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Brief dark exposure restored ocular dominance plasticity in aging mice and after a cortical stroke

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ABSTRACT

In the primary visual cortex (V1), monocular deprivation (MD) induces a shift in the ocular dominance (OD) of binocular neurons towards the open eye (Wiesel and Hubel, 1963; Gordon and Stryker, 1996). In V1 of C57Bl/6J mice, this OD-plasticity is maximal in juveniles, declines in adults and is absent beyond postnatal day (PD) 110 (Lehmann and Löwel, 2008) if mice are raised in standard cages. Since it was recently shown that brief dark exposure (DE) restored OD-plasticity in young adult rats (PD70-100) (He et al., 2006), we wondered whether DE would restore OD-plasticity also in adult and old mice and after a cortical stroke. To this end, we raised mice in standard cages until adulthood and transferred them to a darkroom for 10–14 days. Using intrinsic signal optical imaging we demonstrate that short-term DE can restore OD-plasticity after MD in both adult (PD138) and old mice (PD535), and that OD-shifts were mediated by an increase of open eye responses in V1. Interestingly, restored OD-plasticity after DE was accompanied by a reduction of both parvalbumin expressing cells and perineuronal nets and was prevented by increasing intracortical inhibition with diazepam. DE also maintained OD-plasticity in adult mice (PD150) after a stroke in the primary somatosensory cortex. In contrast, short-term DE did not affect basic visual parameters as measured by optomotry. In conclusion, short-term DE was able to restore OD-plasticity in both adult and aging mice and even preserved plasticity after a cortical stroke, most likely mediated by reducing intracortical inhibition.

1. Introduction

Rodent V1 is dominated by input from the contralateral eye (Dräger, 1975, 1978) but MD can induce a shift in the OD of binocular neurons towards the open eye (Wiesel and Hubel, 1963; Gordon and Stryker, 1996; Cang et al., 2005a). In V1 of C57Bl/6J mice, this OD-plasticity is maximal at 4 weeks of age, declines in 2–3 month old animals and is absent beyond PD110 (Espinosa and Stryker, 2012; Levelt and Hübener, 2012), even after longer deprivation times (Greifzu et al., 2012), if animals are raised in standard cages (SC). In critical period mice (PD19-32), 4 days of MD are sufficient to induce OD-shifts towards the open eye (Gordon and Stryker, 1996). This "juvenile" OD-shift is predominantly mediated by a decrease in the cortical responses to visual stimulation of the deprived eye. In contrast, in 2–3 month old SC-raised mice, significant OD-shifts need 7 days of MD and are primarily mediated by increased open eye responses ("adult" OD-plasticity) (Sawtell et al., 2003; Hofer et al., 2006; Heimel et al., 2007; Sato and Stryker, 2008). It has been shown that the balance between excitation and inhibition is important for regulating plasticity in V1: during postnatal development, the maturation of GABAergic (γ-aminobutyric acid) circuitry opens the critical period for OD-plasticity (Huang et al., 1999, 2010; Rozas et al., 2001) and raising mice in an enriched environment (EE) both preserves a juvenile inhibitory tone and juvenile-like OD-plasticity into adulthood (Greifzu et al., 2014). While dark-rearing has been extensively studied (Hooks and Chen, 2007; Berardi et al., 2000), the effects of short periods of dark exposure (DE) during adulthood are less well understood. Recently, it was shown that 10 days of DE in PD70-100 SC-raised rats reactivated OD-plasticity in V1, reduced the level of $GABA_A$ receptors relative to AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) receptors and caused a return to the juvenile form of NMDA (*N*-methyl-D-aspartate) receptors (He et al., 2006). We therefore wondered whether short-term DE would restore OD-plasticity in adult and old mice already beyond their sensitive phase for OD-plasticity and would also preserve OD-plasticity after a cortical stroke. Testing not only young adult but also old animals is rather important since there are

Abbreviations: DE, dark exposure; EE, enriched environment; LR, light reared; MD, monocular deprivation; OD, ocular dominance; ODI, ocular dominance index; PD, postnatal day; PNN, perineuronal net; PT, photothrombosis; PV, parvalbumin; S1, primary somatosensory cortex; SC, standard cage; V1, primary visual cortex.

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numerous age-dependent changes of cortical circuitry such as reduced functional selectivity and signal-to-noise ratio.

Thus a possible therapeutic strategy that increases cortical plasticity in young adult animals must not necessarily be effective during old age. We therefore tested the effect of brief DE on both adult and old mice. Using optical imaging of intrinsic signals, we show that 14 days of DE reactivated OD-plasticity in 4–6 month old mice after 4 and 7 days of MD. The restored OD-shift was mediated by a significant increase of open eye responses in V1 and prevented by diazepam injections. In addition, we observed a significant reduction of the number of parvalbumin expressing (PV^+) cells and perineuronal nets (PNNs) in the visual cortex of DE-mice compared to light-reared (LR) controls. Fourteen days of DE were also sufficient to restore OD-plasticity in old mice (PD535) after 7 days of MD. Finally, brief DE in adulthood (PD150) also maintained OD-plasticity after a stroke in the primary somatosensory cortex (S1). Short-term DE during adulthood did neither impair basic visual acuity nor the experience-dependent increase of visual acuity after MD as measured by optomotry (Prusky et al., 2004). Taken together, our data strongly suggest short-term DE as a highly efficient therapeutic intervention to restore plasticity in both adult and old mice and even after a cortical lesion, and that the DE-effect is most likely mediated via reduced intracortical inhibition.

2. Materials and methods

2.1. Animal treatment

All experimental procedures were approved by the local government (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, registration number 33.9-42502-04-10/0326). Both male and female C57BL/6J mice from the mouse colony of the central animal facility of the University Medical Center, Göttingen (groups I–III, V–VII, see next paragraph) and 10 C57BL/6JRcc mice from the Harlan laboratories (Netherlands; group IV) were raised in SCs on a 12 h light/dark cycle (LR), with food and water available ad libitum.

2.2. Dark exposure

For short-term visual deprivation (dark exposure = DE) animals were housed in SCs placed into a scantainer (Scanbur Technology, Denmark) in a completely light-tight darkroom for 10 or 14 days.

We investigated a total of 7 different experimental groups: I) adult PD138 mice (age range PD125-157; average: PD138) received either 4 days or II) 7 days of MD after 14 days of DE; III) old PD535 mice (PD517-564) received 7 days of MD after 14 days of DE; IV) diazepam or saline-treated adult mice (PD151-157; average: PD154) received 7 days of MD after 14 days of DE as well as daily injections of diazepam/saline during the MD period; V) Chronically imaged adult mice: imaging was performed before (PD116-123; average: PD118) and after 14 days of DE and 7 days of MD (PD141-150; average: PD145); VI) young adult mice (PD57-80; average: PD72) received 4 days of MD after 10 days of DE; VII) Adult mice (PD135-165; average: PD150) received a photothrombotic (PT) lesion in S1 (sham-surgery for controls) and 7 days of MD after 14 days of DE. Experimental groups also contained equally treated light-reared (LR) control mice (groups I, II, VI) and control animals without MD (groups I, II, III, VI, VII).

2.3. Optomotry

To test whether short-term DE modified basic visual abilities, the spatial frequency threshold ("visual acuity") and contrast sensitivity of the optomotor reflex of animals from all experimental groups (except groups IV and V) were determined using the virtual-reality optomotor system (Prusky et al., 2004). Briefly, freely moving animals were exposed to moving vertical sine wave gratings of various spatial frequencies and contrasts and will reflexively track the gratings by head movements as long as they can see them. Spatial frequency at full contrast and contrast at six different spatial frequencies [0.031, 0.064, 0.092, 0.103, 0.192, 0.272 cycles/degree (cyc/deg)] were varied by the experimenter until the threshold of tracking was determined.

2.4. Monocular deprivation (MD)

According to already published protocols, we always sutured the right eye of our mice (Gordon and Stryker, 1996; Cang et al., 2005a; Lehmann and Löwel, 2008) to trigger visual plasticity. DE-mice were transported (immediately after DE) in a light-tight box to the surgical suite and MD-surgery started directly to minimize light exposure after DE. After box-anesthesia with 2% isofluorane in a 1:1 mixture of nitrous oxide (N_2O) and oxygen (O_2), anesthesia was maintained with 1.5% isoflurane, lid margins were trimmed and an antibiotic gel (gentamicin gel) was applied. The eye was closed with two mattress sutures. After MD, the mice were returned to their standard home cages and stayed in normal 12 h light/dark conditions. Animals were checked daily to make sure that the eye remained closed.

2.5. Surgical preparations for optical imaging (Kalatsky and Stryker, 2003; Cang et al., 2005a)

Mice were initially box-anesthetized with 2% halothane in a 1:1 mixture of O_2:N_2O and received atropine (Franz Köhler, 0.3 mg, subcutaneously (s.c.)), dexamethasone (Ratiopharm, 0.2 mg, s.c.), and chlorprothixene (Sigma, 0.2 mg, intramuscularly). Mice were placed in a stereotaxic frame and anesthesia was maintained with 0.6–0.8% halothane in a 1:1 mixture of O_2:N_2O applied through a tube attached to the nose. Body temperature was maintained at 37 °C and the heart rate was monitored throughout the experiment. Lidocaine (2% xylocaine jelly) was applied locally to all incisions. The skin above the skull was incised to expose V1 of the left hemisphere and agarose (2.5% in 0.9% NaCl) and a glass cover-slip were placed over the exposed area. Imaging was performed through the skull. Mouse visual cortical responses were recorded using the imaging method developed by Kalatsky and Stryker (2003) and optimized for the assessment of OD-plasticity (Cang et al., 2005a). In this method, a temporally periodic stimulus is continuously presented to the animal and the cortical responses at the stimulus frequency are extracted by Fourier analysis. Optical images of intrinsic cortical signals were obtained using a CCD (charge-coupled device) camera (Dalsa) controlled by custom software. The surface vascular pattern and intrinsic signal images were visualized with illumination wavelengths set by a green (550 ± 10 nm) or red (610 ± 10 nm) interference filter, respectively. After acquisition of a surface image, the camera was focused 600 μm below the cortical surface. An additional red filter was interposed between the brain and the CCD camera. Frames were acquired at a rate of 30 Hz, temporally binned to 7.5 Hz and stored as 512 × 512 pixel images after spatial binning of the camera image. Drifting horizontal bars (2° wide) were presented to the animal at a distance of 25 cm on a high refresh-rate monitor. The distance between 2 bars was 80° and they were presented at a temporal frequency of 0.125 Hz. The visual stimulus was restricted to the binocular visual field of the left V1 (−5° to +15° azimuth) and animals were stimulated through either the left or the right eye in alternation to assess the OD of the left hemisphere. To assess map quality the stimulus monitor was placed in the right visual field of the animal at a distance of 25 cm with its left edge approximately aligned to the animal to optimally stimulate the right eye (contralateral to the recorded hemisphere). The drifting bars (elevation or azimuth) were shown across the full screen.

2.6. Data analysis

Visual cortical maps were calculated from the acquired frames by Fourier analysis to extract the signal at the stimulation frequency using custom software (Kalatsky and Stryker, 2003). While the phase

component of the signal is used for the calculation of retinotopy, the amplitude component represents the intensity of neuronal activation (expressed as fractional change in reflectance $\times 10^{-4}$) and was used to calculate OD (for details, see Cang et al. (2005a)). Briefly, we calculated the OD-score of each pixel in the binocularly activated region in V1 as $(C - I)/(C + I)$, with C and I representing the raw response magnitudes of each pixel to visual stimulation of the contralateral and ipsilateral eye, respectively. We then calculated an OD-index (ODI) as the average of the OD-scores of all responsive pixels. This ODI ranged from -1 to $+1$, with negative values representing ipsilateral and positive values representing contralateral dominance. We obtained at least three ODIs per animal; experiments with fewer than three ODIs were discarded from further analyses. All activity and phase maps of one animal were averaged for further quantification and data display. The quality of the retinotopic maps was assessed by the calculation introduced by Cang et al. (2005b). Briefly, the 20,000 pixels that had the greatest response magnitude were selected. For each of these pixels, the difference between its position and the mean position of its surrounding 24 pixels was calculated. For maps of high quality, the position differences are quite small because of smooth progression. The standard deviation of the position difference was then used as an index of the quality of retinotopic maps with a small standard deviation indicating high map quality and high values indicating low map quality.

2.7. Diazepam/saline administration

To increase GABAergic inhibition we applied diazepam (Rotexmedica, intraperitoneally, i.p.), an allosteric $GABA_A$ receptor modulator, in one additional experimental group. Adult DE-mice (PD151-157; average: PD154) received either diazepam (0.25 μg/μl diluted in 0.9% NaCl; 1 mg per kg mouse) or as control saline (0.9% NaCl) 7 h prior to MD and continued for 6 days during MD with one injection per day. The applied dosage of diazepam allowed the normal activity and exploratory behavior of the animals.

2.8. Induction of a photothrombotic stroke

A PT-lesion was induced in the left S1 neighboring V1 by using the Rose Bengal technique introduced by Watson et al. (1985). Briefly, mice (PD150) were initially box-anesthetized with 2% isoflurane, and anesthesia was maintained with 1% isoflurane in 1:1 O_2:N_2O. The animals were placed in a stereotaxic frame and body temperature was maintained at 37 °C. The skin above the skull was incised and an optic fiber bundle (aperture: 1.0 mm) mounted on a cold light source (Schott KL 1500) was positioned 2 mm lateral to the midline and 1 mm posterior to the bregma. Next, 100 μl Rose Bengal (Sigma-Aldrich; 10 mg/ml in 0.9% NaCl) was injected into the tail vein. After waiting for 5 min, the illumination period of 15 min with the cold light source was performed. All control animals were treated identically but the light source was not switched on (sham-treatment). Finally, the skin was sutured and the animals recovered in their home cages. For the DE-group, mice were transferred to the darkroom for 14 days immediately after waking up from anesthesia. Directly after DE, the right eye was deprived in half of the animals (see above) and the mice were transferred to their home cage in a normally lit room for the following 7 days. Finally, we imaged V1-activity in both deprived and non-deprived animals.

2.9. Perfusion and tissue processing

After optical imaging, all mice were deeply anesthetized with an overdose of 0.3 ml 30% chloral hydrate (i.p.) and were perfused transcardially with 1% heparin in 0.9% NaCl for 2 min followed by 4% paraformaldehyde (PFA, pH 7.4) for 3 min. The brain was removed and postfixed in 4% PFA (pH 7.4) for one day and then transferred to a cryoprotectant solution (10% sucrose, 20% glycerol). The brains were frozen in methylbutane and stored at −80 °C. Coronal 40 μm thick brain sections were cut on a sledge microtome.

2.10. Immunofluorescence

The number of wisteria floribunda agglutinin (WFA)-positive PNNs and of PV^+-cells was examined in coronal brain sections of the visual cortex (2.5 mm to 3.5 mm posterior to bregma) of both DE- and LR-mice. Free floating sections were incubated for 30 min in a blocking solution (10% donkey serum, 0.3% Triton X-100 in PB, pH 7.4) at room temperature. Brain sections were incubated overnight at 4 °C with mouse anti-PV (Immunological Science, 1:500) and biotin-conjugated lectin WFA (Sigma, 1:1000) in 0.1 M PB including 0.3% Triton X-100. Antibodies were revealed with Cy2-conjugated donkey anti-mouse (Biotium, 1:200) and Cy3-conjugated streptavidin (Jackson ImmunoResearch, 1:1000) in 0.1 M PB including 0.3% Triton X-100 (2 h incubation at room temperature). Sections were then mounted with Fluoromount containing 4′,6-diamidino-2-phenylindole (DAPI) to stain and visualize cell nuclei. Four 40 μm thick visual cortex sections (approximately every 300 μm) per mouse were analyzed. The visual cortex was localized using the Paxinos mouse brain atlas (Paxinos and Franklin, 2001). Stained sections were examined with a fluorescence microscope (Axioskop, Carl Zeiss) and images of the visual cortex of each hemisphere were acquired using a 20× objective (AxioVision 40 4.8.2.0., Zeiss). Individual images had a size of 335.64 μm × 448.38 μm and were analyzed using ImageJ (Rasband). Background brightness was subtracted from the brightness measurement to reduce noise. All cells specifically stained for PV or WFA were counted in layers II–IV of the visual cortex of both hemispheres using the particle analysis cell counter function. In every group, sections of 3 animals containing the visual cortex of both hemispheres were analyzed. Each hemisphere was analyzed by taking 3 images (335.64 μm × 448.38 μm) of the visual cortex.

2.11. Lesion analysis

To determine size and location of the cortical PT-lesions, coronal brain sections were Nissl-stained and every third section was analyzed under the microscope (Axioskop, Carl Zeiss). Quantitative parameters were measured using AxioVision (40 4.8.2.0., Zeiss).

2.12. Statistical analyses

All intra- and intergroup comparisons were analyzed by a two-tailed Student *t*-test (with Bonferroni correction). The intergroup comparison of the enhancement of visual acuity was analyzed by ANOVA with repeated measurements and Bonferroni correction. The levels of significance were set as $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Data are represented as means ± SEM.

3. Results

3.1. Short-term DE restored OD-plasticity in adult mice (PD138)

Since OD-plasticity is absent in SC-mice beyond PD110 (Lehmann and Löwel, 2008), we tested whether short-term DE can rescue OD-plasticity in animals of this age group. To this end, we raised mice in SCs to an average age of PD118 (age range PD104-136), exposed them to complete darkness (DE) for 14 days and subsequently imaged V1-activities after either 4 or 7 days of MD or without MD for controls. In both LR- and DE-mice without MD, V1-activity was dominated by input from the contralateral eye: the activity spot induced by contralateral eye stimulation was darker than the spot induced by ipsilateral eye stimulation, the 2-dimensional OD-map showed warm colors indicating contralateral dominance and the histogram of all OD-indices peaked at positive values (Fig. 1A,C). After 7 days of MD, the OD shifted towards the open eye only in the adult DE-mice (Fig. 1D; 2A) while V1-activation

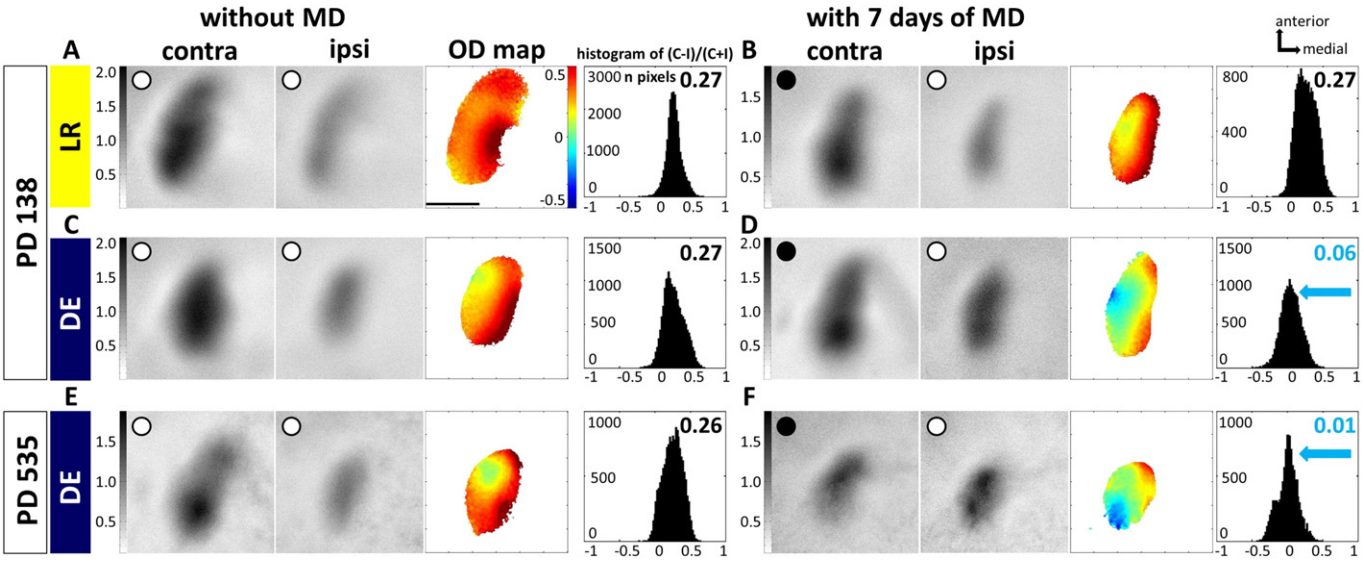

Fig. 1. Short-term dark exposure (DE) restored ocular dominance (OD)-plasticity in adult (PD138) and old (PD535) mice. (A) Optically recorded activity maps of the contralateral (contra) and ipsilateral (ipsi) eye in the binocular region of mouse V1 in both light-reared (LR; A, B) and DE-mice (C, D, E, F). Maps of mice without MD are displayed in A, C and E, maps of mice after 7 days of MD in B, D and F. Grayscale-coded response magnitude maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. (A, C, E) Without MD, activity patches evoked by stimulation of the contralateral eye were darker than those of the ipsilateral eye, the average ODI was positive and warm colors prevailed in the OD-maps, indicating contralateral dominance. (B) Seven days of MD did not induce a significant OD-shift towards the open eye in adult LR mice (B), whereas it induced a strong OD-shift in adult (PD138, D) and old DE-mice (PD535, F): the contra- and ipsilateral eye activated V1 about equally strongly, colder colors appeared in the OD-map, and the histogram of OD-scores shifted to the left. Scale bar: 1 mm.

of LR-mice was still dominated by the deprived (contralateral eye) (Fig. 1B): in DE-mice, the activity spot induced by stimulation of the open (ipsilateral) eye was nearly as dark as the one induced by stimulation of the formerly stronger contralateral (deprived) eye, colder colors predominated in the 2-dimensional OD-map and the ODI-histogram was shifted to the left (Fig. 1D).

The clearly restored OD-plasticity after DE and 7 days of MD prompted us to additionally investigate whether 4 days of MD would also be sufficient to induce significant OD-shifts in these mice, which has been shown for 4-week-old SC-mice. This was indeed the case. Quantification of V1-activation showed that in DE-mice, the average ODI decreased highly significantly from 0.28 ± 0.02 (n = 10) without MD to 0.09 ± 0.03 after 4 days of MD (n = 4; P < 0.001, Bonferroni-adjusted (B) *t*-test) and to 0.06 ± 0.02 after 7 days of MD (n = 8; P < 0.001, *t*-test) (Fig. 2A). In contrast, in adult LR-mice of the same age (PD138), OD-plasticity was absent, consistent with previous

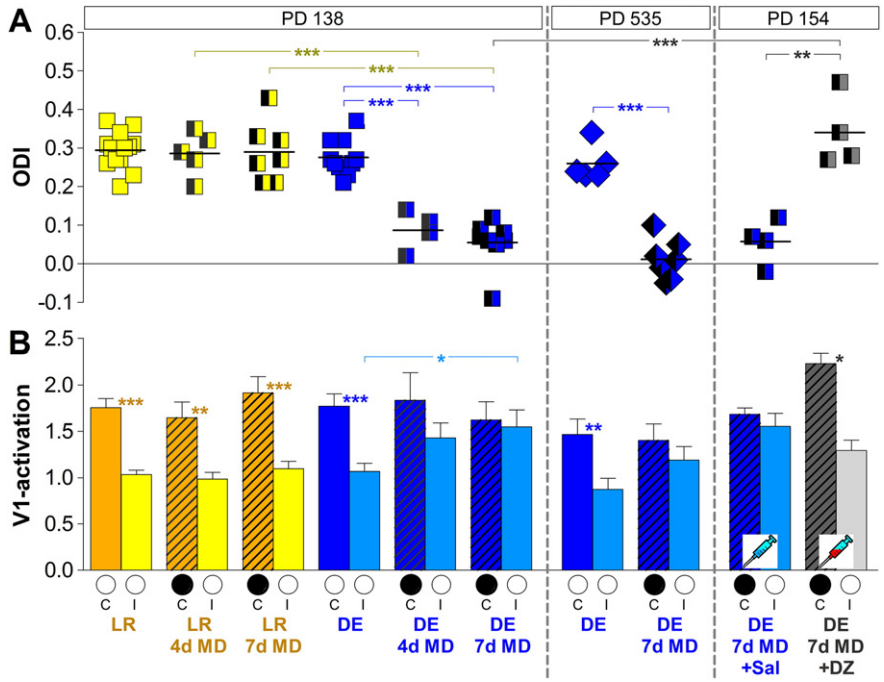

Fig. 2. OD-plasticity was restored in both adult and old mice after short-term DE and abolished after diazepam treatment. (A) Optically imaged OD-indices of control animals and after MD of the contralateral eye in LR- (yellow) and DE-mice (blue) and after diazepam-treatment (DZ, gray). Symbols represent ODI values of individuals, means are marked by horizontal lines. (B) V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye in control animals and after MD (black circle indicates MD eye). In adult DE-mice, both 4 and 7 days (d) of MD induced a significant OD-shift towards the open eye, which was abolished after diazepam-treatment.

publications (Lehmann and Löwel, 2008; Sato and Stryker, 2008). Neither 4 nor 7 days of MD induced significant OD-shifts in LR-mice (Figs. 1A,B; 2A) and ODIs did not differ between groups (LR: no MD: 0.29 ± 0.01, n = 13; with 4 days of MD: 0.29 ± 0.03, n = 5; with 7 days of MD: 0.29 ± 0.03, n = 7; comparison between all groups: P > 0.05, B *t*-test; comparison between LR- and DE-mice without MD: P > 0.05, *t*-test) (Fig. 2A). Thus, 14 days of DE were sufficient to restore OD-plasticity of adult mice (up to an age of at least PD157) after only 4 days of MD. Quantitative analyses of V1-activation further showed that the OD-shift of adult DE-mice was mediated by a significant increase in open (ipsilateral) eye responses in V1 while deprived eye responses remained unchanged. While V1-activities induced by visual stimulation of the open eye increased already slightly after 4 days of MD, 7 days of MD were necessary to induce significant changes of open eye responses in V1: V1-activation through the ipsilateral (open) eye increased from 1.07 ± 0.09 (n = 10) to 1.43 ± 0.16 (n = 4; P > 0.05, *t*-test) after 4 days of MD and to 1.55 ± 0.18 after 7 days of MD (n = 8; P < 0.05, *t*-test) (Fig. 2B). In contrast, deprived eye activities in V1 were not changed after MD (no MD: 1.77 ± 0.13, n = 10; with 4 days of MD: 1.83 ± 0.30, n = 4; P > 0.05, *t*-test; with 7 days of MD: 1.62 ± 0.20, n = 8; P > 0.05, *t*-test). In LR-mice, V1-responses of both eyes were not significantly different between the control group without MD and mice after either 4 or 7 days of MD (contra: no MD: 1.76 ± 0.10, n = 13; with 4 days of MD: 1.65 ± 0.17, n = 5; with 7 days of MD: 1.91 ± 0.17, n = 7; P > 0.05 for all comparisons, B *t*-test; ipsi: no MD: 1.03 ± 0.05, n = 13, with 4 days of MD: 0.98 ± 0.07, n = 5; with 7 days of MD: 1.1 ± 0.08, n = 7; P > 0.05 for all comparisons; B *t*-test, Fig. 2B).

3.2. Chronic optical imaging of V1-activity changes in DE-mice

To unambiguously determine the mechanism underlying the restored OD-plasticity in adult mice after DE, we additionally performed chronic optical imaging experiments in another group of adult mice before and after DE and MD. To this end, we raised mice beyond PD116, imaged V1-activities after visual stimulation of the ipsi- and the contralateral eye (first imaging) and then transferred the animals to a dark room for 14 days. Immediately after DE, we deprived the contralateral eye of vision (MD) and transferred the animals back to a normally lit room (12 h light/dark cycle) for the following 7 days. Finally, V1-activation of all mice was imaged again after 14 days of DE and 7 days of MD (three weeks after the first imaging session–second imaging) (Fig. 3A). Our chronic imaging experiments clearly confirmed that brief DE can rescue OD-plasticity in V1 of SC-mice that were already beyond their sensitive phase for OD-plasticity. In particular, the chronic data showed i) that 14 days of DE restored OD-plasticity after 7 days of MD in adult SC-mice (PD145) (Fig. 3B,C) and ii) that the restored OD-shifts were mediated by an increase of open eye responses in V1 while deprived eye responses were not significantly different before and after DE and MD (Fig. 3D). In the illustrated representative example (first imaging: before DE and MD), visual stimulation of the contralateral eye activated V1 more strongly than stimulation of the ipsilateral eye (Fig. 3B), warm colors prevailed in the 2-dimensional OD-map and the histogram of the OD indices was centered to the right of zero. The average ODI of all chronic mice before DE and MD was 0.28 ± 0.02 (n = 5) (Fig. 3C), and V1-activation was significantly higher after visual stimulation of the contralateral eye (1.92 ± 0.29) compared to the ipsilateral eye stimulation (1.14 ± 0.19, n = 5, P < 0.001; paired *t*-test) (Fig. 3B, D). After 14 days of DE, 7 days of MD induced a strong and highly significant OD-shift towards the open eye in all adult DE-mice (PD141-150): the average ODI decreased to −0.04 ± 0.04 (n = 5; P < 0.001, paired *t*-test) (Fig. 3C). Actually, the OD-shifts of the chronically imaged adult mice were as strong as usually seen in 4-week-old LR-animals after 4 days of MD (Hofer et al., 2006; Heimel et al., 2007; Lehmann and Löwel, 2008), and the visually driven activity in V1 could even become dominated by input from the formerly weaker, ipsilateral eye (Fig. 3B): in the illustrated case, the activity spot in V1 induced by stimulation of the open (ipsilateral) eye was slightly darker than the one induced by stimulating the previously closed (contralateral) eye, the ODI became negative, the ODI-histogram was shifted to the left and colder colors dominated in the 2-dimensional OD-map. On average, after DE and 7 days of MD, V1-activation was no longer significantly different between the contralateral (1.8 ± 0.21) and ipsilateral eye (1.84 ± 0.13, n = 5, P > 0.05; paired *t*-test). Thus, the restored OD-shift was clearly mediated by an increase of open-eye responses (ipsilateral eye) (P < 0.05, paired *t*-test) while closed eye responses (contralateral eye) did not change (P > 0.05, paired *t*-test) (Fig. 3D).

3.3. Short-term DE restored OD-plasticity in old mice (PD535)

Since we could clearly demonstrate that short-term DE restored already lost plasticity in about 4–5 month old SC-mice, we wondered

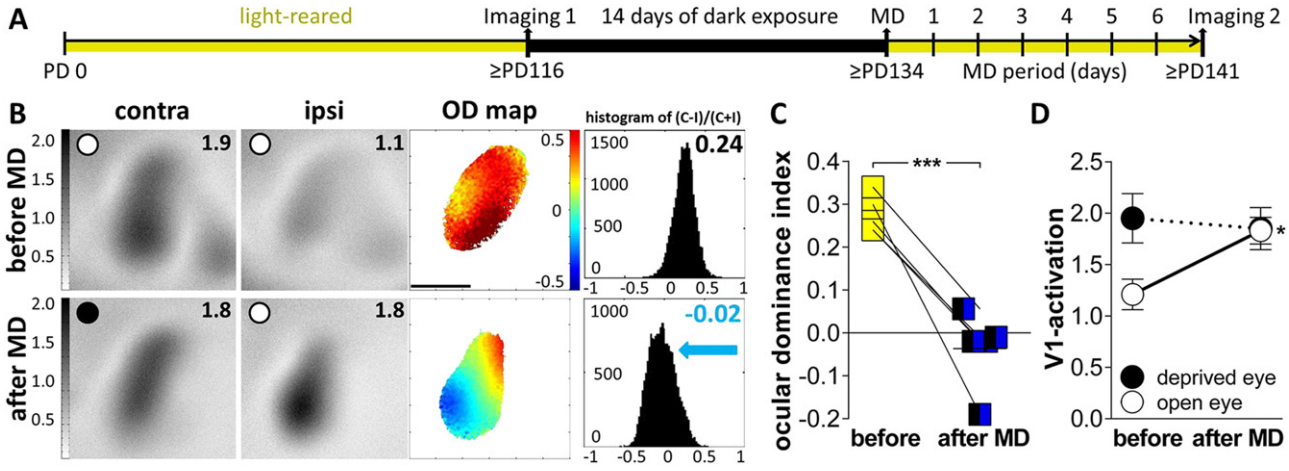

Fig. 3. Chronic imaging in adult mice revealed that the DE-induced restoration of OD-plasticity was mediated by an increase of open eye responses in V1. (A) Time-line of imaging experiments: Mice were raised in standard cages to an age of ≥PD116 and then V1-activity induced by visual stimulation of the ipsi- and contralateral eye was visualized (imaging 1). Directly after imaging 1, mice were dark-exposed for 14 days, the right eye was closed and a second imaging was performed 7 days later in the same animals (imaging 2). (B) Optically imaged activity maps after stimulation of the contralateral (contra) and ipsilateral (ipsi) eye in the binocular region of V1 before (upper row) and after DE and 7 days of MD (lower row). Data display as in Fig. 1. Before DE and MD, V1 was dominated by input from the contralateral eye. After DE and 7 days of MD, there was a significant OD-shift towards the open eye. (C) OD-indices of mice before (yellow) and after MD (blue) (n = 5). (D) V1-activation after visual stimulation of the deprived (contralateral) and open (ipsilateral) eye before and after MD: after DE and MD, open eye responses increased significantly while deprived eye responses did not change. Scale bar: 1 mm.

whether DE would be sufficient to also rescue OD-plasticity in really old mice. This is an important question since there are many age-related changes in V1-circuitry (Lehmann et al., 2012) and it is not clear whether nearly 2 years old mice would react similarly to the DE-intervention as e.g. 4 month old mice. To test the therapeutic potential of DE to restore plasticity also in late adulthood, we raised mice to an average age of PD514 (age range PD461-543), exposed them to complete darkness for 14 days and subsequently imaged V1-activities 7 days later with or without MD. As in the 4–5 month old mice, 7 days of MD in the old DE-mice were sufficient to induce a significant OD-shift towards the open eye: the activity spot in V1 induced by stimulation of the open (ipsilateral) eye was as dark as the one induced by stimulation of the deprived (contralateral) eye, the ODI decreased significantly, the ODI-histogram was shifted to the left and colder colors predominated in the 2-dimensional OD-map (Fig. 1E,F). On average, the ODI of the old DE-mice decreased from 0.26 ± 0.02 (n = 5) without MD to 0.01 ± 0.02 after 7 days of MD (n = 7; P < 0.001, *t*-test) (Fig. 2A). Hence, 14 days of DE completely restored OD-plasticity in V1 of old mice with as little as 7 days of MD and up to an age of at least PD564 (oldest animal tested). Quantitative analyses of V1-activation further showed that open eye responses in V1 increased slightly (but not significantly) from 0.87 ± 0.12 (n = 5) to 1.19 ± 0.15 after 7 days of MD (n = 7; P > 0.05, *t*-test) while deprived eye responses remained nearly unchanged (no MD: 1.47 ± 0.17, n = 5; with 7 days of MD: 1.40 ± 0.18, n = 7; P > 0.05, *t*-test) (Fig. 2B). Sensory responses in V1 of PD535 mice were slightly but not significantly lower compared to PD138 mice (P > 0.05 for both eyes, *t*-test).

3.4. Diazepam prevented OD-shift in adult DE-mice (PD154)

Since it was previously shown that short-term DE modified inhibitory circuitry in young adult rat V1 (He et al., 2006; Huang et al., 2010), we wondered whether the restored OD-plasticity in adult DE-mice was mediated by a similar mechanism. Therefore, we pharmacologically enhanced GABAergic inhibition by injecting diazepam in vivo. One daily injection of diazepam (1 mg per kg mouse, i.p.) during the 7 day MD-period reliably prevented the OD-shift in the adult DE-mice but not in saline-treated controls: ODI-values after 7 days of MD in diazepam-treated mice were 0.34 ± 0.05 (n = 4, PD155-157) and thus significantly higher than ODI-values of saline-treated DE-mice (0.06 ± 0.03, n = 4, PD151-156; P < 0.01, *t*-test) (Fig. 2A). Values of saline-treated DE-mice after MD were indistinguishable from untreated DE-mice with MD (P > 0.05, B *t*-test). Thus, diazepam treatment reliably prevented the restoration of OD-plasticity after DE in adult mice, suggesting that the DE-effect is most likely mediated by a reduction of intracortical inhibition.

3.5. The number of PV^+-cells and PNNs was reduced by short-term DE in adult mice (PD138)

Since inhibitory PV^+-interneurons and PNNs are thought to play a crucial role for OD-plasticity (Pizzorusso et al., 2002; Sugiyama et al., 2008; Carulli et al., 2010; Beurdeley et al., 2012), we visualized the number of PV^+-cells and PNNs per mm^2 in the visual cortex of the same DE- and LR-mice that were used for optical imaging (age range PD125-157) as an additional signature for modified inhibitory circuitry. We used triple immunofluorescence staining for PV^+-cells-, PNNs and DAPI to visualize cell nuclei and cortical layers in animals without and with 7 days of MD. PV^+-cells and PNNs were found throughout layers II–IV in the visual cortex of both LR- and DE-mice. The number of PV^+-cells in adult DE-mice was significantly lower compared to the age-matched LR-mice regardless of whether they were monocularly deprived (DE: 49 ± 1 vs. LR: 66 ± 1, n = 3 mice in both; P < 0.001, *t*-test) or not (DE: 55 ± 1 vs. LR: 63 ± 1, n = 3 in both; P < 0.05, *t*-test; Fig. 4). In addition, the percentage differences in PV^+-cell numbers between the two hemispheres of deprived mice were similar to non-deprived animals (P > 0.05 between all groups, *t*-test). Since there was no significant difference between PV^+-cell numbers in animals without and with 7 days of MD for either rearing condition (DE and LR; P >0.05, *t*-test), we pooled DE and pooled LR values. Pooled PV^+-cell numbers were significantly lower in DE- compared to LR-mice (Fig. 5B; DE: 52 ± 1; LR: 64 ± 0.3, n = 6 in both; P < 0.001, *t*-test).

Similarly, the number of PNNs was lower in DE-mice compared to LR-mice after 7 days of MD (Fig. 4C,D) (with MD: LR: 84 ± 2; DE: 68 ± 3, n = 3 in both; P < 0.05, *t*-test; without MD: LR: 106 ± 11; DE: 73 ± 1, n = 3 in both; P > 0.05, *t*-test). Pooling the numbers of PNNs in animals with and without MD for both rearing conditions (percentage differences in the two hemispheres of deprived mice were similar to non-deprived animals; P > 0.05, *t*-test; DE and LR with and without MD; P > 0.05, *t*-test) again revealed significantly lower numbers in DE-mice compared to LR-mice (Fig. 4D) (LR: 95 ± 3, DE: 71 ± 1, n = 6 in both; P < 0.01, *t*-test).

Diazepam treatment during MD did neither modify PV^+-cell numbers nor PNN numbers compared to saline-treated or untreated DE-mice with MD (Fig. 4B,D) (PV^+: DE + MD + diazepam: 50 ± 1 vs. DE + MD + saline: 50 ± 2, n = 3 in both; P > 0.05, *t*-test; vs. DE + MD: 49 ± 1, n = 3; P > 0.05, *t*-test; PNNs: DE + MD + diazepam: 60 ± 2 vs. DE + MD + saline: 71 ± 2, n = 3 in both; P > 0.05, *t*-test; vs. DE + MD: 67 ± 3, n = 3; P > 0.05, B *t*-test).

Taken together, short-term DE not only restored OD-plasticity in adult and old mice, but additionally reduced both the number of PV^+-expressing cells and PNNs in the visual cortex of adult mice, supporting the conclusion of reduced intracortical inhibition as a major mechanism underlying the rescued OD-plasticity.

3.6. Short-term DE enhanced OD-plasticity in young adult mice (PD72)

Since it was recently shown that DE earlier in postnatal development did not facilitate OD plasticity in young adult rats (Huang et al., 2010), we additionally analyzed the effect of DE on OD-plasticity in young adult mice (age range: PD57-80; average: PD72). Usually, SC-mice of this age do not show a significant OD-shift after 4 days of MD (Lehmann and Löwel, 2008). We therefore tested whether DE can induce OD-plasticity after only 4 days of MD also in young adult mice. Indeed, after short-term DE for 10 days, 4 days of MD induced a strong and highly significant OD-shift towards the open eye. The OD-shift was as strong as usually only seen in 4-week-old animals (Hofer et al., 2006; Heimel et al., 2007; Lehmann and Löwel, 2008). Quantitative analyses of V1-activation showed that the average ODI decreased from 0.27 ± 0.03 (n = 5) without MD to 0.00 ± 0.02 (n = 6) after 4 days of MD (P < 0.001, *t*-test) (Fig. 5A) in DE-mice. The OD-shift was again mediated by a significant increase of open eye responses in V1: V1-activation after stimulation of the open eye increased from 0.91 ± 0.14 (n = 5) to 1.35 ± 0.08 after MD (n = 6; P < 0.05, *t*-test), while V1-activation after deprived eye stimulation did not significantly change (no MD: 1.61 ± 0.2; n = 5; with MD: 1.31 ± 0.09, n = 6; P > 0.05, *t*-test) (Fig. 5B). In contrast, in age-matched young adult LR-mice, 4 days of MD were not sufficient to induce an OD-shift towards the open eye (Fig. 5A) (ODI, without MD: 0.31 ± 0.01, n = 5; with MD: 0.28 ± 0.02, n = 5; P > 0.05; *t*-test) and the visually driven activity in V1 remained dominated by input from the deprived, contralateral eye: V1-responses were not different between mice without and with MD (contra, no MD: 1.75 ± 0.13, n = 5; with MD: 1.61 ± 0.22, n = 5; P > 0.05; *t*-test; ipsi, no MD: 0.93 ± 0.08, n = 5; with MD: 0.93 ± 0.15, n = 5; P > 0.05; *t*-test).

3.7. Short-term DE maintained OD-plasticity after a cortical stroke in adult mice which were already beyond their sensitive phase for OD-plasticity (PD150)

As we have previously shown, a photothrombotically induced small stroke lesion in S1 prevented OD-plasticity in V1 of 3-month-old SC-mice (Greifzu et al., 2011) while similarly old mice raised in an EE

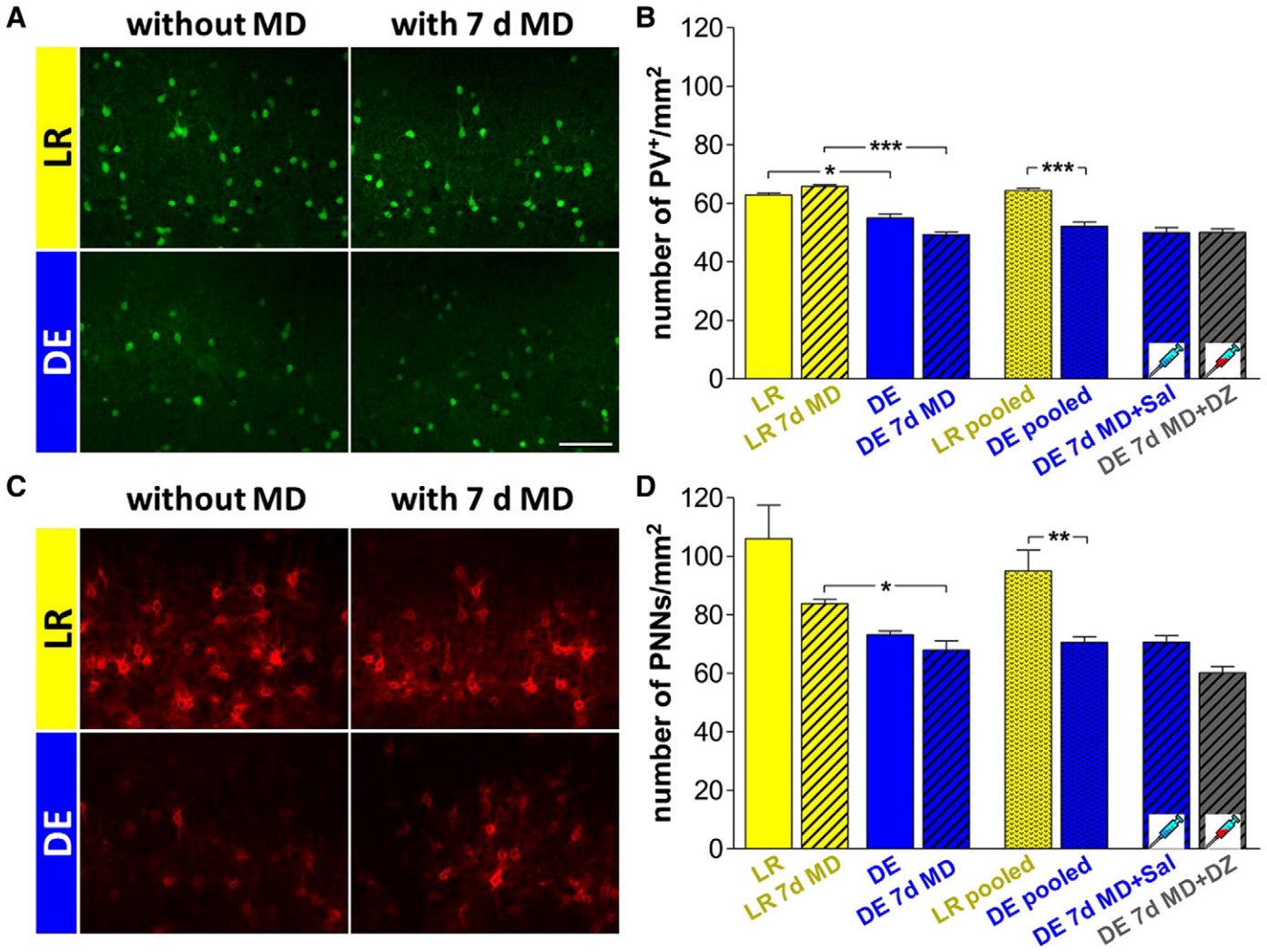

Fig. 4. Fourteen days of DE in adult mice (PD138) induced a significant decrease of the number of both parvalbumin (PV^+) cells and WFA-positive perineuronal nets (PNNs) compared to LR-controls. (A) Representative examples of immunofluorescence images of Cy2-labeled PV^+-cells (green, A) and Cy3-labeled PNNs (red, C) in the visual cortex of LR- and DE-mice before and after 7 days of MD. Scale bar: 100 μm. Number of PV^+-cells (B) or PNNs (D) per mm^2 visual cortex in all LR- (yellow) and DE-groups (blue), and in monocularly deprived DE-mice after saline (blue) or diazepam-treatment (DZ, gray). The number of both PV^+-cells (B) and PNNs (D) was significantly reduced in DE-mice compared to LR-mice. Saline- or diazepam-treatment during the 7 days of MD in DE-mice did not modify cell numbers compared to non-treated controls.

continued to display OD-plasticity after this lesion (Greifzu et al., 2014). To test whether short-term DE might also be used therapeutically to restore plasticity after a cortical stroke lesion, we raised another group of mice in SCs to an average age of PD128 (age range PD114-144). We used mice already beyond their sensitive phase for OD-plasticity to explicitly test whether the plasticity promoting effects of DE can also be exploited in the presence of a cortical lesion. To this end, we first induced a PT-lesion in S1 (or sham-treatment for control) and then transferred the animals into a darkroom for 14 days. Immediately after DE, animals were monocularly deprived (or not deprived for controls) and then transferred back to a normally lit room for the following 7 days to allow normal visual experience of the non-deprived eye. Finally we imaged V1-activity in both deprived and non-deprived DE-animals with or without the PT-lesion (age range PD135-165; average PD150).

PT-lesions were located in the left S1 and induced as described in Greifzu et al. (2011). In the PT-lesioned adult DE-mice, 7 days of MD induced a significant OD-shift towards the open eye. In effect, the OD-shift of PT-lesioned DE-mice was as strong as in sham-treated DE-mice with MD (Fig. 6). In the PT-lesioned DE-mice, the ODI decreased significantly from 0.28 ± 0.02 (n = 6) to 0.06 ± 0.03 after 7 days of MD (n = 7; $P < 0.001$, *t*-test). Likewise, in sham-treated DE-mice, the ODI decreased significantly from 0.26 ± 0.02 (n = 4) to 0.09 ± 0.01 after 7 days of MD (n = 3; $P < 0.001$, *t*-test). Thus, after MD in both PT- and sham-treated DE-mice, both eyes activated V1 more equally strong than without MD and V1-activation was no longer significantly different between the eyes ($P > 0.05$, *t*-test). Therefore, DE not only restored OD-plasticity in mice already beyond their sensitive phase for OD-plasticity, but additionally could counteract the lesion-induced impairments of cortical plasticity typically associated with a stroke lesion in S1 (Greifzu et al., 2011).

3.8. Visual cortical maps and basic visual performance were similar in DE- and LR-mice

3.8.1. V1-activation

To analyze a possible influence of DE on stimulus-driven activity in V1 we visualized elevation maps after visual stimulation with moving horizontal bars and azimuth maps after stimulation with moving vertical bars in PD72 and PD138 mice. Despite the reduced intracortical inhibition, the magnitude of sensory-driven activity in V1 was indistinguishable between DE- and LR-mice for both, elevation and azimuth maps at both ages ($P > 0.05$, B *t*-test for all comparisons; elevation: PD138 DE/LR: 2.2 ± 0.1/2.5 ± 0.2, n = 9/12; azimuth: PD138 DE/LR: 1.8 ± 0.1/1.9 ± 0.2 n = 9/12).

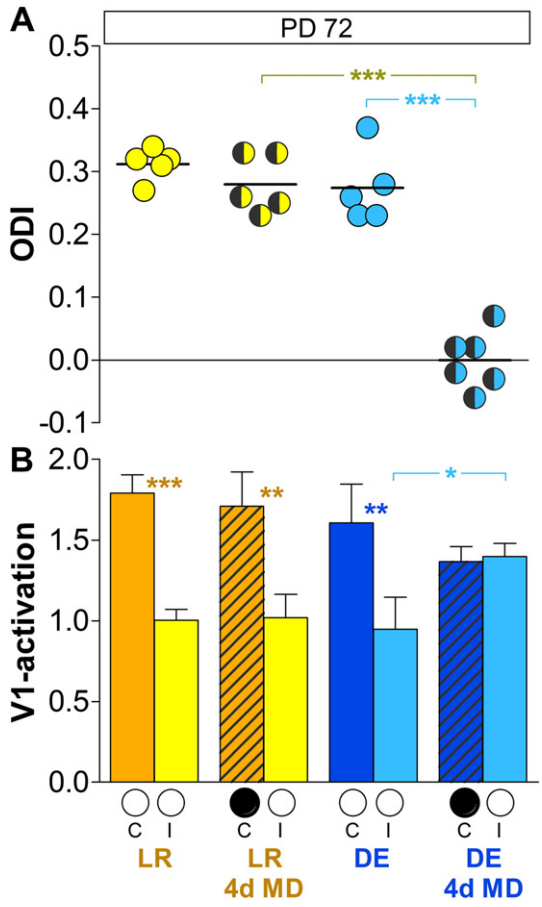

Fig. 5. Short-term DE restored OD-plasticity after 4 days of MD in young adult (PD72) mice. (A) Optically imaged OD-indices of control animals and after MD of the contralateral eye in LR- (yellow) and DE-mice (blue). Data display as in Fig. 2. (B) V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye in control animals and after MD. In DE- but not LR-mice, 4 days of MD were sufficient to induce a significant OD-shift towards the open eye, mediated by an increase of open eye responses in V1.

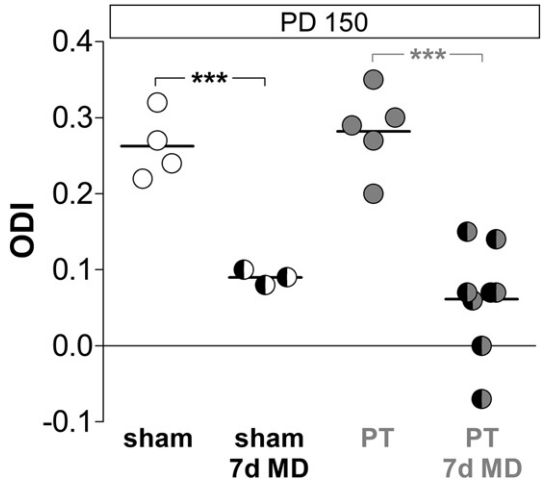

Fig. 6. Short-term DE rescued OD-plasticity in adult mice even after a stroke in the primary somatosensory cortex. Data are displayed as in Fig. 2. Optically imaged OD-indices of DE adult (PD150) sham-treated mice (white) or mice after a photothrombotically induced stroke (PT, gray) are illustrated. In spite of the PT-lesion, OD-plasticity was maintained in adult DE-mice.

3.8.2. Retinotopic maps

Quantification of retinotopic maps showed that short-term DE did also not affect map quality. Retinotopic map scatter was indistinguishable between DE- and LR-mice for both azimuth and elevation maps at both ages ($P > 0.05$ for all comparisons, B *t*-test; elevation: PD138 DE/LR 2.7 ± 1.2/1.7 ± 0.3, n = 9/12; azimuth: PD138 DE/LR 11.9 ± 1.8/13.5 ± 1.5, n = 9/12).

3.8.3. Visual acuity and contrast sensitivity

We used a virtual-reality optomotor setup (Prusky et al., 2004) to test whether short-term DE affected basic visual parameters compared to light-reared controls. In particular, we determined i) the highest spatial frequency ("visual acuity") gratings and ii) lowest contrast (contrast sensitivity) that elicited an optomotor response in animals of all experimental groups and iii) the experience-dependent increase of open eye acuity/contrast sensitivity after MD with daily training in the optomotor setup (Prusky et al., 2006). DE did not have any effect on baseline visual acuity (PD72: DE/LR: 0.39 ± 0.001/0.39 ± 0.002 cyc/deg, n = 5/5; $F_{2,11} = 0.71$, $P > 0.05$, B ANOVA; PD138: DE/LR: 0.38 ± 0.000/0.38 ± 0.001 cyc/deg, n = 10/13; $F_{2,27} = 0.30$, $P > 0.05$, B ANOVA). As reported previously (Lehmann et al., 2012), age had an effect on basic visual acuity of the tested mice: while values were not significantly different between PD72 and PD138 DE-mice ($F_{2,16} = 1.02$, $P > 0.05$, B ANOVA), visual acuity was significantly reduced in the old DE-mice (PD535: 0.35 ± 0.006 cyc/deg, n = 5; $F_{2,19} = 67.41$, $P < 0.001$, B ANOVA).

3.8.4. Experience-enabled increase in visual acuity and contrast sensitivity after MD

After MD and daily training in the optomotor setup, both the spatial frequency threshold and the contrast sensitivity threshold of the optomotor reflex of the open eye increased similarly in age-matched DE- and LR-mice ($P > 0.05$, *t*-test for all comparisons). In DE PD138 mice, visual acuity of the open eye increased by 21 ± 1% from 0.39 ± 0.001 cyc/deg to 0.47 ± 0.001 cyc/deg after 4 days of MD (n = 4; $P < 0.001$, *t*-test) and by 27 ± 2% to 0.49 ± 0.006 cyc/deg on day 7 (n = 8; $P < 0.001$, *t*-test); this enhancement was not different from LR-mice (increase by 21 ± 0.2%: from 0.39 ± 0.001 cyc/deg to 0.47 ± 0.001 cyc/deg after 4 days of MD (n = 5, $F_{2,25} = 0.375$; $P > 0.05$, B ANOVA) and by 27 ± 2% to 0.49 ± 0.07 cyc/deg after 7 days of MD (n = 7; $P < 0.001$, *t*-test)). Likewise, thresholds increased similarly in PD72 DE- and LR-mice ($F_{2,12} = 56.79$, $P > 0.05$, B ANOVA).

In line with our previous observations (Lehmann et al., 2012), the experience-dependent increase of the spatial frequency threshold of the optomotor reflex of the open eye after MD was significantly reduced in old mice. In PD535 DE-mice, visual acuity values of the open eye increased by only 12 ± 1% from 0.35 ± 0.006 cyc/deg on day 0 to 0.39 ± 0.004 cyc/deg on day 7 (n = 7; $P < 0.001$, *t*-test). This increase was significantly different from values in PD138 DE-mice in which acuity increased by 27 ± 2% ($F_{2,19} = 37.85$, $P < 0.001$, B ANOVA).

3.8.5. Contrast sensitivity

Short-term DE did neither have an effect on baseline contrast sensitivity nor on the experience-dependent increase of contrast thresholds of the open eye after MD. Values were similar in both DE- and LR-mice for all measured frequencies (baseline contrast sensitivity: $P > 0.05$, B *t*-test, in all groups; MD-induced increase: at least $P < 0.01$, *t*-test in all groups).

3.9. Adult DE-mice had normal visual capabilities after a stroke (PD150)

In PT-lesioned adult DE-mice, visual acuity values of the open eye increased significantly from 0.38 ± 0.001 cyc/deg on day 0 to 0.48 ± 0.003 cyc/deg after 7 days of MD (n = 7; $P < 0.001$, *t*-test), corresponding to an increase of 24 ± 1% on baseline. This increase was as high as in sham-treated non-lesioned DE-mice with 7 days of MD (25 ± 1%; $P > 0.05$, *t*-test) in which visual acuity values of

the open eye increased from 0.39 ± 0.000 cyc/deg on day 0 to 0.49 ± 0.001 cyc/deg after 7 days of MD ($n = 3$; $P < 0.001$, *t*-test). Since it was previously shown that after PT and late MD (14 days after inducing the lesion), the increase in visual acuity of the open eye was indistinguishable from sham-treated animals (Greifzu et al., 2011), our results of normal visual capabilities and normal enhancement of the spatial frequency threshold of the optomotor reflex after PT are perfectly in line with the previous data. Contrast sensitivity values were also not significantly different between PT- ($n = 6$) and sham-treated mice without MD ($n = 4$, $P > 0.05$, *t*-test) and increased similarly in both groups after 7 days of MD.

4. Discussion

Our results demonstrate that brief DE restored OD-plasticity in V1 of adult and aging SC-mice until at least PD564. The rescued OD-shift in DE-mice was most likely mediated by reduced intracortical inhibition since application of diazepam during the MD-period completely abolished the rescue of plasticity, and the numbers of both PV^+-cells and PNNs were reduced in DE-mice compared to LR-mice. Finally, DE preserved OD-plasticity in aging mice even after a cortical stroke in S1. In contrast, visual cortical maps and basic visual performance were similar in DE- and LR-mice, and DE also did not influence experience-dependent sensory learning after MD.

These findings are consistent with studies showing that DE in young adult rats (<PD100) promoted OD-plasticity most likely by reducing intracortical inhibition (He et al., 2006) and reactivated synaptic plasticity in adult V1 (Montey and Quinlan, 2011). Extending these observations, we show here that DE restored OD-plasticity in much older animals and even after a cortical stroke. To understand the mechanisms underlying the restored OD-plasticity in aging mice after DE, we applied diazepam during the MD period. Diazepam has previously been used to increase $GABA_A$-receptor mediated inhibition in mouse V1 (Hensch et al., 1998; Fagiolini et al., 2004). In V1 of SC-mice, the inhibitory tone increases during postnatal development and GABA-levels exceeding a certain limit may preclude plasticity in adult animals (Espinosa and Stryker, 2012; Levelt and Hübener, 2012). Application of diazepam with a dosage that reliably blocked OD-shifts in 3-month-old SC-mice (Greifzu et al., 2014) in fact blocked OD-shifts in V1 of our DE-mice, indicating that DE exerts its effect most likely by reducing intracortical inhibition. This interpretation is supported by the data of He et al. (2006) showing that restored OD-plasticity was accompanied by reduced numbers of $GABA_A$-receptors relative to AMPA-receptors and also by the reappearance of the juvenile form of NMDA receptors in V1.

To further analyze signatures of altered inhibitory circuits in the visual cortex of aging DE-mice, we quantified PV^+-interneurons that might play a crucial role in OD-plasticity (Espinosa and Stryker, 2012). It was hypothesized that the maturation of PV^+-cells opens the critical period for OD-plasticity (Hensch, 2005) which is then closed by the formation of PNNs around these cells (Beurdeley et al., 2012). Interestingly, short-term DE was accompanied by reduced numbers of both PV^+-cells and PNNs in our DE-mice compared to LR-controls. Reduced numbers of PV^+-cells most likely indicate a reduction of PV-expression rather than a disappearance of the cells. Whether reduced PV-expression correlates with reduced firing-rates of PV^+-cells is not yet clear; nevertheless, it may indicate reduced intracortical inhibition which in turn could promote OD-plasticity. Indeed, pharmacological reduction of PV^+-cell firing rates was recently shown to extend the critical period for OD-plasticity, while enhanced inhibition was shown to block it (Kuhlman et al., 2013). Active inhibition of PV^+-cells is also involved in the initial phases of reinforced associative learning in S1 (Froemke et al., 2007; Letzkus et al., 2011) and reduced numbers of GAD^+-cells accompanied the restoration of OD-plasticity by short-term EE-housing (Sale et al., 2007; Baroncelli et al., 2010; Scali et al., 2012).

The prominent role of reduced intracortical inhibition for promoting OD-plasticity after DE does not rule out additional mechanisms. Enzymatic degradation of extracellular matrix components which arrange into PNNs at the end of the critical period restored OD-plasticity in adult rats (Pizzorusso et al., 2002). PNNs preferentially enwrap PV^+-cells (Ye and Miao, 2013) and PNN formation may limit structural plasticity by forming a physical barrier creating a non-permissive state for plasticity (Moon et al., 2001; Sugiyama et al., 2009). Since short-term DE reduced the number of PNNs compared to LR-controls, the reduced expression of PNNs most likely has contributed to the increased OD-plasticity after DE. A reduced PNN-density was recently also reported after short-term EE-housing in rat V1 which increased plasticity (Sale et al., 2007; Scali et al., 2012). Likewise, orthodenticle homeobox 2 homeoprotein binding to PNNs might regulate plasticity: a transient loss of Otx2 lead to a reduction of both PV^+ and PNN expression in adult mouse V1 and reopened plasticity of visual acuity after MD (Beurdeley et al., 2012). Furthermore, neuromodulatory desensitization and an increase in structural factors that inhibit neurite remodeling have also been implicated in closing the sensitive phase for OD-plasticity (reviewed in Bavelier et al., 2010; Espinosa and Stryker, 2012; Levelt and Hübener, 2012).

Surprisingly, DE also rescued OD-plasticity in adult mice (PD150) after a cortical stroke. In particular, V1 of lesioned adult DE-mice reacted to an MD like sham-treated adult DE-animals, which was previously only observed in juvenile SC-mice and in adult EE-mice (Greifzu et al., 2014). Thus, V1 of adult DE-mice reacted to the stroke lesion like 4-week-old SC-raised mice. This cannot be due to a delay between PT and MD in the present study (2 weeks of DE in between) since we have previously shown that OD-plasticity remained absent in PT-lesioned mice even if MD was induced with a delay of two weeks after the PT (Greifzu et al., 2011). Since both juvenile SC- and adult EE-mice continue to show OD-plasticity after a S1-stroke and have a juvenile GABA/AMPA ratio, our new observations thus indicate that a V1 with a reduced inhibitory tone has a reduced susceptibility for stroke-induced impairments of cortical plasticity. In addition, short-term DE is highly effective: just 2 weeks of DE were sufficient to rescue OD-plasticity after MD, even in the presence of a cortical stroke lesion, and in aging animals already very far beyond their sensitive phase for OD-plasticity.

While it was recently shown that DE earlier in postnatal development (in PD35 rats), did not facilitate OD-plasticity (Huang et al., 2010), short-term DE was able to enhance OD-plasticity in our PD72 mice: at this age, 4 days of MD are not sufficient to induce a significant OD-shift in LR-mice (this study and Lehmann and Löwel, 2008). In contrast, a brief dark exposure (10 days) rescued OD-plasticity after 4 days of MD. Thus, DE promotes OD-plasticity in mice of any age between PD57 and at least PD564. Whether DE would facilitate OD-plasticity at even younger ages was not tested, since even LR-mice may show OD-plasticity after 4 days of MD. In addition, increasing OD-plasticity at an age where it is still present but reduced may depend on different mechanisms than restoring plasticity in aging mice already beyond their sensitive phase for plasticity.

Interestingly, the restored OD-shifts of our adult and aging DE-mice were always mediated by an increase of open eye responses after both 4 and 7 days of MD, without accompanying reductions of deprived eye responses in V1. While this is consistent with literature findings reporting increases in open eye responses in V1 after 5–7 days of MD in adult SC-raised mice ("adult" plasticity; Sawtell et al., 2003; Pham et al., 2004; Tagawa et al., 2005; Hofer et al., 2006; Mrsic-Flogel et al., 2007; Sato and Stryker, 2008) brief dark exposure in P70-P100 rats has recently revealed both a rapid depression of the V1-responses to stimulation of the deprived eye after 3 days of MD and a simultaneous potentiation of non-deprived eye responses (He et al., 2006). Decreases of deprived eye responses have previously been observed in 4-week-old SC-raised mice after 4 days of MD (Frenkel and Bear, 2004; Hofer et al., 2006; Sato and Stryker, 2008), and are generally taken as a signature of "juvenile" OD-plasticity. Interestingly, adult mice raised in an EE also displayed reductions of deprived eye responses after 7 days of MD, in addition to

having a juvenile GABA/AMPA ratio (Greifzu et al., 2014). Taken together, our new data indicate that short-term DE in adult and aging mice restored OD-plasticity of the adult form.

It has recently been shown that the elimination of inhibitory synapses on distal apical dendrites of layer 2/3 pyramidal neurons is a major component of adult OD-plasticity and resulted in increased responsiveness of V1 to stimulation of the non-deprived eye (van Versendaal et al., 2012). This raises the question whether short-term DE might promote structural changes in inhibitory boutons on dendritic spines and thus contributes to the disinhibition of inputs serving the non-deprived eye. While inhibition is reduced in V1 after DE, additional changes of excitatory circuitry like the reported reduction in the ratio of NR2A to NR2B subunits in dark reared or DE-rats that can be reversed rapidly upon re-exposure to light (Quinlan et al., 1999; Bear, 2003; Guo et al., 2012) could contribute to the observed increases in open eye responses after MD in the present study. In fact, a low level of inhibition relative to excitation may be required to allow V1 to respond rapidly to manipulations in visual input (He et al., 2006) and establish a milieu that is more permissive to Hebbian types of plasticity that are normally quite limited in the adult compared with developmental critical periods (Fagiolini and Hensch, 2000; Rozas et al., 2001; Chen and Nedivi, 2013). According to the sliding threshold hypothesis, short-term DE could thus lower the "modification threshold" to enable visual experience to drive an increase of the non-deprived (ipsilateral) eye responses in V1 (Bear, 1995; Kirkwood et al., 1996).

Surprisingly, reduced intracortical inhibition did not lead to an overall enhancement of visual responses in the DE-mice. We have recently reported a similar observation in mice raised in an enriched environment that also had reduced intracortical inhibition (Greifzu et al., 2014). Our interpretation of both findings is that homeostatic mechanisms must have compensatorily downregulated the excitatory drive.

The slight reduction of sensory activation in V1 of PD535 mice compared to PD138 mice is consistent with a previous study of our group (Lehmann et al., 2012) that showed a significantly reduced V1-activation in 23-month-old mice compared to 7-month-old mice. Thus, a significant dampening of visual responses is indeed expected to occur in mouse V1, but only at a later time during aging.

4.1. Conclusion

Taken together, our data clearly show that brief DE not only helped to enhance and restore OD-plasticity in adult and aging mice, and thus presumably throughout life, but additionally also protected V1 from lesion-induced impairments of OD-plasticity. Thus, DE offers a promising, non-pharmacological and highly effective tool for restoring and preserving plasticity of adult and especially aging neuronal circuits.

Conflict of interest

The authors have read the disclosure of potential conflicts of interest and have nothing to declare.

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