausible that different subsets of inhibitory synapses underwent large but opposite adjustments that canceled one another in the average.

Of course, optical stimulus-locked IPSCs may represent compound events if multiple presynaptically connected interneurons are activated simultaneously. Because the mean integrated current depends on the number and the individual strengths of all contributing synapses, it cannot be equated with the strength of a monosynaptic connection. In several instances, however, the two variables of synapse number and average synapse strength could be disambiguated by considering changes in charge transfer in the context of simultaneously occurring changes in the number of connected locations. For example, sensory deprivation greatly reduced the number of connections from layers 4 and 5 but left the charge transfer per remaining connection unchanged. If it is reasonable to assume, in light of this general trend toward synapse elimination, that persisting connections will be made through a constant or smaller rather than a larger number of synapses, then the average strength of these synapses must remain level or increase after deprivation. Our morphometric finding that L5B-derived axonal varicosities in deafferented whisker columns retained their pre-deprivation size or even expanded slightly (Figure 8G) reinforces this conclusion, as bouton size and synaptic strength trend to be tightly correlated [34].

An analogous argument applies to L1, but of the spared whisker-related row C. The charge transfer per IPSC in these barrel-related columns increased significantly after whisker trimming, while the number of connected locations fell marginally (Figure 7 and Figure 10). This constellation of changes indicates that the average L1-derived synapse gained in strength. In L1 of deprived columns, in contrast, both the average charge transfer per IPSC and the number of connected locations rose insignificantly. The statistically significant increase in laminar charge flow from L1 after whisker trimming (Figure 3) thus remains an unresolved consequence of combined increases in monosynaptic connection probability and monosynaptic connection strength.

Figure 6. Sensory deprivation causes motif-specific changes in inhibitory input numbers. Absolute number of locations in the indicated source layers (rows) giving rise to IPSCs in L2/3 pyramidal neurons in barrel-related columns representing intact (left, n = 23), trimmed (center left, n = 23), and previously deprived whiskers after regrowth for 1 mo (center right, n = 19) and 3 mo (right, n = 17). Colored columns represent group averages. Red columns mark significant differences associated with whisker trimming (p < 0.05; ANOVA); dark yellow columns indicate groups whose means differ from the whisker-trimmed state (Bonferroni-corrected t test). No significant differences exist between control and regrowth conditions (p > 0.05; ANOVA). doi:10.1371/journal.pbio.1001798.g006
From hindsight, hints of motif-specific plasticity are already evident in some earlier studies of excitatory [21,40,41] and even inhibitory cortical connections. For example, although the net elimination rate of boutons originating from L2/3 interneurons was found to increase after visual deprivation, inhibitory synapses onto L2/3 pyramidal cells—as opposed to those targeting dendrites of layer 5 pyramidal cells—appeared exempt from elimination [6,7]. These results are consistent with the stability of home layer-derived inhibition in our hands (Figure 3, Figure 6, and Figure 9). Another example is the distinct behavior of different populations of inhibitory axons in superficial layers of barrel cortex. After whisker plucking, some axons in deprived barrel-related columns sprout, while others in the same column suffer bouton losses [39]. In light of our observations it is likely that the sprouting axons derive from L1 interneurons, while axons suffering bouton losses originate from interneurons in deep layers that target the apical dendrites of L2/3 pyramidal cells (Figure 3B).

The adaptive changes displayed by different inhibitory circuit motifs offer some clues to the possible roles of these motifs in normal cortical function. Interneurons in L2/3 are thought to be driven by L2/3 excitatory neurons and the thalamocortical input layers 4 and 5A [27,35]. The amount of feed-forward inhibition these neurons impose on L2/3 pyramidal cells is thus expected to scale with the excitatory drive to the column: the smaller the intensity of sensory stimulation, the smaller the inhibitory counter-force generated by the local feed-forward circuit (Figure 11) [35]. This autoregulatory feature may obviate the need for plasticity of L2/3-derived inhibitory connections and explain their relative stability in the face of our experimental perturbations (Figure 3 and Figure 6).

The inhibitory L5B-to-L2/3 motif, in contrast, is all but deleted from deprived whisker columns (Figure 3 and Figure 6). This striking adaptation suggests that the inhibition imposed by L5B interneurons on L2/3 pyramidal cells would prove excessive if the connection were left in place unaltered. The loss of significant excitatory drive from nonprincipal whiskers may explain why the same adaptation occurs, albeit to a lesser extent, also in spared columns that border deprived cortical tissue (Figure 5 and Figure 7). Most L5B interneurons—including Martinotti cells, the putative mediators of ascending translaminar inhibition [30,31]—lack an autoregulatory mechanism that couples their activity directly to the intensity of sensory stimulation [42]. Instead, L5B interneurons are likely to integrate signals from L2/3 networks spanning more than one cortical column [43] and relay this information back to L2/3 in the form of recurrent inhibition [31]. This type of supralinear feedback inhibition [31,44], triggered by spontaneous activity or sensory input to nearby columns representing intact whiskers, would be expected to extinguish any residual sensory signals reaching columns whose associated principal whiskers have been trimmed. Unplugging the inhibitory feedback connection may be necessary to enable these columns to process feeble thalamic input (Figure 11).

Qualitatively similar considerations may apply to inhibition originating in layers 4 and 5A. L5A and septal regions of L4 form part of a cortico-thalamo-cortical loop involving the posterior medial nucleus [POM] [45–47]. Residual sensory input, relayed via POM to inhibitory neurons in layers 4 (septum) and 5A, might cause excessive inhibition in deprived barrel-related columns. The adaptations we observe may help to rebalance excitation and inhibition (Figure 11).

Inhibitory interneurons in L1, in contrast, are likely mediators of top-down control of cortical areas by hierarchically higher regions [48–50]. The increase in L1-derived inhibition after whisker trimming (Figure 3, Figure 6, and Figure 9) could

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**Figure 7. Sensory deprivation causes motif-specific changes in inhibitory input numbers also in spared barrel-related columns.** Absolute number of locations in the indicated source layers (rows) giving rise to IPSCs in L2/3 pyramidal neurons in barrel-related columns representing intact (left, *n* = 13, column C), spared (spared, *n* = 12, column C), or deprived whiskers (right, *n* = 23, columns A, B, D, and E; same data as in Figure 6, center left). Colored columns represent group averages. Red columns mark significant differences between the spared barrel-related column in deprived cortex and the comparison groups indicated in dark yellow (*p*<0.05, *t* test).

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previous reports of experience-dependent inhibitory plasticity in adult neocortex [6–9,39]. With one exception [39], all of these studies have examined the dynamics of structural changes in visual cortex following retinal lesions or monocular deprivation. Chronic imaging of the dendritic and axonal arbor or fluorescently tagged synapses of L1 and L2/3 interneurons revealed a seemingly general, rapid, and lasting loss of dendritic spines or branch tips [6,7], axon terminals [7], and gephyrin-labeled postsynaptic puncta [8,9] after deprivation. These morphological changes were taken to indicate a broad adaptive downscaling of inhibition. Our direct measurements of activity-induced functional changes in inhibitory-to-excitatory connections across the entire depth of somatosensory cortex paint a more differentiated picture. Although we do find a general decrease in the number of cortical locations providing inhibitory inputs to L2/3 pyramidal neurons (from 67.7±17.0 in control columns to 46.4±19.4 in whisker-deprived columns; means ± 1 SD; *p*<0.001, *t* test; Figure 3B), the scale and specificity of cortical remodelling become apparent only when individual wiring motifs are disentangled and analyzed separately (Figure 3).
**Figure 8. Sensory deprivation reduces the density of presynaptic boutons formed by L5B interneurons in L2/3.** (A) Slices of barrel cortex were immunolabeled with antibodies against GFP (left), parvalbumin (PV, center), and somatostatin (Sst, right). The examples show raw confocal images of L5B after staining with fluorescently labeled secondary antibodies. (B) Densities of cells expressing ChR2-GFP, PV, and Sst in L1 (left), L4 (center), and L5B (right) of barrel-related columns representing intact, trimmed, or previously deprived whiskers after regrowth for 1 mo (mean ± 1 SD; n = 5–16 confocal stacks from 2 mice per condition). No significant differences exist between any of these conditions in any of the layers (p > 0.05; ANOVA). (C) Experimental configuration for axonal morphometry. Interneurons located in L5B were filled with neurobiotin to visualize their axonal arbors in L2/3 (reconstructed in red in the example shown). (D) Examples of axon segments in L2/3 deriving from four different neurobiotin-filled interneurons located in L5B. The linear density of presynaptic varicosities differs between control (left panels) and deprived (right panels) conditions. (E) Higher magnification view shows that individual presynaptic varicosities (red circles) can be distinguished unambiguously. (F) Average linear density of presynaptic varicosities along L5B interneuron-derived axon segments in L2/3 (n = 10 segments each in control and deprived conditions; total reconstructed axon length 7,071 and 3,204 μm, respectively). Black symbols, control condition; gray symbols, deprived condition; triangles, nonaccommodating non-fs interneurons; squares, accommodating non-fs interneurons. The asterisk indicates a significant difference (p < 0.05, t test). (G) Average volume (left), surface area (middle), and diameter (right) of presynaptic varicosities along L5B interneuron-derived axon segments in L2/3 (n = 10 segments and 600 boutons each in control and deprived conditions). Black symbols, control condition; gray symbols, deprived condition; triangles, nonaccommodating non-fs interneurons; squares, accommodating non-fs interneurons. No significant differences exist between the groups (p > 0.05, t tests).

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**Materials and Methods**

**Ethics Statement**

All procedures complied with the UK Animals (Scientific Procedures) Act 1986.

**Experimental Animals and Whisker Deprivation**

Experimental animals were knock-in mice homozygous for R26::CAG-lox-STOP-lox-ChR2-EGFP responder and GAD::CreER<sup>ts</sup> driver transgenes at both targeted loci [5]. Following tamoxifen induction of Cre recombinase activity, these animals express channelrhodopsin-2 (ChR2; GenBank accession number AF461397, [13,14]) comprehensively in all main subclasses of GABAergic interneurons defined cytochemically [5]. Mice were maintained in top-open cages on a 12 h light/dark cycle and fed a custom diet based on Teklad 2018, but with vitamin A levels elevated to 100 IU/g (Harlan Laboratories). At 8–10 wk of age, whiskers in rows A, B, D, and E on the right side of the snout were trimmed every other day for 2–3 wk. Whisker trimming was performed under transient anesthesia induced by subcutaneous (s.c.) injection of ~20 μl of a 3:5 mixture of ketamine (100 mg/ml; Fort Dodge) and medetomidin (1 mg/ml; Pfizer) and reversed by s.c. injection of 15–20 μl atipamezole (5 mg/ml; Pfizer). Control animals underwent the same anesthetic regimen as did whisker-trimmed animals.

Slices of acutely deprived somatosensory cortex were harvested no later than 36 h after the last whisker trimming session. To examine the effects of recovery from deprivation, whiskers were allowed to regrow for 4–5 wk (1-mo regrowth) or 12–14 wk (3-mo regrowth). Starting at 6–8 d before slices were cut, mice were injected intraperitoneally (i.p.) on 5 consecutive days with 0.3–0.5 mg 4-OH-tamoxifen (Sigma-Aldrich), which was dissolved in sterile sunflower oil at 5 mg/ml.
Cortical Wiring Motif Plasticity

Figure 9. Sensory deprivation causes motif-specific changes in inhibitory connection strength. Voltage-clamp recordings at a holding potential of 0 mV from L2/3 pyramidal neurons in barrel-related columns representing intact (left, n = 23), trimmed (center left, n = 23), or previously deprived whiskers after regrowth for 1 mo (center right, n = 19) or 3 mo (right, n = 17). IPSCs were evoked by optical stimulation of interneurons in the indicated cortical layers; traces of all individual IPSCs (gray) were aligned to the time at which the rising IPSC reached half-maximal amplitude. Bold black traces indicate group averages. Colored columns represent the mean integrated current (charge transfer) per IPSC. Red columns indicate significant differences associated with partial whisker regrowth (p < 0.05; ANOVA); dark yellow columns indicate groups whose means differ from the state of partial whisker regrowth (Bonferroni-corrected t test). Asterisks mark pairwise differences remaining after whisker regrowth for 3 mo (p < 0.05; ANOVA followed by Bonferroni-corrected t test). See also Figure S3 and Figure S4.

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Electrophysiology and Optical Stimulation

Experiments were performed on mice 2–4 d after the last 4-OH-tamoxifen injection. Animals were anesthetized by i.p. injection of 150 µl of a 3:5 mixture of ketamine (100 mg/ml; Fort Dodge) and medetomidine (1 mg/ml; Pfizer) and perfused cardiacly with ice-cold solution containing (in mM): 2.5 KCl, 1.25 NaH2PO4, 25 NaHCO3, 10 glucose, 240 sucrose, 0.5 CaCl2, 7 MgCl2, pH 7.4, 320 mOsm. The brain was recovered into perfusion solution, and columns corresponding to whisker rows A through E. Patch All extracellular solutions were bubbled with 95% O2/5% CO2. The internal solution for current-clamp recordings contained (in mM): 120 K-gluconate, 10 KCl, 10 Hepes, 4 MgATP, 0.3 Na2GTP, 10 phosphocreatine, 0.2% neurobiotin. Signals were amplified and low-pass-filtered at 2 kHz by a Multiclamp 700a amplifier (Molecular Devices) and digitized at 5–10 kHz (Digidata 1440, Molecular Devices).

Three criteria were used to distinguish fast-spiking (fs) from non-fast-spiking (non-fs) interneurons. Fs neurons (i) attained firing rates >90 Hz during a 1,000-ms depolarizing current step, (ii) exhibited a ratio of >0.7 of the average interspike interval (ISI) at the beginning and end of the depolarizing current step (averages of 3 ISIs each), and (iii) displayed a spike width of ≤1 ms at half-maximal amplitude. Cells that met all three criteria were classified as fs and cells that failed all three criteria as non-fs.

Optical stimulation experiments were performed on a Zeiss Axioskop 2FS microscope. A 40 ×, 0.3 NA water immersion objective with DIC optics was used for electrode placement and a 10 ×, 0.3 NA water immersion objective, without DIC optics, for optical stimulation. The output of a continuous-wave solid-state laser with a maximum power of 325 mW at 473 nm (LR 473-AH-300-10, Laserglow) was digitally switched and intensity-modulated by an acousto-optic deflector (IntraAction model 3000).

Figure 10. Sensory deprivation causes motif-specific changes in inhibitory connection strength also in spared barrel-related columns. Voltage-clamp recordings at a holding potential of 0 mV from L2/3 pyramidal neurons in barrel-related columns representing intact (left, n = 13, column L), spared (center, n = 12), or deprived whiskers (right, n = 23; same data as in Figure 9, center left panels). IPSCs were evoked by optical stimulation of interneurons in the indicated cortical layers; traces of all individual IPSCs (gray) were aligned to the time at which the rising IPSC reached half-maximal amplitude. Bold black traces indicate group averages. Colored columns represent the mean integrated current (charge transfer) per IPSC. Red columns mark significant differences between the spared barrel-related column in deprived cortex and the comparison groups indicated in dark yellow (p < 0.05, t test).

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Encompassing 14 Leica TCS SP5 confocal microscope. Slices were mounted in VectaShield (Vector Labs) and imaged on a secondary antibodies (Invitrogen). After four rinses in TBS, the sections were rinsed 4 times in TBS and stained in TBS-T containing 1% (v/v) horse serum, or anti-somatostatin (rabbit, 1:500, Millipore). The GFP (chicken, 1:500, AbCam), anti-parvalbumin (mouse, 1:2,000, Swant), or anti-somatostatin (rabbit, 1:500, Millipore). The sections were converted to outside-out after neurobiotin infusion to allow the plasma membrane to reseal. Slices were incubated in modified aCSF for 1 h and then overnight in PBS containing 4% (w/v) PFA and 0.2% (v/v) picric acid. The slices were rinsed in TBS and stained in TBS-T containing 1% (v/v) horse serum, 4 μg/ml Alexa546-labeled streptavidin (Invitrogen), and 0.0001% DAPI (Sigma) for 12–24 h. After four rinses in TBS, the slices were mounted in VectaShield (Vector Labs) and imaged on a Leica TCS SP5 confocal microscope.

Immunohistochemistry

Animals (n = 2 in each condition) were perfused with phosphate-buffered saline (PBS, pH 7.4) containing 4% (w/v) parafomaldehyde (PFA) and 0.2% (v/v) picric acid under general ketamine-xylazine anesthesia. The brain was removed, incubated for 24 h in perfusion solution, and infiltrated with 30% (w/v) sucrose in PBS for at least 24 h. Coronal sections of 50 μm were cut on a Leica SM 2000R sliding microtome. The sections were rinsed three times in Tris-buffered saline (TBS, Sigma), three times in TBS containing 3% (w/v) Triton X-100 (TBS-T), and once for 1 h in TBS-T containing 20% (v/v) horse serum (Vector Labs) and then incubated for 48 h at 4°C in TBS-T containing 1% horse serum and combinations of the following primary antibodies: anti-GFP (chicken, 1:500, AbCam), anti-parvalbumin (mouse, 1:2,000, Swant), or anti-somatostatin (rabbit, 1:500, Millipore). The sections were rinsed 4 times in TBS and stained in TBS-T containing 1% horse serum and Alexa488- and Alexa546-labeled secondary antibodies (Invitrogen). After four rinses in TBS, the slices were mounted in VectaShield (Vector Labs) and imaged on a Leica TCS SP5 confocal microscope.

Axonal Morphometry

To estimate the density of presynaptic varicosities along axon segments, the neurobiotin concentration in the internal solution for current-clamp recordings was raised to 1%. Patch configurations were converted to outside-out after neurobiotin infusion to allow the plasma membrane to reseal. Slices were incubated in modified aCSF for 1 h and then overnight in PBS containing 4% (w/v) PFA and 0.2% (v/v) picric acid. The slices were rinsed in TBS and stained in TBS-T containing 1% (v/v) horse serum, 4 μg/ml Alexa546-labeled streptavidin (Invitrogen), and 0.0001% DAPI (Sigma) for 12–24 h. After four rinses in TBS, the slices were mounted in VectaShield (Vector Labs) and imaged on a Leica TCS SP5 confocal microscope.

Data Analysis

Data were analyzed as described [5], using Igor 6 (Wavemetrics) and SPSS 17 (IBM). Briefly, maps of inhibitory inputs were

Figure 11. Plasticity of inhibitory wiring motifs in a functional context. (A) L2/3 pyramidal cells in barrel cortex of mice with intact whiskers receive feed-forward (yellow), feedback (blue), and top-down inhibition (red). Interneurons providing these different forms of inhibition are located in different cortical layers, where they are driven, respectively, by thalamocortical afferents and cortical excitatory neurons (L4 and L2/3 interneurons, yellow); by intracortical or cortico-thalamo-cortical loops (L4 septal and L5B interneurons, blue); and by interareal excitatory connections terminating in L1 (red). (B) Whisker trimming leads to motif-specific changes in connection probabilities: existing inhibitory connections from L4 and L5B are eliminated, whereas new inhibitory connections from L1 are formed. (C) Partial whisker regrowth leads to overcompensation of deprivation-induced changes in connection probabilities and a large reduction in the strength of inhibitory connections from L1 and L2/3. (D) Full whisker regrowth restores connection probabilities to baseline. The strength of inhibitory connections from L1 and L2/3 recovers only partially.

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ASN-802832 with ME-802 driver), positioned by a pair of galvanometric mirrors (GSI Lumonics VM500 with MiniSAX servo controllers), and merged with the epi-illumination path of the microscope via custom-built optics. Light pulses carried 0.3–1.8 mW of optical power at the exit pupil of the objective. To generate maps of inhibitory inputs, a virtual instrument written in LabVIEW 8.5 delivered focused stimulation light pulses (spot size 3–5 μm, 20 ms duration) at intervals of 680 ms to 60-μm grids encompassing 14 × 20 locations in pseudorandomized order.

Axonal Morphometry

To estimate the density of presynaptic varicosities along axon segments, the neurobiotin concentration in the internal solution for current-clamp recordings was raised to 1%. Patch configurations were converted to outside-out after neurobiotin infusion to allow the plasma membrane to reseal. Slices were incubated in modified aCSF for 1 h and then overnight in PBS containing 4% (w/v) PFA and 0.2% (v/v) picric acid. The slices were rinsed in TBS and stained in TBS-T containing 1% (v/v) horse serum, 4 μg/ml Alexa546-labeled streptavidin (Invitrogen), and 0.0001% DAPI (Sigma) for 12–24 h. After four rinses in TBS, the slices were mounted in VectaShield (Vector Labs) and imaged on a Leica TCS SP5 confocal microscope.

Interneurons were identified from their spiking responses to step-current pulses during the neurobiotin infusion as well as on the basis of morphological criteria after filling (dendrites with beaded appearance; absence of mushroom spines; absence of a prominent apical dendrite). The axonal arbors of 11 out of 49 neurobiotin-filled neurons (five cells in control conditions, and six cells in deprived conditions) showed high-contrast axonal labeling in L2/3 that could be traced back to the filled soma in L5B; these arbors were chosen for morphometric analysis. Ten L2/3 axon segments in each condition, ranging in length from 125 to 2,063 μm, were reconstructed manually using the freeware Neuromatic [56]. Varicosities were identified as focal swellings that appeared larger and brighter than the neighboring stretches of axon and could, due to their size and brightness and the axial resolution of the microscope, also be seen in at least two adjacent confocal image planes [57]. Objects with a maximum diameter of 2 μm were counted as single varicosities if no constriction of the circumference was evident; the rare objects whose maximum diameter exceeded 2 μm were counted as two varicosities.
columns of mice with intact (black, capacitance (right) of L2/3 pyramidal cells in barrel-related deprived whiskers after regrowth for 1 mo (second from bottom) representing intact (top), trimmed (second from top), or previously trimmed (bottom). The amplitude of the IPSC evoked at each location is represented in gray scale, according to the look-up table at the bottom. The selected neurons represent deciles in the frequency distribution of the number of L5B-derived inputs to L2/3 pyramidal cells. Maps are scaled to the size of a standard whisker-related barrel (yellow outline).

Figure S3 Variability of IPSC amplitudes. Coefficients of variation of the individual IPSC amplitudes shown in Figure 9. IPSCs were evoked by optical stimulation of interneurons in the indicated cortical layers of barrel-related columns representing intact, trimmed, and previously deprived whiskers after regrowth for 1 mo and 3 mo.

Figure S4 Distribution of IPSC amplitudes. Histograms display the number of verified input sources (x-axis) of a given amount of charge transfer (y-axis) grouped in bins of 1 pC (layer 1–4) or 0.5 pC (layer 5). Data correspond to IPSC traces in Figure 9, and bars of average charge flow are shown for reference as displayed in Figure 9. Histograms are shown for inhibitory input sources of layer 1 to 5B (top to bottom) in barrel-related columns representing intact (top), trimmed (center-left), or previously deprived whiskers after regrowth for 1 mo (center-right) or 3 mo (right). Absolute number of locations in the indicated source layers (rows) giving rise to IPSCs of the indicated charges in L2/3 pyramidal neurons. Same data as in Figure 9 (barrel-related columns A, B, D, and E) and Figure 10 (barrel-related column C). Dark yellow columns representing average charge flow are reproduced for reference from Figure 9 and Figure 10.

Table S1 Estimate of the number of interneurons activated per stimulation site. The number of ChR2-expressing interneurons activated by a single optical pulse was calculated using two estimates of interneuron densities in specific layers of primary somatosensory cortex (S1) of the mouse [24,25] and the empirically supported assumptions of a lateral optical resolution of ~60 μm FWHM (Figure 1A and Figure 2E) [5], a response reliability of >90% (Figure 2B) [5], a slice thickness of 310 μm, and a scatter coefficient of ~10 mm^-1 [23].

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Author Contributions

The author(s) have made the following declarations about their contributions: Conceived and designed the experiments: DK GM. Performed the experiments: DK. Analyzed the data: DK GM. Wrote the paper: DK GM.

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