Global impairment and therapeutic restoration of visual plasticity mechanisms after a localized cortical stroke

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We tested the influence of a photothrombotic lesion in somatosensory cortex on plasticity in the mouse visual system and the efficacy of anti-inflammatory treatment to rescue compromised learning. To challenge plasticity mechanisms, we induced monocular deprivation (MD) in 3-mo-old mice. In control animals, MD induced an increase of visual acuity of the open eye and an ocular dominance (OD) shift towards this eye. In contrast, after photothrombosis, there was neither an enhancement of visual acuity nor an OD-shift. However, OD-plasticity was present in the hemisphere contralateral to the lesion. Anti-inflammatory treatment restored sensory learning but not OD-plasticity, as did a 2-wk delay between photothrombosis and MD. We conclude that (*i*) both sensory learning and cortical plasticity are compromised in the surround of a cortical lesion; (*ii*) transient inflammation is responsible for impaired sensory learning, suggesting anti-inflammatory treatment as a useful adjuvant therapy to support rehabilitation following stroke; and (*iii*) OD-plasticity cannot be conceptualized solely as a local process because nonlocal influences are more important than previously assumed.

visual cortex | in vivo imaging | ocular dominance index | optometry | network

Learning and brain plasticity are most strongly required when the brain suffers from a sudden lesion, such as following a stroke: brain circuits need to be rearranged and compensatory mechanisms have to be established. It is therefore highly desirable to support and optimize this brain plasticity (1). A stroke not only kills some brain regions, it also disturbs the surrounding and remote—and to some extent even the contralateral—hemisphere, causing a transhemispheric diaschisis (2, 3). A number of studies demonstrated that focal brain injury alters the properties of the entire brain in many ways (4): GABAergic inhibition is decreased (5–7), neuronal activity is enhanced (5), and NMDA receptor binding is increased (8). The functional consequences of such changes are as yet not well understood: long-term potentiation (LTP), a cellular paradigm for learning, was increased in brain tissue surrounding a focal lesion (9, 10). In accordance with this, an early time window for stroke rehabilitation was observed: animals receiving early behavioral rehabilitative therapy recovered better than those in which the therapy started with a delay of several weeks (11). On the other hand, a decrease of whisker representation plasticity following stroke was recently observed (12). In the light of the finding that early forced activity might cause an enlargement of the original brain lesion (13, 14), there is an ongoing discussion in rehabilitation literature as to when treatment should commence (15, 16).

The mechanisms underlying stroke-induced changes in the plasticity of the structurally intact cortex are also not well understood. One likely candidate, which might modify brain plasticity following stroke, are inflammatory reactions: stroke initiates a cerebral inflammatory reaction. Antagonizing this inflammation might reduce lesion size (17, 18), although this has not been proved in patients yet. Furthermore, no systematic data are available on how an anti-inflammatory treatment regime might modify brain plasticity.

The present study was designed to investigate the impact of a cortical stroke and the efficacy of anti-inflammatory treatment on two different plasticity paradigms in mice: (*i*) the enhancement of visual acuity and contrast sensitivity (19), and (*ii*) ocular dominance (OD) plasticity. To probe plasticity mechanisms we used the monocular deprivation (MD) paradigm, a well-established model for experience-dependent plasticity in the visual system (20). Deprivation of vision in one eye in adult mice induces a "sensory learning" process: a use-driven improvement of visual acuity and contrast sensitivity through the nondeprived eye after daily testing in a virtual-reality optomotor setup (19, 21). In this setup, freely moving mice display reflexive head-tracking behavior induced by slow rotation of full-field vertical grating stimuli. By modulating both spatial frequency and contrast of these gratings, one can determine the animals' performance limits: after MD, the optomotor response through the open eye is enhanced by about 30% (19, 21), and this "sensory learning" is dependent on the cortex (19). We started MD either immediately or 1 to 2 wk after a focal cortical lesion induced by photothrombosis (PT) (22) in the neighboring somatosensory cortex. Ibuprofen-treatment was started directly after MD and continued for 7 d. In addition, MD causes classical OD plasticity, i.e., a shift in the OD of neurons in the binocular part of the visual cortex toward the open eye (23, 24). To assess OD-plasticity in both the lesioned and nonlesioned hemisphere, we visualized cortical activity maps using optical imaging of intrinsic signals (25, 26).

Our data showed that a small cortical lesion in the somatosensory cortex was sufficient to completely abolish the sensory learning. Anti-inflammatory treatment with ibuprofen restored improvement of both visual acuity and contrast sensitivity after MD to control values. In agreement with this finding, a delay of 2 wk between PT and MD also rescued sensory learning. OD-plasticity was also disrupted after PT. In contrast to sensory learning, however, neither anti-inflammatory treatment nor a delay between PT and MD restored OD-plasticity. Thus, inflammation was responsible for reductions in sensory learning but lesion-induced impairment of OD-plasticity was mediated by a different cellular mechanism, supporting the view that distinct

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mechanisms underlie sensory learning and classical OD-plasticity. In addition, our results indicate that OD-plasticity cannot be conceptualized solely as a local process: Apparently, changes in the activity of the major thalamo-cortical inputs were not sufficient to induce OD-plasticity so that nonlocal influences must play a much more important role for these learning phenomena than previously assumed. Although anti-inflammatory treatment only rescued plasticity in one of the studied designs, it might prove to be a useful adjuvant therapy to support rehabilitation following stroke.

Results

Localization of the PT Lesion. The PT-lesions were in the left hemisphere, 1.3 ± 0.06-mm anterior to the primary visual cortex (V1) (Fig. S1), measured 1.1 ± 0.06-mm medio-laterally, 0.9 ± 0.05-mm anterio-posteriorly, and were 1.8 ± 0.06-mm lateral to the midline (Fig. S1 *A* and *B*). The lesion center was located 0.85 ± 0.06-mm posterior to the bregma primarily in the hindlimb region (27). Notably, lesion size and location correlated with neither the enhancement of visual acuity nor the OD-index (ODI) (*SI Text, Data S1*). Moreover the size of the lesion was neither influenced by ibuprofen treatment nor by time (1, 2, or 3 wk after lesion induction, respectively; all $P > 0.05$, t test).

PT Abolished Enhancement of Vision After MD. Because Prusky et al. (19) showed that visual acuity improvement depends on the cortex contralateral to the open eye, while the ipsilateral cortex is necessary for the persistence of the effect, we separately tested the influence of an MD ipsi- and contralateral to the PT-lesion. MD was induced directly after PT. In the following 7 d, both visual acuity and contrast sensitivity were measured behaviorally.

MD right eye. In control animals, visual acuity was 0.38 ± 0.003 cycles per degree (cyc/deg; $n = 9$) for the left and 0.38 ± 0.003 cyc/deg for the right eye ($n = 9$). Because there was no significant difference between values of the two eyes ($P > 0.05$, t test, $n = 9$), we pooled values for further analyses. In PT-animals without MD, visual acuity was also not significantly different between left (0.37 ± 0.003 cyc/deg) and right (0.37 ± 0.003 cyc/deg) eyes ($P < 0.05$, t test, $n = 13$), and values were also pooled. In control animals, baseline visual acuity (without MD) was 0.38 ± 0.003 cyc/deg on day 1 and remained stable for 7 d ($P > 0.05$, t test, $n = 9$) (Fig. 1*A*). After MD, visual acuity of the open eye increased significantly from 0.39 ± 0.003 cyc/deg on day 1 to 0.46 ± 0.01 cyc/deg on day 7 ($P < 0.001$, t test, $n = 10$). The increase was significantly different from control animals without MD ($F_{1,17} = 110.61$, $P < 0.001$, ANOVA). In contrast, animals with a PT-lesion showed no significant enhancement of visual acuity after right eye MD (day 1: 0.38 ± 0.01 cyc/deg; day 7: 0.39 ± 0.01 cyc/deg; $P > 0.05$, t test, $n = 9$), although the hemisphere contralateral to the open (left) eye was not lesioned. In addition, visual acuities were not significantly different from PT-animals without MD (day 1: 0.37 ± 0.003 cyc/deg; day 7: 0.37 ± 0.003 cyc/deg; $n = 13$; $F_{1,20} = 5.23$, $P > 0.05$, ANOVA). Likewise, in PT-animals, in contrast to control animals, MD had no reinforcing effect on contrast sensitivity (*SI Text, Data S2* and Fig. S2).

MD left eye. In PT-animals, visual acuity improvement was also significantly reduced after left eye MD ($n = 10$) compared with control animals ($F_{1,18} = 60.71$, $P < 0.001$, ANOVA) and was indistinguishable from PT-animals with right eye MD ($F_{1,17} = 3.61$, $P > 0.05$, ANOVA). Similarly, enhancement of contrast sensitivity was also significantly diminished after left eye MD ($n = 10$) compared with control animals ($F_{1,18} = 56.96$, $P < 0.001$, ANOVA) and was indistinguishable from PT-animals with right eye MD ($F_{1,17} = 8.61$, $P > 0.05$, ANOVA).

Thus, irrespective of the site of MD, visual acuity and contrast-sensitivity enhancement were significantly reduced, suggesting a nonlocal, possibly brain-wide disturbance caused by the PT.

Ibuprofen Restored Enhancement of Vision After MD. Because stroke has been shown to be accompanied by inflammation processes in the brain (28), we next tested whether treatment with the cyclooxygenase-inhibitor ibuprofen might rescue diminished plasticity. In lesioned mice treated with ibuprofen ($n = 12$) during MD, visual acuity of the open eye indeed increased significantly (day 1: 0.40 ± 0.003 cyc/deg; day 7: 0.46 ± 0.01 cyc/deg; $P < 0.001$, t test) (Fig. 1*A*). This increase was indistinguishable from nonlesioned animals ($F_{1,20} = 0.50$, $P > 0.05$, ANOVA). In contrast, vehicle-injected PT-animals ($n = 6$) did not improve (day 1: 0.39 ± 0.005 cyc/deg; day 7: 0.40 ± 0.01 cyc/deg; $P > 0.05$, t test) and were indistinguishable from nontreated PT-mice ($F_{1,13} = 1.51$, $P > 0.05$, ANOVA). Ibuprofen treatment had no effect on the visual acuity of control mice (day 1: 0.38 ± 0.002 cyc/deg; day 7: 0.38 ± 0.002 cyc/deg; $P > 0.05$, t test, $n = 6$), which was indistinguishable from nontreated animals ($F_{1,13} = 0.39$, $P > 0.05$, ANOVA). Contrast sensitivity also increased significantly in ibuprofen-treated but not in vehicle-treated PT-animals (*SI Text, Data S3* and Table S1).

Hence, ibuprofen-treatment completely rescued the improvement of both visual acuity and contrast sensitivity of the open eye after MD in PT-animals.

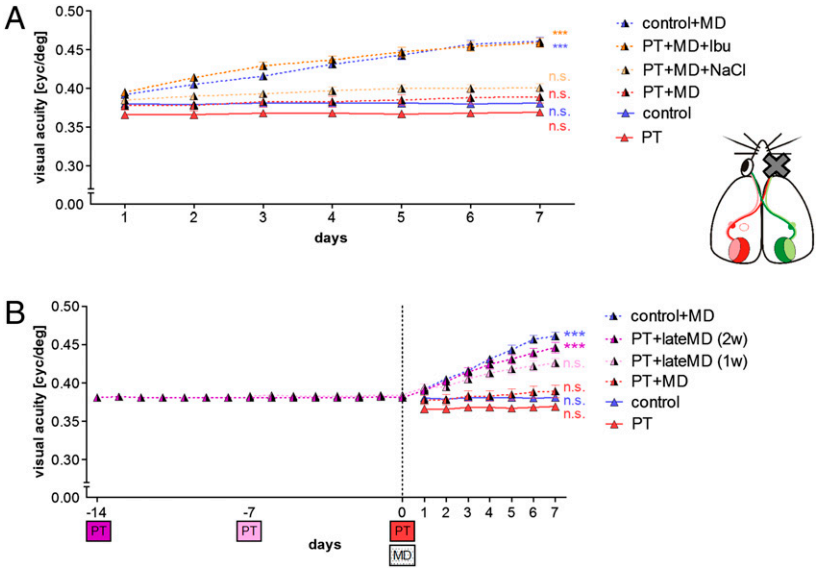

Fig. 1. A PT-lesion outside the visual cortex abolished visual acuity improvement after MD in mice. Both ibuprofen and late MD rescued this form of visual learning. (*A*) Spatial frequency selectivity of the optokinetic response in cycles per degree (cyc/deg) plotted against days after MD. In control animals, during 7 d of MD and daily testing (control+MD), visual acuity of the nondeprived (open) eye significantly increased. In contrast, in PT-animals, MD failed to induce improvement (PT+MD). Treatment with the anti-inflammatory drug ibuprofen (PT+MD+Ibu) but not vehicle (PT+MD+NaCl) rescued enhancement to control values (control+MD). (*B*) Spatial frequency selectivity in the "late MD" paradigm: MD-induction either 1 (1w) or 2 wk (2w) after PT. Note that after a 2-wk delay [PT+lateMD (2w)], visual acuity was significantly enhanced compared with animals with an MD directly after the lesion (PT+MD), and that values were rather indistinguishable from controls (control+MD).

Late MD Restored Enhancement of Vision After MD. To analyze whether the diminished plasticity after a PT-lesion was an acute effect or a longer-lasting modification of neuronal circuits, we next introduced a delay of 1 or 2 wk between the lesion and MD. When MD was started 1 wk after PT, visual acuity improved only slightly (day 1: 0.40 ± 0.01cyc/deg; day 7: 0.43 ± 0.01 cyc/deg; $P < 0.05$, t test, $n = 5$) (Fig. 1*B*), while the increase was significant after a 2-wk delay (day 1: 0.39 ± 0.00 cyc/deg; day 7: 0.45 ± 0.01 cyc/deg; $P < 0.001$, t test, $n = 11$) (Fig. 1*B*). This improvement was significantly different from mice with MD directly after the PT-lesion ($F_{1,18} = 19.46$, $P < 0.001$, ANOVA) and indistinguishable from control animals with MD ($F_{1,19} = 1.72$, $P > 0.05$, ANOVA). Contrast sensitivity also significantly increased in PT-animals deprived 2 wk but not 1 wk after the lesion (*SI Text, Data S4* and Table S2).

Thus, a delay of 2 wk between lesion induction and MD was sufficient to completely restore the enhancement of both visual acuity and contrast sensitivity, indicating that the reduced sensory learning was a transient phenomenon.

Because MD also induces classical OD-plasticity in the visual cortex, we next tested whether the PT-lesion in somatosensory cortex also influenced this plasticity paradigm.

OD-Plasticity Was Impaired in the Surround of a PT-Lesion. In control animals, visual stimulation of the contralateral eye induced stronger cortical activation than ipsilateral eye stimulation (Figs. 2*A* and 3*A*). Warm colors in the OD-map visualize this contralateral dominance, and the histogram of ODIs was centered to the right of zero (average ODI, 0.28 ± 0.03, $n = 7$). After MD in control animals, the OD-map was dominated by colder colors (Fig. 2*B*) and the ODI decreased to 0.03 ± 0.02 ($n = 9$), indicating a significant OD-shift toward the open eye ($P < 0.001$, t test) (Fig. 3*A*). In contrast, in PT-animals, MD did not induce a significant OD-shift: binocular visual cortex remained dominated by input from the contralateral eye, irrespective of whether this input was deprived or not (Fig. 2 *C* and *D*). After MD, activity patches induced by stimulation of the deprived eye were still darker than those of the open eye. The ODI was not significantly different from PT-animals without MD (PT+MD: 0.17 ± 0.02; PT: 0.19 ± 0.01; $n = 10/13$; $P > 0.05$, t test) (Fig. 3*A*), but different from control animals after MD ($P < 0.05$, t test). Our optical imaging data were confirmed by extracellular recordings in four control and five PT-animals (*SI Text, Data S5* and Fig. S3).

Changes in Response Strength After MD. To better understand the mechanisms underlying the difference in OD-plasticity between control and PT-animals, we compared maximum response strength of the two eyes (Fig. S4). In control animals, cortical activation was significantly higher after visual stimulation of the contralateral (2.42 ± 0.16) compared with the ipsilateral eye (1.34 ± 0.08; $P < 0.001$, t test, $n = 7$). After MD of the contralateral eye, response strengths of the two eyes were no longer significantly different ($P > 0.05$, t test): Open-eye responses were slightly but nonsignificantly enhanced (1.84 ± 0.23; $P > 0.05$, t test, $n = 9$) and the response strength after deprived-eye stimulation was slightly decreased (1.92 ± 0.16; $P < 0.05$, t test). In contrast, in PT-mice, cortical activation after deprived-eye stimulation remained significantly higher than after open-eye stimulation (deprived/open: 2.22 ± 0.18/1.53 ± 0.12, $n = 10$; $P < 0.001$, t test), as in PT-mice without MD (contra/ipsi: 2.31 ± 0.17/1.57 ± 0.13, $n = 13$; $P < 0.001$, t test) and irrespective of "treatment" regime.

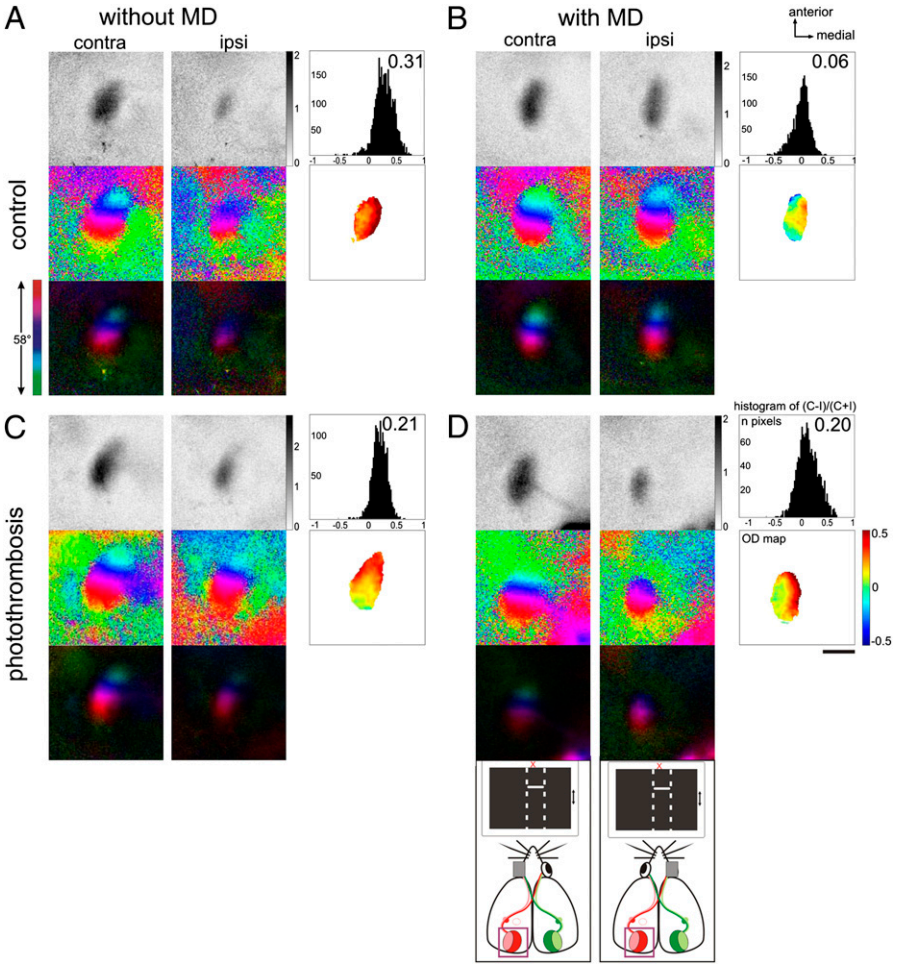

Fig. 2. A PT-lesion outside the visual cortex prevented OD plasticity. Optically imaged maps of responses to the contra- and ipsilateral (ipsi) eye (see *Insets*) in the binocular region of mouse visual cortex in both control animals (*A* and *B*) and in mice with PT (*C* and *D*). Maps without MD are illustrated in the left column (*A* and *C*), maps after MD in the right column (*B* and *D*). Both gray-scale coded response-magnitude maps and their quantification (*Top*), and color-coded phase and polar maps of retinotopy (*Middle, Bottom*) are illustrated. The magnitude of the optical responses is illustrated as fractional change in reflection $\times 10^4$. For each experiment, the histogram of OD-scores, the average OD-index (ODI), and the corresponding 2D OD-maps are included (ODI values are color-coded: blue represents negative, red positive values). Note that without MD, activity patches evoked by stimulation of the contralateral eye were consistently darker than those after stimulation of the ipsilateral eye, 2D OD-maps display warm colors, and the average ODI is positive, indicating contralateral dominance (*A* and *C*). In contrast, MD for 7 d in control (*B*) but not PT-animals (*D*) induced a significant OD-shift toward the open eye: the response-magnitude maps of both contra- (deprived) and ipsilateral (open) eye were equally dark, the histogram of OD-scores shifted to the left, the ODI was 0.06 compared with 0.31 without MD, and colder colors prevailed in the OD-map. In PT-animals with MD (*D*), the response-magnitude maps of the deprived eye were still darker than those of the open eye; the histogram of OD-scores and the average ODI were essentially unchanged and warm colors still prevailed in the OD-map. (Scale bar, 1 mm.)

Taken together, these results clearly show that a PT-lesion impedes enhancement of visual acuity and contrast sensitivity of the open eye and OD-plasticity after MD.

OD-Plasticity in the Nonlesioned Hemisphere. To check whether the reduction of plasticity in the lesioned hemisphere was caused by global mechanisms affecting the entire brain, we next analyzed OD-plasticity in the nonlesioned hemisphere. In control animals with MD of the ipsilateral eye, the ODI was 0.36 ± 0.03 ($n = 10$), and thus slightly but not significantly higher than in nondeprived control animals (0.22 ± 0.05, $n = 6$, $P > 0.05$, t test) (Fig. 3*B*). In contrast, in PT-animals, MD of the ipsilateral eye induced a significant OD-shift: the ODI was 0.36 ± 0.03 ($n = 7$), significantly higher than in PT-animals without MD (0.20 ± 0.02; $n = 10$; $P < 0.01$, t test) (Fig. 3*B*). After MD of the contralateral eye, we also observed a significant OD-shift in the nonlesioned hemisphere: the ODI was 0.11 ± 0.02 ($n = 10$), and thus significantly lower than in PT-mice without MD ($P < 0.05$, t test) (Fig. 3*B*). Thus, OD-plasticity was present in the nonlesioned hemisphere, indicating that the reduction of OD-plasticity in the lesioned hemisphere must be a rather specific process and cannot be caused by a general decline of plasticity mechanisms in the entire brain.

Simultaneous Imaging in Both Hemispheres. What differences might exist between the two hemispheres that could account for this difference in OD-plasticity? To approach this problem, we simultaneously recorded visual cortical activity in the two hemispheres. In control animals, response magnitudes in the left and right hemisphere were almost identical (Fig. S5*A*): the hemisphere activation quotients were 0.90 ± 0.06 ($n = 8$, without MD) and 1.00 ± 0.05 ($n = 10$, with MD). Activation quotients were not significantly different ($P > 0.05$, t test). In PT-animals with MD, the quotient was 1.06 ± 0.04 ($n = 10$). In contrast, in PT-animals without MD, the quotient was 1.15 ± 0.08 ($n = 14$), indicating a higher activation of the nonlesioned compared with the lesioned visual cortex (Fig. S5*B*). This difference was significant compared with controls ($P < 0.05$, t test) (Fig. S5*C*). Thus, on average, visual stimulation induced a significantly higher cortical activation in the nonlesioned compared with the lesioned hemisphere in PT-animals.

Ibuprofen Did Not Restore OD-Plasticity. Visual cortical activity was imaged directly after the behavioral measurements. In ibuprofen-treated PT-animals ($n = 10$), MD did not induce a significant OD-shift and the visual cortex remained dominated by the deprived (contralateral) eye (Fig. 3*A*): The ODI was 0.14 ± 0.03, not significantly different from vehicle-treated mice (0.16 ± 0.02, $n = 6$; $P > 0.05$, t test) nor from mice without MD ($P > 0.05$, t test). Analyses of maximum response strength also revealed that cortical activation was indistinguishable from untreated PT-animals: cortical activation after deprived-eye stimulation remained higher than after open eye stimulation, irrespective of whether animals were injected with ibuprofen (deprived/open: $2.52 \pm 0.20/1.93 \pm 0.12$; $P < 0.001$, t test) or vehicle (deprived/open: $2.22 \pm 0.10/1.63 \pm 0.02$; $P < 0.01$, t test) (Fig. S4). In control animals, ibuprofen treatment had neither an effect on the ODI (0.21 ± 0.02, $n = 6$; $P > 0.05$, t test) nor on the maximum response strength (contra/ipsi: $2.36 \pm 0.21/1.56 \pm 0.12$, $n = 6$; $P > 0.05$, t test).

Late MD Did Not Restore OD-Plasticity. We also imaged visual cortical activity of animals with late MD to test whether OD-plasticity was restored, which was not the case (Fig. 3*A*). After both a 1-wk or a 2-wk delay, MD did not induce a significant OD-shift: the ODI was 0.19 ± 0.04 (1 wk, $n = 5$) or 0.18 ± 0.01 (2 wk, $n = 10$), not significantly different from PT-mice without MD (1 wk/2 wk: $P > 0.05$/$P > 0.05$, t test). Maximum response strength after deprived-eye stimulation also remained significantly higher than after open-eye stimulation (1 wk: deprived/open: $2.08 \pm 0.24/1.42 \pm 0.14$; $P < 0.05$, t test; 2 wk: deprived/open: $2.24 \pm 0.12/1.67 \pm 0.07$; $P < 0.001$, t test) (Fig. S4). Thus, OD-plasticity was not restored by a 1- or 2-wk delay between lesion induction and MD.

Discussion

We observed that a PT-lesion outside the visual cortex prevented visual plasticity in mice: after 7 d of MD in 3-mo-old mice, lesioned animals showed neither improvement of visual acuity and contrast sensitivity of the open eye nor an OD-shift toward this eye in the lesioned hemisphere. Although sensory learning was compromised irrespective of whether the lesion was ipsi- or contralateral to the open eye, classical OD-plasticity was present in the nonlesioned hemisphere, alluding to a more specific disturbance and arguing against some "whole-brain" sickness preventing plasticity. In contrast to OD-plasticity, sensory learning was rescued by both anti-inflammatory treatment and when MD was induced 2 wk after the lesion (late MD), highlighting that distinct mechanisms underlie these two forms of visual plasticity. Our results suggest, that—although anti-inflammatory treatment only rescued plasticity in one of the studied designs—it might prove to be a useful adjuvant therapy to support rehabilitation following stroke. Concerning OD-plasticity, the surprising finding is that changes in the input statistics of the major thalamo-cortical afferents to V1 were not sufficient to induce a shift in the OD of visual cortical neurons: changes in the target circuitry can obviously alter V1-sensitivity to modified afferent activity patterns and the altered sensitivity is not restored by ibuprofen-treatment or late MD. We therefore conclude that visual cortical plasticity cannot be conceptualized solely as a local process and that nonlocal influences are much more important than previously assumed.

PT-Effects and Treatment. Stroke is known to be associated with inflammation (28). Both the susceptibility to anti-inflammatory treatment and the functional recovery after late MD indicate

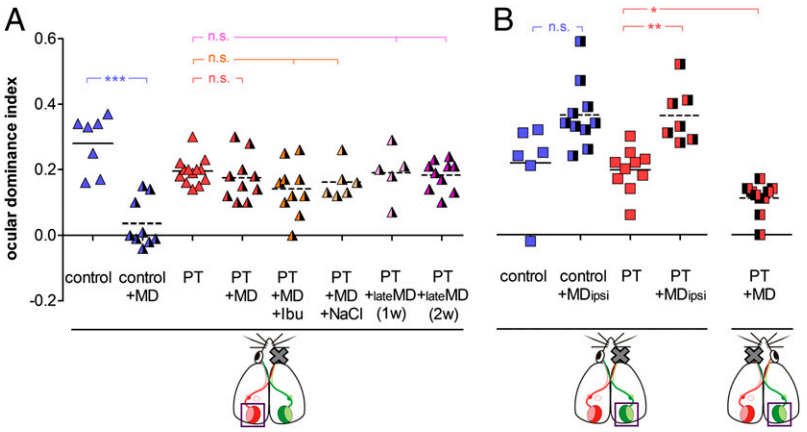

Fig. 3. OD-plasticity was absent in the lesioned and present in the nonlesioned hemisphere. Neither ibuprofen (Ibu) nor late MD restored OD-plasticity. OD-indices of the left (lesioned) and right (nonlesioned) hemisphere after MD of either the contralateral (MD) or the ipsilateral eye (MDipsi). Symbols represent ODI values of individuals; means are marked by horizontal lines. (*A*) ODIs in the lesioned hemisphere. In control animals, MD induced a significant reduction of ODIs (control+MD). In contrast, in PT-animals, ODIs were not changed after MD (compare PT with PT+MD), not even after various "treatments" (Ibu/NaCl, late MD). w, week. (*B*) ODIs in the nonlesioned hemisphere. In control animals, MD of the ipsilateral eye increased ODIs non-significantly (control+MDipsi) while it led to a significant OD-shift toward the open eye in PT-animals (PT+MDipsi). OD-plasticity in the right hemisphere was also present after MD of the contralateral eye: ODIs of nondeprived (PT) and deprived PT-animals (PT+MD) were significantly different.

that the sensory learning impairment was caused by a transient, but nonlocal inflammatory process. This conclusion is underscored also by the fact that neither lesion location nor size influenced the magnitude of the effect, and sensory learning was always abolished immediately after PT, irrespective of whether the lesion was ipsi- or contralateral to the open eye. Thus, although it has been shown that the monocular visual cortex contralateral to the open eye is needed for this form of sensory learning (19), an intact contralateral cortex is not sufficient because even a small PT-lesion in the somatosensory cortex of the opposite hemisphere can abolish learning, most probably mediated by a nonlocal inflammatory reaction affecting both hemispheres. To antagonize the inflammation, we tested the therapeutic effect of the cyclooxygenase-inhibitor ibuprofen (29). Cyclooxygenase-2 is rapidly induced in inflamed tissues, whereby its reaction products are responsible for many of the cytotoxic effects of inflammation (30). It was shown previously that ibuprofen reduced injury from global and focal ischemia (31, 32) and restored plasticity in the somatosensory cortex after a PT-lesion (33). In our experiments, ibuprofen treatment completely rescued the enhancement of both visual acuity and contrast sensitivity after MD in PT-animals but had no effect on OD-plasticity. In the ischemic cortex, levels of proinflammatory mediators, including cytokines and adhesion molecules, increase about 1 h after stroke, and return to basal values after 5 d (34, 35). To analyze whether the inhibited plasticity in our study was an acute effect or a longer-lasting modification of neuronal circuits, we inserted a delay between the PT-lesion and MD. When MD was induced 2 wk after stroke, sensory improvement was restored to control values, as after anti-inflammatory treatment, but OD-plasticity in the lesioned hemisphere remained absent. Hence, the reduced sensory learning after a PT-lesion was an acute effect, most probably due to a nonlocal inflammation affecting both hemispheres. This conclusion is also supported by the fact that lesion size changed neither after ibuprofen treatment nor after the 2-wk delay period. In contrast, the impaired OD-plasticity was longer-lasting and mediated by different mechanisms.

Different Networks? Our results of a different susceptibility of sensory learning and OD-plasticity to ibuprofen-treatment or late MD further demonstrates that these two forms of visual plasticity are mediated by different neuronal subsystems. The enhancement of visual acuity of the open eye after MD is restricted to the monocular visual field, despite the dependence of the plasticity on binocular interactions (19). In contrast, OD-shifts happen in the binocular region of V1 (23, 26, 36). In addition, OD-plasticity after the critical period mostly takes place in superficial cortical layers (37–39), while the enhancement of the optokinetic response involves the cortical control of the accessory optic system triggering the reflex, presumably from deep-layer efferents (19).

In Vitro, in Vivo. Our observation that OD-plasticity was reduced in the vicinity of a cortical lesion was surprising, given that several in vitro studies have provided evidence for enhanced plasticity in the surround of a cortical stroke, e.g., easier induction of LTP, enhanced activity, and reduced inhibition (4, 40). Because OD-plasticity after MD is mediated by impaired connections of the deprived eye and enhanced connections of the open eye (41, 42), a disturbed balance of LTP and long-term depression in the first week after a lesion (10) could impair coordinated synaptic strengthening and weakening and affect neuronal plasticity. However, these in vitro experiments may have only limited validity for complex changes taking place in nerve cell networks in vivo. Indeed, a recent in vivo study provided evidence for impaired plasticity in the rat somatosensory cortex after a PT near the barrel field (12); it was, however, not analyzed whether this was accompanied by a functional impairment, as shown in our study.

Interhemispheric Interactions. It has been argued previously that both hemispheres inhibit each other (43). A lesion on one side of the brain would disrupt this balance, leading to decreased inhibition and increased excitation in the intact hemisphere (44–47). Similarly, the inhibitory influence of the intact hemisphere on the lesioned hemisphere would increase (43, 48), additionally depressing the activity and thereby possibly reducing plasticity in the lesioned hemisphere (49). Indeed, we observed that maximal response strength in the lesioned hemisphere of PT-mice was significantly weaker compared with the nonlesioned hemisphere. Because enhanced activity supports synaptic plasticity and learning (50) and reduced activity might prevent experience-dependent changes in neuronal circuitry, diminished plasticity in the lesioned, and present or even enhanced plasticity in the nonlesioned hemisphere of PT-animals, might be at least partly due to changes in activity levels. Recently, Failor et al. (51) also concluded that OD-plasticity may be uniquely dependent upon precise regulation of cortical activation: In mice with moderate cortical injury following neonatal hypoxia-ischemia, impaired OD-plasticity was correlated with a loss of parvalbumin expression, modifying excitation-inhibition balance.

Network Effect. Why is OD-plasticity compromised although the visual cortex is not lesioned? Von Monakow (2) coined the term "diaschisis" to characterize remote effects and transient alterations of brain functions after lesions (3). Our results indicate that such nonlocal influences might also play a much more important role for neuronal plasticity phenomena than previously assumed. In early postnatal development, changes in the input statistics gate experience-dependent OD-plasticity only after sufficient maturation of specific inhibitory circuits (52). Likewise, here, lesions outside the visual cortex altered V1-sensitivity to changes in afferent inputs. Thus, OD-plasticity cannot be conceptualized solely as a local process but rather as a network phenomenon. This conclusion is underscored by the recent observation that OD-plasticity in the rat is crucially dependent on callosal connections, and the thalamo-cortical pathway alone is not responsible for the OD of neurons in the binocular part of V1 (53). By inactivating V1 in one hemisphere, it was shown that callosal connections predominantly contributed ipsilateral eye inputs and were critically involved in weakening deprived eye inputs during MD. In our PT-animals, callosal inputs from the nonlesioned hemisphere were intact but nevertheless, deprived-eye inputs were not weakened and OD-plasticity was not observed after MD. Whether this finding indicates that callosal inputs are less important in mice is not yet known. A recent in vivo study in mice presents evidence in favor of this possibility (54): the OD-shift after MD was expressed mainly at the level of thalamo-cortical synapses. Our results extend these data by showing that activity changes in the thalamo-cortical afferents are, however, not sufficient to modify cortical binocularity under special circumstances, such as a cortical lesion. Thus, our data clearly indicate that OD-plasticity is not a local phenomenon but determined by network interactions, even from outside V1 and different from the major thalamo-cortical inputs.

It was recently observed that the predominant cellular mechanism underlying OD-plasticity after the end of the critical period is a depression of the responses of GABAergic neurons to the deprived eye, while open-eye responses of excitatory neurons were only modestly potentiated (55). When OD-plasticity is absent, like in our PT-animals, one could therefore hypothesize that—by a so far unknown mechanism—the depression of GABAergic neurons to deprived eye stimulation is weaker, which in turn could cause stronger inhibition and preclude plastic changes in V1. This hypothesis is supported by our observation of reduced V1 activation to visual stimulation in the lesioned hemisphere. It is also in line with the emerging idea that intracortical inhibition is a crucial limiting factor for the induction of experience-dependent plasticity after the end of the critical period (56).

Taken together, our results promote the idea that long-ranging interactions play a much more important role for neuronal plasticity than previously assumed: changes in the major thalamo-cortical inputs were not sufficient to induce OD-plasticity in mouse V1, indicating that V1-sensitivity was altered by net-

work interactions. In addition, a lesion-induced inflammation, affecting also the nonlesioned hemisphere, was responsible for reductions in sensory learning but not for impaired OD-plasticity, further supporting the view that distinct cellular mechanisms underlie the two forms of visual plasticity. Finally, although anti-inflammatory treatment only rescued plasticity in one of the studied designs, it might prove to be a useful adjuvant therapy to support rehabilitation in the early poststroke period.

Materials and Methods

Male C57BL/6J mice were between 70 and 110 d of age at the day of optical imaging. All experimental procedures were approved by the local government (Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz) under the registration number 02–003/08. A PT lesion was induced in the left somatosensory cortex using the Rose Bengal technique introduced by Watson et al. (22). All control animals were sham-treated. To test sensory learning and cortical plasticity, the right or left eye was monocularly deprived 5 h after the PT/sham treatment or with a delay of 1 or 2 wk (late MD). MD continued for 7 d according to published protocols (23, 26). For anti-inflammatory treatment, mice received daily intraperitoneal injections of ibuprofen (60 mg/kg) or NaCl as vehicle, starting directly after MD. Visual acuity and contrast sensitivity were determined using a virtual-reality optomotor system (57). Mouse visual cortical responses were recorded and analyzed as described previously (21), using the imaging method developed by Kalatsky and Stryker (25), and optimized for the assessment of OD-plasticity (26). Size and location of the cortical lesions was determined on Nissl-stained coronal brain sections. For details, see *SI Materials and Methods*.

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