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Experience-Dependent Plasticity of the Barrel Cortex in Mice Observed with 2-DG Brain Mapping and c-Fos: Effects of MMP-9 KO

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Modifications of properties of the adult sensory cortex by elimination of sensory input (deprivation) serves as a model for studying plasticity in the adult brain. We studied the effects of short- and long-term deprivation (sparing one row of vibrissae) upon the barrel cortex. The response to stimulation (exploration of a new environment) of the spared row was examined with [14C]-2-deoxyglucose autoradiography and c-Fos immunohistochemistry. Both methods found large increases of the functional cortical representation of the spared row of vibrissae, extending into parts of the barrel cortex previously activated by the deprived vibrissae. With both methods, the greatest expansion of spared input was observed in cortical layer IV. In this way, we established a model, which was applied for examining involvement of matrix metalloproteinase 9 (MMP-9), upon experience-dependent cortical plasticity. MMP-9 is an enzyme implicated in plastic modification of the neuronal connections. We found that MMP-9 activity was increased in response to stimulation, and furthermore, MMP-9 knockout mice showed a modest but significant decrease of plasticity in layer IV with 2-DG mapping and in layers II/III with c-Fos mapping. Thus, in adult mouse brain experience-dependent plasticity is in part supported by the activity of MMP-9.

Keywords: barrel cortex, c-Fos, [^{14}C]-2-deoxyglucose mapping, matrix metalloproteinase 9, sensory deprivation

Introduction

Restriction of input to sensory receptors results, in both neonatal and adult mammals, in alterations in functional activation and anatomical connectivity of cortical sensory areas. Cutting off or plucking out selected facial vibrissae serves as an important model of sensory deprivation in rodents. Local elimination of excitatory input to some of the cortical barrels disturbs the normal balance of excitation and inhibition. This leads to retraction of thalamocortical afferents from barrels deprived of their sensory input (Wimmer et al. 2010). Corticocortical circuits also undergo rewiring: axons and dendrites are remodeled (Hickmott and Steen 2005; Tailby et al. 2005; De Paola et al. 2006; Cheetham et al. 2007, 2008; Marik et al. 2010), spinogenesis is stimulated (Knott et al. 2002; Holtmaat et al. 2005, 2006, 2008; Knott et al. 2006), and new connections are formed.

The dynamics of deprivation-induced changes is influenced by the pattern of deprivation (removal of all vibrissae, a single one, all except one, a single row, all rows of vibrissae except one, chessboard deprivation etc.). The effects may also vary with age and duration of the deprivation procedure. Glazewski and Fox (1996) in adolescent rats observed first increases of responses to inputs from spared vibrissae in the deprived barrel (deprivation of a single vibrissa) after 20 days. Maier et al. (2003) in spared row C preparation in young adult hamsters found, with 2-DG autoradiography, expansion of the spared input after 6 days of deprivation. Bender et al. (2003) observed depression of L4 -> L2/3 input in deprivation after 5, but not 3, days in young rats. In mice, both electrophysiological and 2-DG studies reported effects of 7 days long deprivation (Wallace and Fox 1999; Liguz-Lecznar et al. 2011).

In the current study, plasticity of the functional representation of spared whiskers was estimated by 2-DG autoradiography and c-Fos immunohistochemistry in animals actively using their whiskers while exploring new environment. In this experimental model, 2-DG is incorporated into cells activated by stimulation of the spared vibrissae following 2-DG injection (Kossut and Hand 1984). c-Fos is an immediate early gene considered to be a marker of recent neuronal activity, and therefore, it has been widely used for functional brain mapping (Herrera and Robertson 1996; Herdegen and Leah 1998; Wirtshafter 2005). In the barrel cortex, c-Fos expression is triggered by exploration of new environment (Filipkowski et al. 2000; Staiger et al. 2000; Bisler et al. 2002). Since 2-DG uptake and c-Fos expression may provide distinct and complementary information (Duncan et al. 1993, 1998; McCown et al. 1995; Eells et al. 2000; Montag-Sallaz et al. 2003), we used both approaches to visualize functional changes induced by deprivation. We examined the effects of 7 and 28 days long deprivation. We used the spared row of vibrissae preparation where all whiskers except row C were repeatedly removed. This procedure leaves the centrally situated row of barrels activated, while on both sides of the active row, the barrels do not receive input from their principal whiskers and their territory is available for the spared input, freed from lateral inhibition.

With this procedure, we established a model that was applied for examining effects of genetic manipulation upon experience-dependent cortical plasticity. Recent studies suggest a role of matrix metalloproteinase 9, MMP-9, in brain plasticity. Engagement of MMP-9 in the adult brain synaptic plasticity, learning, and memory has only recently been appreciated (Kaczmarek et al. 2002; Nagy et al. 2006; Okulski et al. 2007; Wilczynski et al. 2008). Knockouts of MMP-9 show impairment of LTP in the CA1 region (Nagy et al. 2006). In rats, MMP-9 levels in the hippocampus increase during spatial memory learning and inhibitors of metalloproteinases impair acquisition of the task (Meighan et al. 2006) as well as impair LTP in the prefrontal cortex (Okulski et al. 2007). Matrix metalloproteinase 9 has been identified as a novel synaptic enzyme (Wilczynski et al. 2008) secreted upon stimulation and cleaving extracellular matrix to allow reshaping of synaptic

connections. We describe here that exploration of a new environment results in MMP-9 activation and in the knockouts of MMP-9 gene, plasticity evoked by sensory deprivation is less pronounced than in the wild-type (WT) mice.

Materials and Methods

Subjects

Twenty-four C57BL/6, 12 MMP-9 KO, and 10 of their WT littermates of both sexes, aged 8–9 weeks were used in the study. MMP-9 homozygous knockout (MMP-9 KO) mice in a C57BL/6 background were obtained from Dr Z. Werb (University of California at San Francisco, San Francisco, CA). The mice were reared in a 12:12 light/dark cycle in standard cages and they had ad libitum access to water and food. All experimental procedures were approved by the First Ethical Commission in Warsaw, Poland and were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Sensory Deprivation

Mice were deprived under isoflurane anesthesia by plucking out all whiskers except the row C on one side of the snout (referred as an experimental side later in the text). This experimental model of sensory deprivation leaves intact the centrally situated row C of vibrissae, with symmetrical space for remodeling of the cortex in the medial and lateral direction. Every second day regrowing vibrissae were removed. On the 6th or 27th day, the other side of snout (control side) was subjected to the same deprivation procedure, so that mice were left with rows C of whiskers on both sides intact.

Experimental Groups

Following groups of animals were used in the study:

- C57BL/6 mice deprived for 1 week and used in a 2-DG study, $n = 5$
- C57BL/6 mice deprived for 4 weeks and used in a 2-DG study, $n = 5$
- C57BL/6 mice deprived for 1 week and used for immunohistochemical experiments, $n = 4$
- C57BL/6 mice deprived for 4 weeks and used for immunohistochemical experiments, $n = 4$
- MMP-9 KO mice, deprived for 4 weeks and used in a 2-DG study, $n = 6$
- WT littermate control mice, deprived for 4 weeks and used in a 2-DG study, $n = 6$
- MMP-9 KO mice, deprived for 4 weeks and used for immunohistochemical experiments, $n = 6$
- WT littermate control mice, deprived for 4 weeks and used for immunohistochemical experiments, $n = 4$
- C57BL/6 mice deprived for 1 week and used for gel zymography, $n = 6$

Enriched Environment Exploration

On the day 7th or 28th, mice were allowed to explore a stimulatory cage (with all the walls and floor made of bars, equipped with mouse wheels and plastic toys and maze-like constructions). Before the exploration, mice for 2-DG study were administered IM a 40 μCi/100 g injection of [^{14}C]-2-deoxyglucose (American Radiolabeled Chemicals, ARC, specific activity 55 mCi/mmol). Immediately after the injection, animals were placed in the enriched environment and left for 30 min in darkness. Next, animals were killed with an overdose of Vetbutal (Biowet, Puławy) and briefly perfused with ice-cold 4% paraformaldehyde in phosphate buffer. The brains were dissected out, cortices were flattened (Strominger and Woolsey 1987), and frozen in −70 °C isopentane.

Animals used for immunohistochemical experiments were moved to the enriched environment directly from their home cages and allowed to explore for 2 h. After that time, animals were killed with an overdose of Vetbutal, perfused with 0.9% NaCl followed by 4% paraformaldehyde in phosphate buffer. The brains were dissected, cortices were flattened, and postfixed in the same fixative overnight. Then, cortices were cryoprotected in 30% sucrose and frozen in −70 °C isopentane.

Mice used for gel zymography experiments were moved to the enriched environment directly from their home cages and allowed to explore for 10 min. Next, they were killed by cervical dislocation. Immediately after brain removal from the skull, barrel cortices from both hemispheres were dissected on ice according to coordinates given by Strominger and Woolsey (1987) and stored at −70 °C until use.

2-DG Autoradiography and Data Analysis

Flattened cortices were cryosectioned (30 μm) at −20 °C tangentially to the brain surface. The serial sections were collected onto glass slides, dried on the hot plate, and then exposed against Kodak mammography X-ray film together with sets of [^{14}C] standards. The autoradiograms were analyzed using custom-written application for ImageJ. In each autoradiogram, the width of spared row C functional representation was measured using previously established criterion (Kossut et al. 1988), which considered as activated the regions with a level of 2-DG labeling ([^{14}C] concentration) 15% higher than in the surrounding cortex. It was found that 15% difference corresponds to 2 standard deviations above the mean value of labeling in the surrounding cortex (Chmielowska et al. 1986). Intensity of labeling within the row with spared inputs was also measured. The measurements were performed in all collected sections. After obtaining autoradiograms, sections were Nissl stained to allow visualization of the barrel field and assigning slices to cortical layers.

c-Fos Immunohistochemistry

Cortices were cryosectioned (30 μm) into phosphate-buffered saline (PBS) containing thiomersal as a preservative and stored in 4 °C upon usage. After several washes in PBS, endogenous peroxidase activity was blocked by incubation in 0.3% H_2O_2 in PBS. Sections were incubated in 4 °C overnight with polyclonal anti-c-Fos antibody (1:10 000, sc-52, Santa Cruz). Next, they were washed in PBS-T (PBS with Triton X-100, 0.3%) and incubated with goat anti-rabbit biotinylated secondary antibody conjugated with HRP (1:1000, BA-1000, Vector) followed by ABC kit (PK-6100, Vector). Reaction was developed by using DAB/urea H_2O_2 tablets (SigmaFAST, D4293, Sigma) with addition of nickel ammonium sulfate hexahydrate (0.02%), resulting in black nuclear staining. Slide-mounted sections were dehydrated, cleared in xylene, and embedded in DePeX.

Microscope pictures were taken using a Nikon Eclipse 80i microscope equipped with a ×10 objective and Wikom Evolution WF camera. Sections (10–12) from each animal from layers II/III, IV, and Vb were analyzed. Rows B, C, D were outlined in sections from layer IV. These outlines were superimposed onto microphotographs of slices from layers II/III and Vb. Image-J software was used for automated analysis of pictures. c-Fos positive nuclei were counted within whole outlined regions. Results are presented as number of immunopositive nuclei in 0.1 mm^2.

Gel Zymography

Extraction of MMPs was performed by affinity chromatography using gelatin-Sepharose 4B (GE Healthcare). Tissues were homogenized with a blade homogenizer at 20 500 rpm in a working buffer composed of 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, with the addition of 0.02% NaN_3. The homogenates was centrifuged for 15 min at 4 °C at 12 000 × *g*. A 5-μL aliquot of the recovered supernatants was saved and assayed for total protein, and the rest was incubated with gelatin-Sepharose 4B. After incubation and centrifugation, the gelatin-Sepharose pellet was incubated with elution buffer consisting of working buffer plus 10% dimethylsulfoxide. The samples normalized for protein concentration were mixed with 4× sample buffer (0.25 M Tris–HCl, pH 6.8, 8% SDS, 40% glycerol, 0.008% bromophenol blue) and loaded on a polyacrylamide gel copolymerized with 2 mg/mL gelatin as a substrate and run for 3 h at 90 V. After electrophoresis, SDS was removed from the gels by washing with 2 changes of zymogram renaturating buffer (2.5% Triton X-100, 30 min each), and the gels were then incubated at 37 °C in a developing buffer containing 50 mM

Tris–HCl, pH 7.5, 1% Triton-X 100, 10 mM $CaCl_2$, and 0.02% NaN_3, 1 μM $ZnCl_2$ in 37 °C, for 96 h. Following incubation, the gels were stained with 0.5% Coomassie Blue R-250 for 30 min and then destained appropriately. ImageJ software was used to measure the mean optical density of MMP-9 and MMP-2 band within a fixed area.

Statistics

Statistical analysis was performed using the GraphPad Prism 5 software (GraphPad Software, Inc.). Interhemispherical comparisons in 2-DG and c-Fos studies were performed using paired *t*-test. To compare the effect of deprivation in different groups of animals in 2-DG study, ratios of labeling width in experimental and control hemisphere were determined and analyzed using Mann–Whitney test. To compare the effect of deprivation upon c-Fos labeling in MMP-9 KO mice and their WT littermates, one-way analysis of variance followed by Bonferroni correction for deprived and spared regions was performed. For interhemispherical comparisons in gel zymography, intensity of the band in the experimental hemisphere was normalized to the control hemisphere, and they were compared by Wilcoxon matched-pairs test.

Results

2-Deoxyglucose Study

One Week and 4 Weeks of Deprivation

Comparison of labeling of cortical representation of row C vibrissae in control hemispheres of animals subjected to deprivation for either 1 or 4 weeks demonstrated no differences in the width of the labeled representation for any cortical layers ($P > 0.05$). The control hemispheres in fact received 24 h of sensory deprivation. This was done so as to make the experimental protocols for 2DG and c-Fos comparable, and c-Fos requires a temporary elimination of sensory inputs so that no accidental labeling from previous stimulations, especially the effects of whisker plucking, would obscure labeling evoked by the experiment. Consequently, we are not concerned here with the effects of very short-lasting deprivation and make no statements about it, but observe the progress of effects due to longer durations of deprivation.

Following 1 week and 4 weeks of deprivation, the functional representation of the spared row C vibrissae in the experimental hemisphere increased when compared with the control hemisphere (Fig. 1*A*). In layers II/III, the width of labeling in the experimental hemisphere surpassed labeling in the control hemisphere by 52.7% (597 vs. 391, $P < 0.01$) after 1 week of deprivation and by 94.4% (704 vs. 362 μm, $P < 0.001$) after 4 weeks of deprivation (Fig. 1*B*). Similarly, in layer IV, representation of the spared row C in the experimental hemisphere was wider than in the control hemisphere in both groups: in the group deprived for 1 week by 143.4% (691 vs. 482 μm, $P < 0.01$) and in the group deprived for 4 weeks by 163.3% (1289 vs. 489 μm, $P < 0.001$). In the infragranular layers, functional representation of the spared row C also invaded neighboring regions deprived of their sensory input in the experimental hemisphere. It exceeded the width of labeling in the control hemisphere by 54.2% (693 vs.449 μm, $P < 0.01$) after 1 week of deprivation and by 120.8% (974 vs. 441 μm, $P < 0.001$) after 4 weeks of deprivation. To evaluate the effect of length of deprivation, we determined the ratio of labeling width in the experimental hemisphere to labeling width in the control hemisphere and compared the ratios for groups deprived for 1 week and 4 weeks. In layers II/III, longer deprivation produced bigger change than the shorter deprivation (the ratios: 1.58 ± 0.32—1 week of deprivation; 1.95 ± 0.11—4 weeks of deprivation, $P < 0.05$). Time dependence was especially pronounced in layer IV (the ratios: 1.44 ± 0.15—1 week of deprivation; 2.63 ± 0.27—4 weeks of deprivation, $P < 0.01$). Layers V/VI in the experimental hemisphere were also more affected by longer deprivation (ratio 2.25 ± 0.42) than by the shorter deprivation (ratio 1.58 ± 0.33, $P < 0.05$).

After 1 week of deprivation, intensity of labeling in layer IV within the spared row C increased by 3.7% ($P < 0.05$) in comparison with homotopic region in control hemisphere, while in other layers labeling in control and experimental hemisphere did not differ. Longer deprivation affected intensity of labeling in all cortical layers. In layers II/III, it increased by 5.8% ($P < 0.05$), in layer IV by 3.0% ($P < 0.05$), and in layers V/VI by 5.3% ($P < 0.01$).

MMP-9 KO and Wild-Type Mice

We evaluated impact of 4 weeks of deprivation upon the spared row functional representation in MMP-9 KO mice and their WT littermates. In both groups, deprivation-induced plasticity could be observed in all cortical layers (Table 1).

Calculating ratios of labeling width in experimental and control hemisphere allowed us to compare effects of deprivation between MMP-9 KO and WT mice. The comparison revealed decreased plasticity in layer IV of MMP-9 KO mice (ratios: MMP-9 KO 1.68 ± 0.23, WT 1.99 ± 0.14, $P < 0.05$, Fig. 2). There were no differences between MMP-9 KO and WT mice in other cortical layers (layers II/III ratios: MMP-9 KO 1.54 ± 0.38, WT 1.54 ± 0.38; layers V/VI ratios: MMP-9 KO 1.65 ± 0.16, WT 1.50 ± 0.43).

c-Fos Immunohistochemistry

One Week and 4 Weeks of Deprivation

We used c-Fos immunohistochemistry to assess restoration of sensory-driven activity in deprived regions because its basal level is low and upon sensory stimulation it is heavily expressed in the barrel cortex, being restricted in layer IV to barrels with active sensory input (Staiger et al. 2000; Bisler et al. 2002). We counted c-Fos positive nuclei within spared rows C and neighboring deprived rows B and D in experimental and control hemispheres. Results obtained for rows B and D were averaged.

In both groups of animals, deprived for a week and deprived for 4 weeks, c-Fos labeling in control hemispheres was similar ($P > 0.05$). In our experimental design, the whiskers contralateral to control hemisphere were plucked except for row C 24 h before placing animal in enriched environment to avoid expression of c-Fos induced by whisker plucking on a day of the experiment. Vibrissal columns B and D, deprived for 24 h, showed similar c-Fos labeling in both experimental groups (layers II/III: 124 ± 28 cells in 0.1 mm^2—1 week, 146 ± 32—4 weeks; layer IV: 99 ± 18—1 week, 132 ± 30—4 weeks; layer Vb: 134 ± 19—1 week, 141 ± 25—4 weeks). So did the C columns, with spared sensory input (layers II/III: 207 ± 33—1 week, 250 ± 60—4 weeks; layer IV: 384 ± 18—1 week, 412 ± 30—4 weeks; layer Vb: 214 ± 15—1 week, 197 ± 33—4 weeks). The difference of labeling of deprived and spared rows in the control hemisphere shows the magnitude of c-Fos activation by stimulation in the enriched environment.

c-Fos labeling increased in deprived barrels B and D after 1 week of deprivation (Fig. 3). In layer IV, difference between

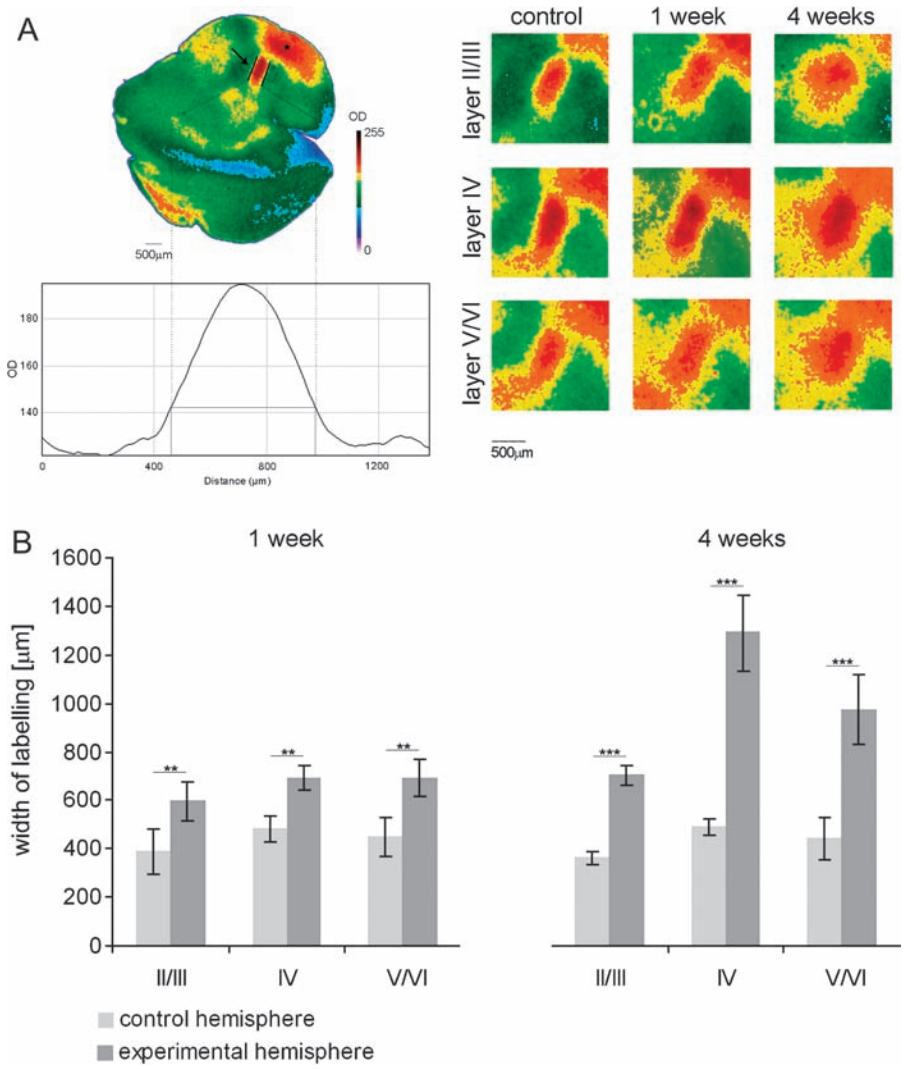

Figure 1. (*A*) Left panel: a typical pseudocolored autoradiogram of tangential section through layer IV, showing 2-DG incorporation during exploration of the enriched environment. The most active regions are represented in red, the less active in blue. All vibrissae except for the row C on the contralateral side were removed on a day preceding the experiment. The arrow indicates the functional representation of the spared row. Visual cortex strongly activated during exploration of enriched environment in the dark is marked by an asterisk. Drawing on the autoradiogram and the graph in lower left panel show where and how measurements of the width of labeling were taken. Right panel: high magnification of labeling of the spared row from the control hemisphere (deprived for 1 day) and experimental hemisphere (after 1 week or 4 weeks of deprivation). Exemplary autoradiograms are presented for all cortical layers. (*B*) Quantification of deprivation-induced changes in the width of the spared row C representation labeling in cortical layers II–III, IV, and V/VI. In the experimental hemisphere, deprivation lasted for 1 or 4 weeks and the control hemisphere was deprived on a day preceding the experiment. In all analyzed layers, the width of 2-DG labeling was always significantly greater in experimental hemispheres than in control hemispheres; mean ± standard deviation, ** $P < 0.01$, *** $P < 0.001$.

Table 1
Width of the spared row C representation labeling (µm) in MMP-9 KO and WT mice

		Layers II/III	Layer IV	Layers V/VI
MMP-9 KO	Control	446 ± 48	542 ± 50	470 ± 125
	Experimental	688 ± 185	914 ± 186	683 ± 175
WT	Control	469 ± 72	510 ± 57	432 ± 73
	Experimental	723 ± 226	1015 ± 161	703 ± 138

control and experimental hemisphere were especially apparent and reached 105.1% ($P < 0.01$, Fig. 4). In other layers, differences were smaller but still significant. In layers II/III, labeling increased by 39.9% ($P < 0.01$) and in layers V/VI by 42% ($P < 0.01$). In layers II/III, longer deprivation produced further increase in labeling density (174 ± 35 cells/0.1 mm^2 in the experimental hemisphere after 1 week of deprivation and 238 ± 27 after 4 weeks of deprivation, $P < 0.05$) up to 62.9% of control side. Duration of sensory deprivation also affected expression of c-Fos in layer IV, as after 4 weeks of deprivation it was higher than after 1 week of deprivation (303 ± 9 vs. 203 ± 19, $P < 0.001$) and reached 129.3% of the control side. Length of deprivation did not exert impact on labeling in deprived regions in layers V/VI (1 week 190 ± 35 vs. 195 ± 32, $P > 0.05$).

Interhemispherical comparisons of c-Fos labeling density in spared rows C in layers II/III demonstrated increased response to stimulation in experimental hemisphere after 1 week of deprivation (255 ± 29 vs. 207 ± 33, $P < 0.05$, Fig. 4). This effect was also observed after 4 weeks of deprivation (287 ± 46 vs. 250 ± 60, $P < 0.05$). However, there was no further increase in labeling density when compared with 1 week of deprivation ($P > 0.05$). In other layers, no differences in density of c-Fos expressing cells were observed in spared rows C in experimental

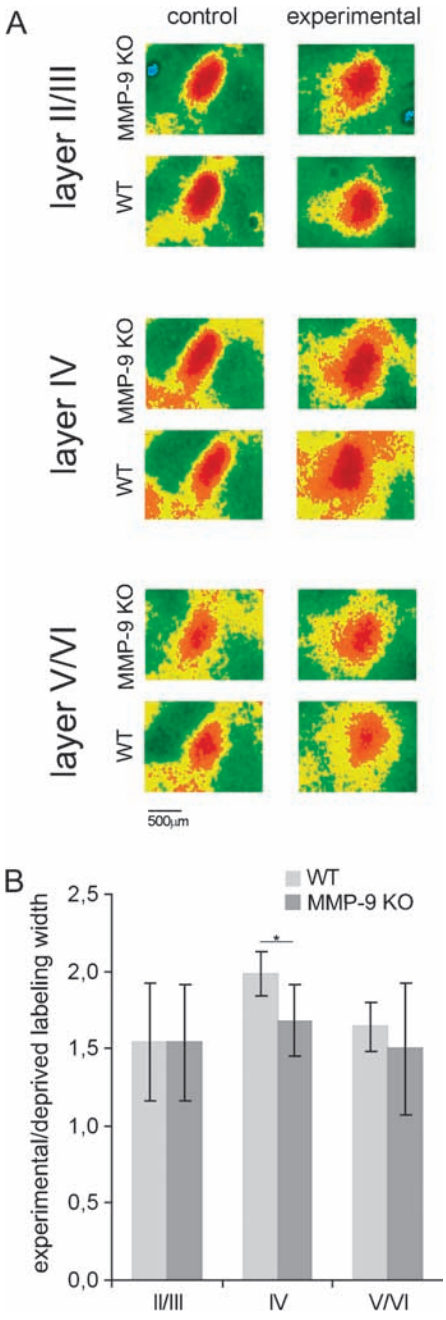

Figure 2. (*A*) Representative pseudocolored 2-DG autoradiograms of the spared row from MMP-9 KO mouse and its wild-type (WT) littermate deprived for 4 weeks. On the left autoradiograms from the control hemispheres (subjected to 1 day of deprivation) are shown. Autoradiograms on the right are from hemispheres deprived for 4 weeks. (*B*) Quantification of 2-DG labeling in MMP-9 KO mice. Ratios of the spared row representation width (experimental/control hemisphere) in MMP-9 KO mice and WT littermates deprived for 4 weeks. In MMP-9 KOs, deprivation-induced broadening of the spared row functional representation is smaller in layer IV. Mean ± standard deviation, $^*P < 0.05$.

hemisphere in comparison with control side, neither after 1 week (layer IV: 384 ± 18 vs. 402 ± 17, layer Vb 214 ± 15 vs. 244 ± 29) nor after 4 weeks of deprivation (layer IV: 412 ± 30 vs. 408 ± 25, layer Vb 197 ± 33 vs. 211 ± 32).

MMP-9 KO and WT Mice

MMP-9 KO and their WT littermates were subjected to deprivation procedure for 4 weeks. The only statistically significant difference concerned deprived rows B and D in layers II/III of experimental hemisphere, where in MMP-9 KO mice, c-Fos expression was less pronounced than in WT mice (MMP-9 KO 184 ± 8, WT 226 ± 20, $F_{11,48} = 41.6$, $P < 0.05$). In other cortical layers, labeling was comparable in deprived B and D columns and also in spared C columns ($P > 0.05$) for both groups of animals (Fig. 5).

Gel Zymography

One week of deprivation followed by 10 min of a novel environment exploration resulted in higher MMP-9 activity in the barrel cortex of the experimental hemisphere when compared with the control hemisphere as assessed with gel zymography ($P < 0.05$, Fig. 6). MMP-2 activity remained unaffected by sensory deprivation ($P > 0.05$).

Discussion

Partial deprivation of the vibrissal system of sensory input results in experience-dependent plasticity marked by pronounced expansion of cortical activation by the spared inputs, as measured by both 2-DG uptake and c-Fos immunochemistry. The greatest changes were observed with both 2-DG and c-Fos in cortical layer IV.

Enhancement of Response in the Spared Barrel Columns

We have observed an increase in density of cells expressing c-Fos in layer 2/3 within regions with spared sensory input following 7 and 28 days of deprivation. This result is consistent with electrophysiological data of Benedetti et al. (2009), who showed that removing of all but one whisker on one side of the snout results in the potentiation of stimulus-evoked firing in layer 2/3 neurons within the cortical representation of the spared whisker in adolescent mice after 7 days of deprivation.

We did not observe further increase in c-Fos expression after 28 days of deprivation, which suggests that enhancement of the spared whiskers input reaches its maximum level already after 7 days of deprivation. Possible mechanism for increase in density of immunolabeled cells in supragranular layers could involve strengthening of excitatory synapses between layer 4 and layers 2/3. This mechanism has, however, been demonstrated only in young mice after removing of all but one whisker (Clem et al. 2008). It may also reflect increased interactions between layer III neurons, as recently described by Finnerty (2011) (IBRO abstract) and increase in the number of axonal varicosities (Cheetham et al. 2008) following short-lasting deprivation.

With 2-DG autoradiography, we observed an increase in intensity of labeling within the row of barrels with spared inputs in layer IV after 1 week of deprivation. Potentiation of layer IV neurons responses in spared barrels was not observed with extracellular recordings in urethane anesthesia (Benedetti et al. 2009). As with the 2-DG method, a significant proportion of the label is ascribed to synaptic activity (Schwartz et al. 1979; Nudo and Masterton 1986), the signal in layer IV may originate from thalamocortical axons (Lübke et al. 2003; Meyer et al. 2010), and interbarrel connections or from axons of layer IV making synapses on basal dendrites of layers II/III neurons (Meyer et al. 2010). It can be presumed that axonal terminals innervating neurons in layers II/III in the same barrel column are remodeled (and therefore their metabolic requirements are higher), which leads to increase in number of activated neurons in layers II/III (as measured with c-Fos expression).

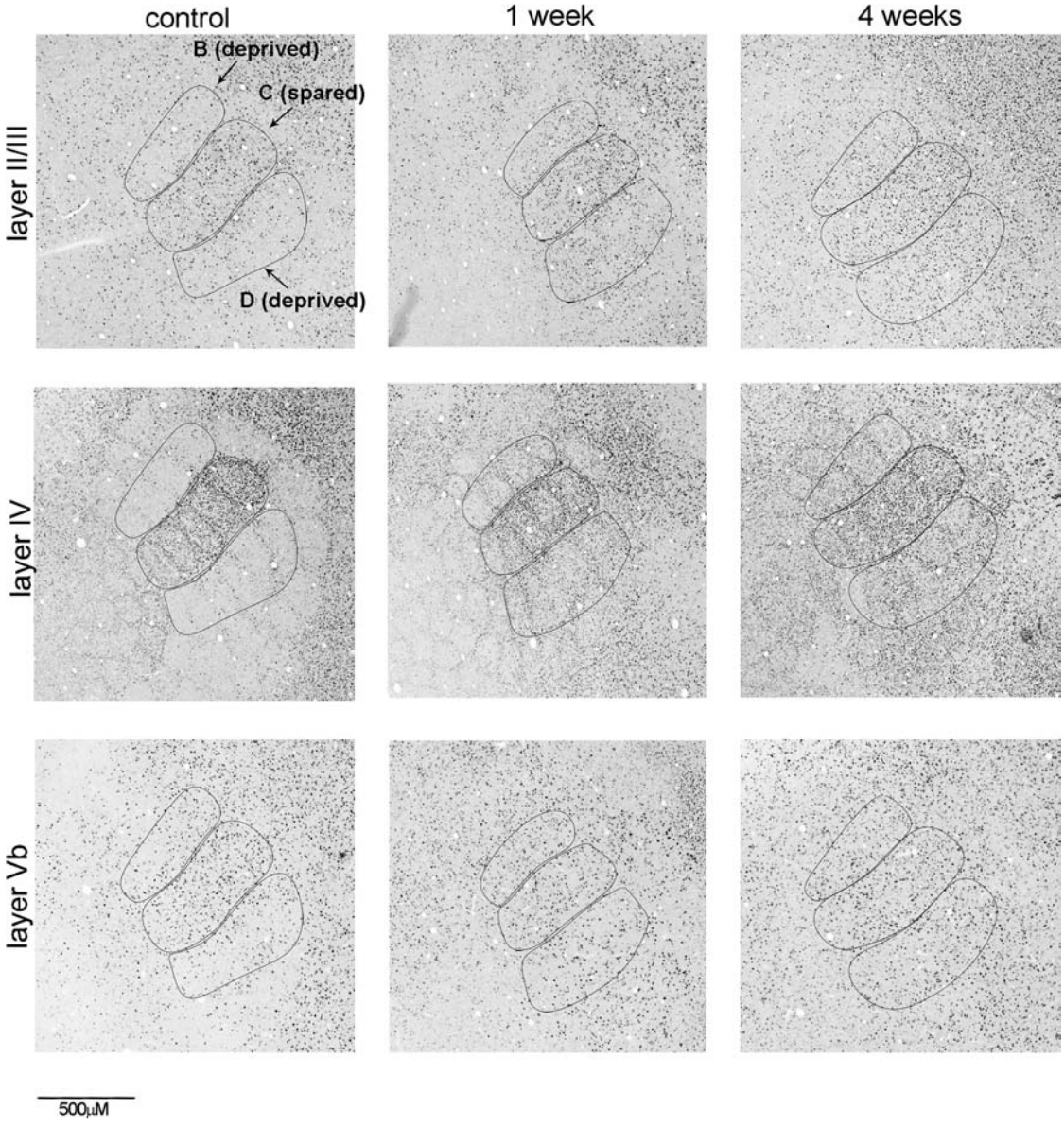

Figure 3. Photographs of c-Fos immunolabeling in the barrel cortex; representative examples for all analyzed layers in control hemisphere (deprived for 1 day) and experimental hemispheres (deprived for 1 or 4 weeks). Rows C (with spared inputs), B, and D (deprived) are outlined in every tangential section.

From this point of view, 2-DG signal would reflect ability of neurons to transmit excitation and c-Fos expression would denote responsiveness of cells upon stimulation, not only the spiking activity but also increased subthreshold depolarizations.

After longer deprivation, we observed an increase of 2-DG signal in all cortical layers within columns with spared inputs, which was not always accompanied by increase in c-Fos expression. It can be hypothesized that the already existing connections within spared barrels are strengthened (which explains higher 2-DG uptake), but new connections are not formed (and number of cells activated remains the same).

Enhancement of Response in Deprived Barrel Columns

Sensory deprivation leads to remapping of cortical topography. Processes leading to reorganization start immediately following deprivation. It has been recently shown in vivo imaging studies that massive axonal remodeling in the barrel cortex starts as early as few hours after deprivation (Marik et al. 2010). Representation of nondeprived sensory input expands into deprived regions, and new sensory maps are eventually formed. Using 2-DG autoradiography and c-Fos immunostaining, we were able to observe recruitment of deprived areas into functional representation of spared row of whiskers after 7 days of deprivation in all cortical layers. The time course of deprivation-induced changes depends on the pattern of deprivation, that is, local interactions between activated and nonactivated cortical columns (Fox 2002). Using SWE paradigm (single-whisker experiment, removal of all whiskers except one) in adolescent rats, Glazewski and Fox (1996) observed increase in response magnitude of cells in deprived barrel columns neighboring with nondeprived barrel upon spared vibrissa stimulation after 18 days of deprivation but not after shorter periods of deprivation. Sparing more than one vibrissa

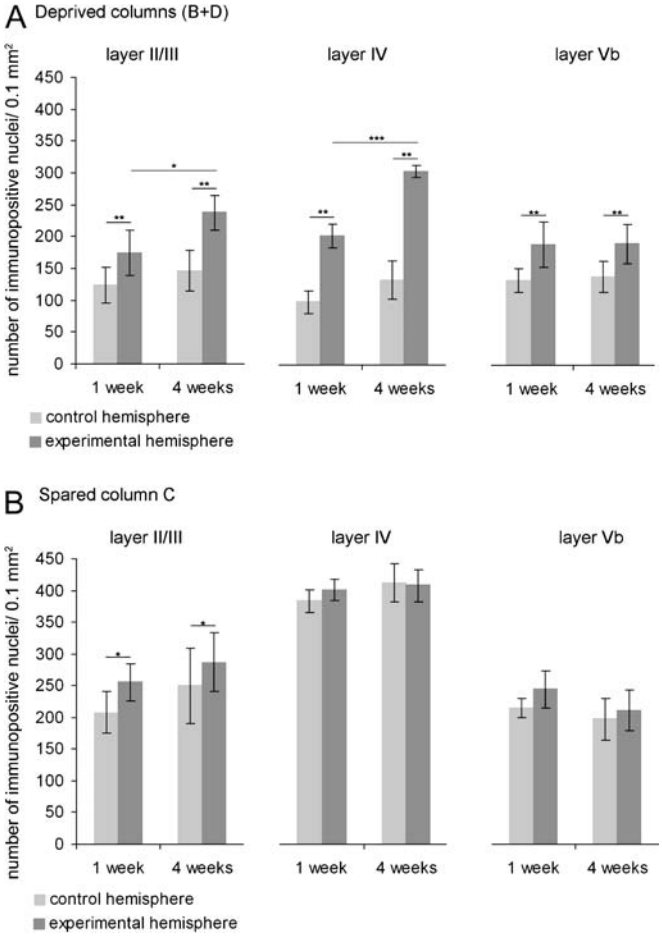

Figure 4. Quantification of c-Fos immunolabeling. (*A*) Density of c-Fos immunopositive nuclei in deprived regions (averaged rows B and D). (*B*) Density of c-Fos immunopositive nuclei in nondeprived regions (rows C). Control hemisphere corresponds to the side of the snout deprived for 1 day and experimental hemisphere to the side of the snout deprived for 1 or 4 weeks. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. In deprived columns B and D, deprivation induced an increase in the number of cells expressing c-Fos. Note that the effect was strongly pronounced in layer IV, especially after 4 weeks of deprivation. In the spared columns C, deprivation induced enhancement of c-Fos expression in layers II/III.

facilitates and accelerates potentiation in adjacent deprived regions, which was demonstrated after 7 days (in layers II/III and IV) using chessboard pattern of deprivation (Wallace and Fox 1999). In mice with the row C spared and other vibrissae deprived, for 7 days, in a situation when the animal is immobilized and spared whiskers are passively stimulated, functional representation of the spared row invades deprived neighboring barrels (Liguz-Lecznar et al. 2011). Whisker pairing results in redistribution of cortical activity and expansion of the representation of the 2 intact vibrissae into the neighboring barrel columns after 3.5 days (Lebedev et al. 2000). So far, the data concerning plastic changes induced in the murine barrel cortex by sensory deprivation have been acquired using anesthetized or at least immobilized animals. Here, we performed experiments in freely moving animals and mapped activity in the barrel cortex of deprived mice during their natural exploratory behavior. Our results from conscious and unrestrained mice show that indeed response to spared inputs potentiates over the first week of deprivation in all cortical layers. This confirms the results of 2-DG study by Maier et al. (2003) in freely moving young adult hamsters.

It is recognized that changes evoked by deprivation in supragranular layers come before those observed in layer IV (Diamond et al. 1994). When compared with postnatal animals, in young adult mice plasticity decreases most in layer IV and no longer shows anatomical plasticity nor LTP nor experience-dependent plasticity with the single spared whisker paradigm (Fox 1992; Fox et al. 1996). In the current experiments, broadening of functional representation of the spared row as seen with 2-DG was comparable for all cortical layers after 7 days of deprivation and strikingly greater in layer IV in comparison with other layers after 28 days of deprivation. After 28 days, functional representation of the spared row in each layer was wider than after 7 days of deprivation, but it looks like plasticity "accelerated" in layer IV or "slowed down" in other layers between 7th and 28th day of deprivation. Even if threshold for inducing plasticity in layer 4 is higher than for the other layers, if once started, plasticity seems to be even greater than in other layers. Layer IV in young adult mice is amenable to changes of synaptic density driven by increased sensory input (Knott et al. 2002) and sensory conditioning (Siucinska and Kossut 1996; Jasinska et al. 2010). Also, it responds to peripheral denervation by a decrease of γ-aminobutyric $acid_a$ receptor binding (Skangiel-Kramska et al. 1994) and transient increase of GAD67 mRNA and decrease of GluR2 mRNA expression (Gierdalski et al. 1999).

It has been recently shown that deprivation of vibrissae causes retraction of thalamocortical axons of the deprived input and a decrease in the number of thalamocortical synapses (Wimmer et al. 2010). No increase of thalamocortical axons linked to spared inputs was observed. However, an in vivo imaging study of intracortical axons showed that sprouting starts over the first few hours after deprivation (Marik et al. 2010). We can hypothesize that axonal sprouting is a kind of compensatory process, and the bigger the loss, the bigger compensation is needed. Sensory input coming via thalamocortical fibers consists 14–17% of the synapses in layer IV, a fraction greater than in other layers (Meyer et al. 2010). After deprivation, layer IV may need more activity from other inputs. It would explain higher metabolic plasticity of deprived layer IV in comparison with other layers. The substrate for plastic changes, widespread axonal connection in layers II/III and V, are well described in mouse barrel field (Bernardo et al. 1990; McCasland et al. 1992; Kossut and Juliano 1999; Frostig et al. 2008). A recent paper by Li et al. (2010) gives, with quantitative connectional mapping, a very good illustration of the sprouting potential of mouse somatosensory axons. Although the extracellular recordings by Glazewski et al. (2007) in spared vibrissa preparation of mice did not register changes in response strength of layer IV neurons in the deprived barrels following 7 days of deprivation, it has to be remembered that the 2-DG uptake is both neuronal and glial and reflects subthreshold activity as well as action potentials (Pellerin and Magistretti 1994; Attwell and Laughlin 2001).

Plasticity in MMP-9 Knockout Mice

We have found changes in the MMP-9 activity to be associated with the barrel cortex plasticity, as already reported for other systems (Nagy et al. 2006; Okulski et al. 2007; Wilczynski et al. 2008; Spolidoro et al. 2011). A significantly higher activity was found in the barrel cortex where only one row received normal afferentation, and the plastic reorganization was well

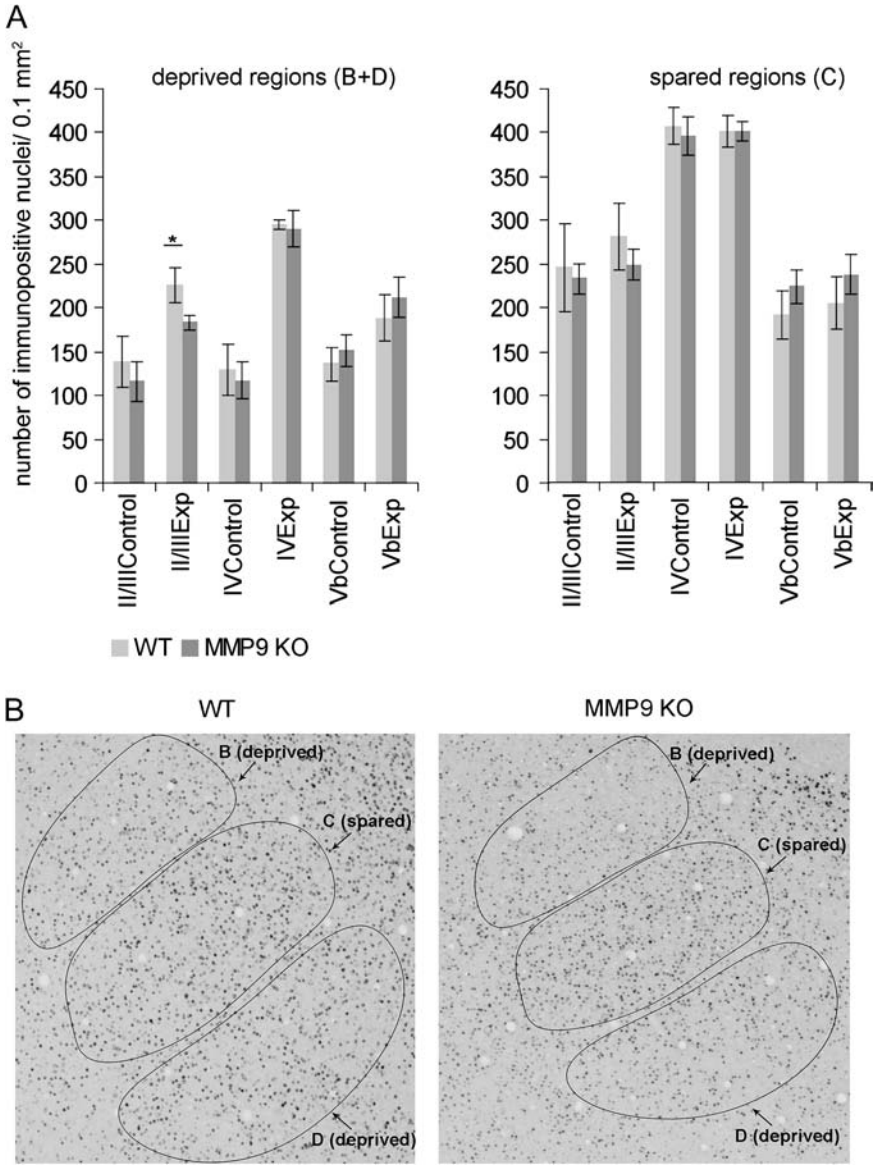

Figure 5. (*A*) Density of c-Fos immunopositive cells in MMP-9 KO and wild-type (WT) mice. In MMP-9 KO mice in layers II/III, there are less immunopositive cells in the experimental hemisphere in regions deprived for 4 weeks (rows B and D). Mean ± standard deviation, $^*P < 0.05$. (*B*) Photographs of c-Fos immunolabeling in layer II/III in WT and MMP-9 KO mice; representative examples for experimental hemispheres (deprived for 4 weeks). Rows C (with spared inputs), B, and D (deprived) are outlined. In MMP-9 KO mice, in layers II/III, there are less immunopositive cells in regions deprived for 4 weeks (rows B and D).

developed. This prompted us to investigate a possible role of this enzyme in the plastic changes observed. Toward this, the MMP-9 KO mice were employed.

We compared effect of deprivation on the spared row functional representation and c-Fos expression in MMP-9 knockout mice and their WT littermates. We chose longer deprivation, that is, 4 weeks for these experiments as changes observed after 4 weeks are more pronounced than after 1 week, so potential differences between WT and knockout mice should be easier to detect. In 2-DG study, we found that broadening of the spared row C representation in layer IV was smaller in case of knockout animals. In c-Fos experiment, in knockout mice, the density of c-Fos immunopositive cells was decreased in layers II/III in deprived rows B and D in the experimental hemisphere in comparison with WT animals.

What are possible reasons of laminar discrepancy between 2-DG and c-Fos? c-Fos immunostaining visualizes nuclei of activated cells, while with the 2-DG autoradiography primarily synapses are labeled, as they represent the major sites of enhanced glucose utilization during increased neuronal activation (Schwartz et al. 1979; Nudo and Masterton 1986). In murine barrel cortex, spiny neurons of the layer IV have vertically oriented axons that target preferentially layer III pyramidal cells (Bureau et al. 2006). Basal dendrites of layer III neurons reach down to layer IV (Lübke et al. 2003; Meyer et al. 2010), so 2-DG signal observed in layer IV partially reflects synaptic activity between layer IV and layer III neurons. In the MMP-9 knockout mice, broadening of functional representation of the spared row of whiskers over adjacent rows of barrels is smaller, so we can suppose that in the deprived columns, synaptic activity between layer IV and layer III neurons is lower. Consequently, there are fewer activated (c-Fos+) cells in the supragranular layers. It should also be taken into consideration that reduced 2-DG signal in layer IV in

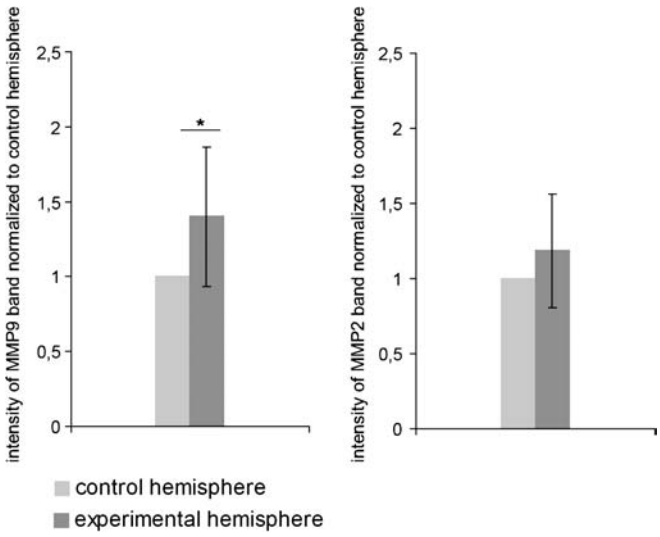

Figure 6. Quantification of MMP-9 and MMP-2 activity in barrel cortices isolated from mice deprived for 1 week and put for 10 min into enriched environment. Intensity of zymogram bands from experimental hemisphere was normalized to control hemisphere. MMP-9 activity is higher in the experimental hemisphere (deprived for 1 week) than in the control hemisphere. Mean ± SD, $*P < 0.05$.

knockout mice could be secondary to reduced activity of layer II/III cells. Schubert et al. (2003) observed that there are synaptic inputs onto pyramidal neurons in layer IV originating in layer III. Reduced activation of layers II/III cells (as assessed with c-Fos immunohistochemistry) could then result in reduced synaptic activation in layer IV.

Reduction of plastic changes in MMP-9 KO mice was not observed in all cortical layers, neither in 2-DG study nor in c-Fos experiment. It indicates that MMP-9 is not a ubiquitous player in plasticity. This has already been described by Nagy et al. (2006), who showed that MMP-9 KO mice are impaired in hippocampal-dependent associative learning but not in hippocampus-independent tasks.

What are Possible Reasons of Reduced Plastic Changes in MMP-9 Knockout Mice?

Functional reorganization of adult somatosensory cortex depends on NMDA receptors (Kano and Iino 1991). Partial blocking of NMDAR suppresses deprivation-induced plasticity in the barrel cortex (Jablonska et al. 1995; Rema et al. 1998). MMP-9 is involved in NMDAR-dependent synaptic plasticity (Nagy et al. 2006; Bozdagi et al. 2007). In cultures of hippocampal neurons, MMP-9 enzymatic activity increases NR1-NMDAR surface diffusion (Michaluk et al. 2009). Possible explanation for reduction of plasticity observed in MMP-9 knockout mice could be then impairment of NMDAR trafficking.

A few hours after sensory deprivation processes of axonal reorganization begin (Marik et al. 2010). MMPs play role in axon guidance, which is attributed to their ability to degrade extracellular matrix and clearing a passage for a growing axon (Dodd and Jessell 1988; Romanic and Madri 1994; Seeds et al. 1997). The MMPs were reported to participate in the controlled proteolytic cascades that remove old synaptic structures during remodeling (Vaillant et al. 1999). After injury, MMP-9 promotes nerve growth factor–induced neurite elongation (Shubayev and Myers 2004). Axonal elongation could be affected in MMP-9 knockout mice, leading to limited remodeling of cortical circuitry after deprivation.

Brain plasticity can be modulated by growth factors (Arancio and Chao 2007), produced within the CNS, and also those delivered to the brain from the circulation. IGF-1 increases branching and total extent of apical and basal dendrites of pyramidal cells in organotypic slices of rat primary somatosensory cortex (Niblock et al. 2000). It has been recently reported that IFG-1 uptake in the somatosensory cortex is elevated following whisker stimulation. This process is mediated by MMP-9, which cleaves IGF binding protein-3 allowing the passage of serum IGF-1 into the CNS (Nishijima et al. 2010). Diminished IGF-1 uptake in MMP-9 knockout mice may impair plastic reorganization after deprivation.

Our experiment demonstrates that in response to enriched environment, neurons in the barrel field undergoing reorganization show higher MMP-9 activity than neurons in the control hemisphere of the same animal. Thus, plasticity inducing experimental situation is linked to the increased activity of MMP-9.

Conclusions

In experience-dependent plasticity induced by deprivation of selected vibrissae, the spared input progressively activates larger extents of the barrel cortex. The broadening of functional representation of the spared row as seen with 2-DG is comparable for all cortical layers after short-lasting deprivation and greater in layer IV after long deprivation. We suggest that the characteristic strong involvement of layer IV is due to depriving this layer of a considerable part of its normal input (information coming via thalamocortical fibers) and compensatory rewiring by active inputs from other layers. Both methods, c-Fos immunohistochemistry and 2-DG mapping, similarly visualize changes in regions deprived of sensory input and undergoing large-scale reorganization. A laminar dissociation of c-Fos and 2-DG results within columns with spared inputs may be due to preferential labeling of different cellular compartments by the 2 methods.

In MMP-9 knockout mice, we observed laminar dissociation of c-Fos expression and 2-DG uptake within deprived cortical columns. It was demonstrated that MMP-9 activity drives dendritic spine remodeling and enlargement (Wang et al. 2008; Michaluk et al. 2011). In the absence of MMP-9, maturation of spines could be impaired and thus transmission of the signal could be diminished. A reduction of 2-DG labeling in the layer IV of MMP9 KO mice indicates that transmission of information to layers II/III is less effective. Consequently, c-Fos activation in layers II/III is decreased. Hence, our data support a possible role of the MMP-9 in experience-dependent plasticity of cortical vibrissal representation.

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Notes

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References

Arancio O, Chao MV. 2007. Neurotrophins, synaptic plasticity and dementia. Curr Opin Neurobiol. 17:325–330.

Attwell D, Laughlin SB. 2001. An energy budget for signaling in the grey matter of the brain. J Cereb Blood Flow Metab. 21:1133–1145.
Bender KJ, Rangel J, Feldman DE. 2003. Development of columnar topography in the excitatory layer 4 to layer 2/3 projection in rat barrel cortex. J Neurosci. 23:8759–8770.
Benedetti BL, Glazewski S, Barth AL. 2009. Reliable and precise neuronal firing during sensory plasticity in superficial layers of primary somatosensory cortex. J Neurosci. 29:11817–11827.
Bernardo KL, McCasland JS, Woolsey TA. 1990. Local axonal trajectories in mouse barrel cortex. Exp Brain Res. 82:247–253.
Bisler S, Schleicher A, Gass P, Stehle JH, Zilles K, Staiger JF. 2002. Expression of c-Fos, ICER, Krox-24 and JunB in the whisker-to-barrel pathway of rats: time course of induction upon whisker stimulation by tactile exploration of an enriched environment. J Chem Neuroanat. 23:187–198.
Bozdagi O, Nagy V, Kwei KT, Huntley GW. 2007. In vivo roles for matrix metalloproteinase-9 in mature hippocampal synaptic physiology and plasticity. J Neurophysiol. 98:334–344.
Bureau I, von Saint Paul F, Svoboda K. 2006. Interdigitated paralemniscal and lemniscal pathways in the mouse barrel cortex. PLoS Biol. 4:e382.
Cheetham CE, Hammond MS, Edwards CE, Finnerty GT. 2007. Sensory experience alters cortical connectivity and synaptic function site specifically. J Neurosci. 27:3456–3465.
Cheetham CE, Hammond MS, McFarlane R, Finnerty GT. 2008. Altered sensory experience induces targeted rewiring of local excitatory connections in mature neocortex. J Neurosci. 28:9249–9260.
Chmielowska J, Kossut M, Chmielowski M. 1986. Single vibrissal cortical column in the mouse labeled with 2-deoxyglucose. Exp Brain Res. 63:607–619.
Clem RL, Celikel T, Barth AL. 2008. Ongoing in vivo experience triggers synaptic metaplasticity in the neocortex. Science. 319:101–104.
De Paola V, Holtmaat A, Knott G, Song S, Wilbrecht L, Caroni P, Svoboda K. 2006. Cell type-specific structural plasticity of axonal branches and boutons in the adult neocortex. Neuron. 49:861–875.
Diamond ME, Huang W, Ebner FF. 1994. Laminar comparison of somatosensory cortical plasticity. Science. 265:1885–1888.
Dodd J, Jessell TM. 1988. Axon guidance and the patterning of neuronal projections in vertebrates. Science. 242:692–699.
Duncan GE, Johnson KB, Breese GR. 1993. Topographic patterns of brain activity in response to swim stress: assessment by 2-deoxyglucose uptake and expression of Fos-like immunoreactivity. J Neurosci. 13:3932–3943.
Duncan GE, Moy SS, Knapp DJ, Mueller RA, Breese GR. 1998. Metabolic mapping of the rat brain after subanesthetic doses of ketamine: potential relevance to schizophrenia. Brain Res. 787:181–190.
Eells JB, Clough RW, Miller JW, Jobe PC, Browning RA. 2000. Fos expression and 2-deoxyglucose uptake following seizures in developing genetically epilepsy-prone rats. Brain Res Bull. 52:379–389.
Filipkowski RK, Rydz M, Berdel B, Morys J, Kaczmarek L. 2000. Tactile experience induces c-fos expression in rat barrel cortex. Learn Mem. 7:116–122.
Finnerty GT. 2011. Neocortical rewiring and memory storage. Workshop W14.1 at 8th IBRO World Congress of Neuroscience Florence; July 14–18.
Fox K. 1992. A critical period for experience-dependent synaptic plasticity in rat barrel cortex. J Neurosci. 12:1826–1838.
Fox K. 2002. Anatomical pathways and molecular mechanisms for plasticity in the barrel cortex. Neuroscience. 111:799–814.
Fox K, Glazewski S, Chen CM, Silva A, Li X. 1996. Mechanisms underlying experience-dependent potentiation and depression of vibrissae responses in barrel cortex. J Physiol Paris. 90:263–269.
Frostig RD, Xiong Y, Chen-Bee CH, Kvasnak E, Stehberg J. 2008. Large-scale organization of rat sensorimotor cortex based on a motif of large activation spreads. J Neurosci. 28:13274–13284.
Gierdalski M, Jablonska B, Smith A, Skangiel-Kramska J, Kossut M. 1999. Deafferentation induced changes in GAD67 and GluR2 mRNA expression in mouse somatosensory cortex. Brain Res Mol Brain Res. 71:111–119.
Glazewski S, Benedetti BL, Barth AL. 2007. Ipsilateral whiskers suppress experience-dependent plasticity in the barrel cortex. J Neurosci. 27:3910–3920.
Glazewski S, Fox K. 1996. Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats. J Neurophysiol. 75:1714–1729.
Herdegen T, Leah JD. 1998. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res Brain Res Rev. 28:370–490.
Herrera DG, Robertson HA. 1996. Activation of c-fos in the brain. Prog Neurobiol. 50:83–107.
Hickmott PW, Steen PA. 2005. Large-scale changes in dendritic structure during reorganization of adult somatosensory cortex. Nat Neurosci. 8:140–142.
Holtmaat A, De Paola V, Wilbrecht L, Knott GW. 2008. Imaging of experience-dependent structural plasticity in the mouse neocortex in vivo. Behav Brain Res. 192:20–25.
Holtmaat A, Wilbrecht L, Knott GW, Welker E, Svoboda K. 2006. Experience-dependent and cell-type-specific spine growth in the neocortex. Nature. 441:979–983.
Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, Svoboda K. 2005. Transient and persistent dendritic spines in the neocortex in vivo. Neuron. 45:279–291.
Jablonska B, Gierdalski M, Siucinska E, Skangiel-Kramska J, Kossut M. 1995. Partial blocking of NMDA receptors restricts plastic changes in adult mouse barrel cortex. Behav Brain Res. 66:207–216.
Jasinska M, Siucinska E, Cybulska-Klosowicz A, Pyza E, Furness DN, Kossut M, Glazewski S. 2010. Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. J Neurosci. 30:1176–1184.
Kaczmarek L, Lapinska-Dzwonek J, Szymczak S. 2002. Matrix metalloproteinases in the adult brain physiology: a link between c-Fos, AP-1 and remodeling of neuronal connections? EMBO J. 21:6643–6648.
Kano M, Iino K. 1991. Functional reorganization of adult cat somatosensory cortex is dependent on NMDA receptors. Neuroreport. 2:77–80.
Knott GW, Holtmaat A, Wilbrecht L, Welker E, Svoboda K. 2006. Spine growth precedes synapse formation in the adult neocortex in vivo. Nat Neurosci. 9:1117–1124.
Knott GW, Quairiaux C, Genoud C, Welker E. 2002. Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. Neuron. 34:265–273.
Kossut M, Hand P. 1984. The development of the vibrissal cortical column: a 2-deoxyglucose study in the rat. Neurosci Lett. 46:1–6.
Kossut M, Hand PJ, Greenberg J, Hand CL. 1988. Single vibrissal cortical column in SI cortex of rat and its alterations in neonatal and adult vibrissa-deafferented animals: a quantitative 2DG study. J Neurophysiol. 60:829–852.
Kossut M, Juliano SL. 1999. Anatomical correlates of representational map reorganization induced by partial vibrissectomy in the barrel cortex of adult mice. Neuroscience. 92:807–817.
Lebedev MA, Mirabella G, Erchova I, Diamond ME. 2000. Experience-dependent plasticity of rat barrel cortex: redistribution of activity across barrel-columns. Cereb Cortex. 10:23–31.
Li S, Overman JJ, Katsman D, Kozlov SV, Donnelly CJ, Twiss JL, Giger RJ, Coppola G, Geschwind DH, Carmichael ST. 2010. An age-related sprouting transcriptome provides molecular control of axonal sprouting after stroke. Nat Neurosci. 13:1496–1504.
Liguz-Lecznar M, Siucinska E, Zakrzewska R, Kossut M. 2011. Impairment of experience-dependent cortical plasticity in aged mice. Neurobiol Aging. 32:1896–1905.
Lübke J, Roth A, Feldmeyer D, Sakmann B. 2003. Morphometric analysis of the columnar innervation domain of neurons connecting layer 4 and layer 2/3 of juvenile rat barrel cortex. Cereb Cortex. 13:1051–1063.
Maier DL, Grieb GM, Stelzner DJ, McCasland JS. 2003. Large-scale plasticity in barrel cortex following repeated whisker trimming in young adult hamsters. Exp Neurol. 184:737–745.
Marik SA, Yamahachi H, McManus JN, Szabo G, Gilbert CD. 2010. Axonal dynamics of excitatory and inhibitory neurons in somatosensory cortex. PLoS Biol. 8:e1000395.
McCasland JS, Bernardo KL, Probst KL, Woolsey TA. 1992. Cortical local circuit axons do not mature after early deafferentation. Proc Natl Acad Sci U S A. 89:1832–1836.

McCown TJ, Duncan GE, Johnson KB, Breese GR. 1995. Metabolic and functional mapping of the neural network subserving inferior collicular seizure generalization. Brain Res. 701:117–128.
Meighan SE, Meighan PC, Choudhury P, Davis CJ, Olson ML, Zornes PA, Wright JW, Harding JW. 2006. Effects of extracellular matrix-degrading proteases matrix metalloproteinases 3 and 9 on spatial learning and synaptic plasticity. J Neurochem. 96:1227–1241.
Meyer HS, Wimmer VC, Oberlaender M, de Kock CP, Sakmann B, Helmstaedter M. 2010. Number and laminar distribution of neurons in a thalamocortical projection column of rat vibrissal cortex. Cereb Cortex. 20:2277–2286.
Michaluk P, Mikasova L, Groc L, Frischknecht R, Choquet D, Kaczmarek L. 2009. Matrix metalloproteinase-9 controls NMDA receptor surface diffusion through integrin beta1 signaling. J Neurosci. 29:6007–6012.
Michaluk P, Wawrzyniak M, Alot P, Szczot M, Wyrembek P, Mercik K, Medvedev N, Wilczek E, DeRoo M, Zuschratter W, et al. 2011. Influence of matrix metalloproteinase, MMP-9 on dendritic spine morphology. J Cell Sci. 124:3369–3380.
Montag-Sallaz M, Baarke A, Montag D. 2003. Aberrant neuronal connectivity in CHL1-deficient mice is associated with altered information processing-related immediate early gene expression. J Neurobiol. 57:67–80.
Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, Costa RM, Silva AJ, Kaczmarek L, Huntley GW. 2006. Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci. 26:1923–1934.
Niblock MM, Brunso-Bechtold JK, Riddle DR. 2000. Insulin-like growth factor I stimulates dendritic growth in primary somatosensory cortex. J Neurosci. 20:4165–4176.
Nishijima T, Piriz J, Duflot S, Fernandez AM, Gaitan G, Gomez-Pinedo U, Verdugo JM, Leroy F, Soya H, Nunez A, et al. 2010. Neuronal activity drives localized blood-brain-barrier transport of serum insulin-like growth factor-I into the CNS. Neuron. 67:834–846.
Nudo RJ, Masterton RB. 1986. Stimulation-induced [14C]2-deoxyglucose labeling of synaptic activity in the central auditory system. J Comp Neurol. 245:553–565.
Okulski P, Jay TM, Jaworski J, Duniec K, Dzwonek J, Konopacki FA, Wilczynski GM, Sanchez-Capelo A, Mallet J, Kaczmarek L. 2007. TIMP-1 abolishes MMP-9-dependent long-lasting long-term potentiation in the prefrontal cortex. Biol Psychiatry. 62:359–362.
Pellerin L, Magistretti PJ. 1994. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A. 91:10625–10629.
Rema V, Armstrong-James M, Ebner FF. 1998. Experience-dependent plasticity of adult rat S1 cortex requires local NMDA receptor activation. J Neurosci. 18:10196–10206.
Romanic AM, Madri JA. 1994. Extracellular matrix-degrading proteinases in the nervous system. Brain Pathol. 4:145–156.
Schubert D, Kotter R, Zilles K, Luhmann HJ, Staiger JF. 2003. Cell type-specific circuits of cortical layer IV spiny neurons. J Neurosci. 23:2961–2970.
Schwartz WJ, Smith CB, Davidsen L, Savaki H, Sokoloff L, Mata M, Fink DJ, Gainer H. 1979. Metabolic mapping of functional activity in the hypothalamo-neurohypophysial system of the rat. Science. 205:723–725.
Seeds NW, Siconolfi LB, Haffke SP. 1997. Neuronal extracellular proteases facilitate cell migration, axonal growth, and pathfinding. Cell Tissue Res. 290:367–370.
Shubayev VI, Myers RR. 2004. Matrix metalloproteinase-9 promotes nerve growth factor-induced neurite elongation but not new sprout formation in vitro. J Neurosci Res. 77:229–239.
Siucinska E, Kossut M. 1996. Short-lasting classical conditioning induces reversible changes of representational maps of vibrissae in mouse SI cortex–a 2DG study. Cereb Cortex. 6:506–513.
Skangiel-Kramska J, Głazewski S, Jabłońska B, Siucińska E, Kossut M. 1994. Reduction of GABAA receptor binding of [3H]muscimol in the barrel field of mice after peripheral denervation: transient and long-lasting effects. Exp Brain Res. 100:39–46.
Spolidoro M, Putignano E, Munafo C, Maffei L, Pizzorusso T. 2011. Inhibition of matrix metalloproteinases prevents the potentiation of nondeprived-eye responses after monocular deprivation in juvenile rats. Cereb Cortex. doi: 10.1093/cercor/bhr158.
Staiger JF, Bisler S, Schleicher A, Gass P, Stehle JH, Zilles K. 2000. Exploration of a novel environment leads to the expression of inducible transcription factors in barrel-related columns. Neuroscience. 99:7–16.
Strominger RN, Woolsey TA. 1987. Templates for locating the whisker area in fresh flattened mouse and rat cortex. J Neurosci Methods. 22:113–118.
Tailby C, Wright LL, Metha AB, Calford MB. 2005. Activity-dependent maintenance and growth of dendrites in adult cortex. Proc Natl Acad Sci U S A. 102:4631–4636.
Vaillant C, Didier-Bazes M, Hutter A, Belin MF, Thomasset N. 1999. Spatiotemporal expression patterns of metalloproteinases and their inhibitors in the postnatal developing rat cerebellum. J Neurosci. 19:4994–5004.
Wallace H, Fox K. 1999. The effect of vibrissa deprivation pattern on the form of plasticity induced in rat barrel cortex. Somatosens Mot Res. 16:122–138.
Wang XB, Bozdagi O, Nikitczuk JS, Zhai ZW, Zhou Q, Huntley GW. 2008. Extracellular proteolysis by matrix metalloproteinase-9 drives dendritic spine enlargement and long-term potentiation coordinately. Proc Natl Acad Sci U S A. 105:19520–19525.
Wilczynski GM, Konopacki FA, Wilczek E, Lasiecka Z, Gorlewicz A, Michaluk P, Wawrzyniak M, Malinowska M, Okulski P, Kolodziej LR, et al. 2008. Important role of matrix metalloproteinase 9 in epileptogenesis. J Cell Biol. 180:1021–1035.
Wimmer VC, Broser PJ, Kuner T, Bruno RM. 2010. Experience-induced plasticity of thalamocortical axons in both juveniles and adults. J Comp Neurol. 518:4629–4648.
Wirtshafter D. 2005. Cholinergic involvement in the cortical and hippocampal Fos expression induced in the rat by placement in a novel environment. Brain Res. 1051:57–65.