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Social experience modulates ocular dominance plasticity differentially in adult male and female mice

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ABSTRACT

Environmental factors have long been known to regulate brain plasticity. We investigated the potential influence of social experience on ocular dominance plasticity. Fully adult female or male mice were monocularly deprived for four days and kept a) either alone or in pairs of the same sex and b) either in a small cage or a large, featureless arena. While mice kept alone did not show ocular dominance plasticity, no matter whether in a cage or in an arena, paired female mice in both environmental conditions displayed a shift of ocular dominance towards the open eye. Paired male mice, in contrast, showed no plasticity in the cage, but a very strong ocular dominance shift in the arena. This effect was not due to increased locomotion, since the covered distance was similar in single and paired male mice in the arena, and furnishing cages with a running wheel did not enable ocular dominance plasticity in cage-housed mice. Confirming recent results in rats, the plasticity-enhancing effect of the social environment was shown to be mediated by serotonin. Our results demonstrate that social experience has a strong effect on cortical plasticity that is sex-dependent. This has potential consequences both for animal research and for human education and rehabilitation.

Introduction

All mammals are social animals. Social interaction is vital for their well-being and optimal neural functioning (Hendrichs, 1978; Liu et al., 2012), and social experience modulates cortex-dependent learning (Goeckner et al., 1973; Sterlemann et al., 2010). Social rearing of mice is sufficient to protect the prefrontal cortex to the same degree as an enriched environment from the detrimental effects of isolated rearing (Makinodan et al., 2012). Most recently, it was shown that colony housing of inbred mice in an enriched environment leads to increasing interindividual variability in hippocampal neurogenesis, which is correlated with exploratory behaviour (Freund et al., 2013). In the classical paradigm of cortical plasticity, i.e. the shift of ocular dominance after monocular deprivation (Gordon and Stryker, 1996; Wiesel and Hubel, 1963), however, an effect of social environment has as yet not been described, and even rather been discarded in rats (Baroncelli et al., 2012).

Ocular dominance plasticity in mice peaks during a critical period between postnatal days (PD) 22 and 35 (Gordon and Stryker, 1996), but can still be found during adolescence (Sawtell et al., 2003; Tagawa et al., 2005), until it ceases in adulthood, i.e. after PD 100 (Lehmann and Löwel, 2008). Beyond that age, we were unable to elicit a shift in ocular dominance even with prolonged periods, i.e. 14 days, of monocular deprivation. However, a variety of influences have been discovered in recent years that reinstate ocular dominance plasticity in adult animals: a period of dark exposure (Duffy and Mitchell, 2013; He et al., 2006, 2007), histone deacetylation (Putignano et al., 2007), fluoxetine treatment (Maya Vetencourt et al., 2008) and housing in an enriched environment (Greifzu et al., 2014; Sale et al., 2007). It has, moreover, been shown that environmental influences and fluoxetine treatment converge at an increased serotonergic transmission via the 5HT1A receptor, which in turn results in decreased GABAergic cortical inhibition enabling cortical plasticity (Harauzov et al., 2010; Maya Vetencourt et al., 2011; Sale et al., 2007).

Enriched environments comprise a multitude of different aspects with potentially different effects on neural function (Lehmann et al., 2009): They provide for visual stimulation, social interaction, learning opportunities and increased locomotion. A study in rats recently attempted to disentangle these factors and showed visual stimulation, increased locomotion and visual training to be effective in reinstating ocular dominance plasticity, whereas social experience was without effect (Baroncelli et al., 2012). In a recent study in fully adult mice, we showed that temporally highly coherent visual stimulation, presumably via spike-timing dependent plasticity, could induce critical period-like plasticity even after two days of monocular deprivation (Matthies et al., 2013). In the present study, we revisited the issue of social experience and reduced it to its fundamental function, i.e. the interaction

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of two individuals. We found a striking effect on ocular dominance plasticity, which was, however, synergistically dependent on spatial conditions.

Materials and methods

Animals and housing conditions

Male and female C57BL/6 mice older than postnatal day (PD) 110 at the start of monocular deprivation (MD) were reared in standard housing conditions, i.e. in sibling groups of the same sex kept in makrolon cages. In order to standardise the animals' previous experience, the animals were separated one week before MD and kept alone in type 2 cages (bottom inside dimension approx 190 mm × 100 mm, 125 mm high). After MD (see below), the animals were randomly assigned to the following two-by-two paradigm of experimental conditions for six hours per day: First, in the Single vs. Paired condition, mice were either kept alone throughout the deprivation period, or paired with a brother or sister, respectively. In this case, one mouse in each pair was shaved on a small spot on the back for distinguishing purposes; Second, in the Cage vs. Arena condition, they were either kept in a type 3 makrolon cage (210 mm × 160 mm × 125 mm) or an open field arena with a side length of 72 cm. After six hours, the animals were put back into their single cages. In the paired conditions, both animals were monocularly deprived. Control animals were treated identically.

Throughout the experiments, food and water were provided *ad libitum*. All experimental procedures have been performed according to the German Law on the Protection of Animals and the corresponding European Communities Council Directive of November 24, 1986 (86/609/EEC), and were approved by the Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (Thuringia State Office for Food Safety and Consumer Protection) under the registration number 02-027/11.

Monocular deprivation

For probing visual cortical plasticity, we monocularly deprived mice according to published protocols (Gordon and Stryker, 1996; Lehmann et al., 2012). In all cases, the right eyes were sutured shut. Animals were checked daily to make sure that the eyes remained closed; animals in which the eye was not completely closed were excluded from the experiments.

Optical imaging

Using the imaging method of temporally encoded maps (Kalatsky and Stryker, 2003), visual cortical responses in the left hemisphere were recorded as described previously (Lehmann and Löwel, 2008; Lehmann et al., 2012; Yeritsyan et al., 2012) under halothane (1% in 1:1 O_2/N_2O) anaesthesia, supplemented by chlorprothixene (0.2 mg/mouse, i.m.), atropine (0.3 mg/mouse, s.c.) and dexamethasone (0.2 mg/mouse, s.c.). With a 135 mm × 50 mm tandem lens configuration (Nikon, Inc., Melville, NY), we recorded optical images of intrinsic signals in a cortical area of 4.6 × 4.6 mm^2 at a wavelength of 610 ± 3 nm.

Horizontal drifting bars (2° wide), spaced 80° apart, were presented at a temporal frequency of 0.125Hz in the binocular visual field of the recorded left hemisphere (- 5° to + 15° azimuth) in front of the animal. Visual stimuli were presented alternately to the left and right eye. Ocular dominance indices (ODIs) were calculated as described previously (Cang et al., 2005; Lehmann and Löwel, 2008). Briefly, activity maps were thresholded at 30% of peak amplitude, and OD was calculated for each pixel in the binocularly responsive region as (contra-ipsi)/(contra + ipsi), and averaged across all selected pixels. At least three ODIs per animal were obtained and averaged; experiments with less than three ODIs were discarded.

Locomotor tracking

To assess locomotor activity in male mice, a video camera was set up to record the first hour in the arena. The movie was binned at 4 frames/second, and out of each quarter of an hour of recorded time, the first five minutes were clipped and used for quantification. Thus, twenty minutes were evaluated for each single and paired mouse in the arena. Freeware software for automated tracking (Tracker, Open Source Physics) was used to determine the position of the mice in each frame, and covered distance was thus calculated.

Behavioural analysis

Using the same recording setup, we quantified the behaviour of paired male animals during four five-minute-intervals in one hour after half-time in the condition. To this end, we defined an ethogramme based on a published template (Olsson and Sherwin, 2006). The following behaviours were defined and their frequency and duration quantified:

Non-social behaviours
- Locomotion – movements that result in a change of position
- Exploration – frequent rearing or active sniffing during locomotion
- Food intake – nibbling on food pellets or drinking

Social behaviours
- Huddling – peaceful body contact while lying
- Attack – jumping at or chasing the other mouse, biting, kicking, wrestling
- Flight – avoidance of contact, direct withdrawal from the other mouse
- Head sniff – sniffing directed to the head (mostly nose) of the other mouse
- Anal sniff – sniffing directed to the anus of the other mouse
- Social grooming – licking and nibbling the other mouse at different areas of the body

Post-mortem HPLC

Additional sets of male Single and Paired Arena animals were used for this experiment. The mice were monocularly deprived and exposed to the social conditions as described above. After optical imaging, the scalp was sutured and the animals were allowed to re-awake. The following day, they were again transferred to their respective condition. After six hours, the animals were killed by cervical dislocation, the brains were quickly dissected and frozen immediately at −40 °C.

Neurotransmitter contents were measured using high performance liquid chromatography (HPLC). Micropunches were taken from 1 mm V1 slices at −3.28 from Bregma and homogenized by ultrasonication in 20 vol of 0.1 N perchloric acid at 4 °C immediately after collection. A total of 100 ml of the homogenate was added to equal volumes of 1 N sodium hydroxide for measurement of protein content. The remaining homogenate was centrifuged at 17 000 g and 4 °C for 10 min. Supernatants were used for immediate measurement of 5HT and its metabolite 5HIAA via HPLC with electrochemical detection as previously described (Enard et al., 2009; Giovanoli et al., 2013; Winter et al., 2009). Briefly, the perchloric acid extracts were seperated on a column (Prontosil 120-3-C18-SH; length 150 mm, inner diameter 3 mm; Bischoff Analysentechnik und -geräte GmbH, Leonberg, Germany) at a flow rate of 0.55 ml/min. The mobile phase consisted of 80 mM sodium dihydrogene phosphate, 0.85 mM octane-1-sulfonic acid sodium salt, 0.5 mM ethylenediaminetetraacetic acid disodium salt, 0.92 mM phosphoric acid and 4% 2-propanol (all chemicals Merck KGaA, Darmstadt, Germany). Monoamines were detected using an electrochemical detector (41 000, Chromsystems Instruments & Chemicals GmbH, Munich, Germany) at an electrode potential of 0.8 V. For calibration, 0.1 M perchloric acid containing 0.1 mM 5HIAA and 5HT was

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injected into the HPLC system before and after sample analysis. Sample analysis was performed based on peak areas using a computer-based chromatography data system (CSW 1.7, DataApex Ltd, Praha, Czech Republic) in relation to the mean of the applied calibration solutions.

Drugs used

To investigate the role of the $5HT_{1A}$ receptor in adult ocular dominance plasticity, both control and MD mice in the Paired Arena condition were injected twice daily with 1 mg/kg b.w. i.p. of the specific antagonist WAY-100635 (Fletcher et al., 1996; Forster et al., 1995) dissolved in saline at the start and half-time of the six-hour arena interval in one set of experiments. For control, animals received equal volumes of vehicle. Rather than assigning one paired animal each to the control and the treatment conditions, we chose to treat both animals in a pair identically for two reasons: First, to avoid potential effects of altered social behaviour patterns if the treated animal's behaviour was changed; and second, to minimize the risk of mixing up the animals.

Statistical analyses

The influence of the independent experimental factors was analysed by a three-way ANOVA (cage type x group size x MD), which was followed by post-hoc testing using Student's t-test, which was Bonferroni-corrected with the number of comparisons in which each sample was used. For analysis of the behavioural data, an ANOVA with repeated measures was performed. The levels of significance were set as *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. All data are represented as means ± s.e.m..

Results

The opportunity for social interaction in a large arena reinstates ocular dominance plasticity in male mice

Male mice older than PD100 do not show ocular dominance plasticity under standard conditions (Lehmann and Löwel, 2008). Accordingly, four days of monocular deprivation (MD) failed to change ocular dominance in male Single Cage mice, and had, on average, no effect in Paired Cage mice, either. The representative maps shown in Fig. 1A illustrate that activity patches in both groups elicited by contralateral eye stimulation appeared darker than those acquired by ipsilateral eye stimulation both in control and 4d MD mice, and the OD map was coded in warm colours indicating a positive ODI. This was also the typical picture seen in Single Arena mice with and without MD. In Paired Arena mice, however, the image was dramatically different after MD: Contralateral eye maps were weaker than ipsilateral eye maps, and OD maps appeared typically in cold, blue-green colours.

Quantification and statistical analysis using three-way ANOVA confirmed that ocular dominance was influenced by MD ($F_{1,39} = 31.157$, $p < 0.001$), housing ($F_{1,39} = 6.184$, $p < 0.05$) and social condition ($F_{1,39} = 23.767$, $p < 0.001$), with significant interactions among all factors (housing x social: $F_{1,39} = 4.709$, $p < 0.05$; housing x MD: $F_{1,39} = 4.128$, $p < 0.05$; social x MD: $F_{1,39} = 15.723$, $p < 0.001$; housing x social x MD: $F_{1,39} = 10.938$, $p < 0.01$). This prompted for further post-hoc analysis (Fig. 1B, Bonferroni-corrected t-tests in all comparisons). In Single Cage mice, MD did not change ocular dominance (ODI control: 0.27 ± 0.01, n = 5, 4d MD: 0.2 ± 0.03, n = 7, $p > 0.3$). In Paired Cage mice, there was a certain decrease in contralateral bias (control: 0.22 ± 0.03, n = 5, 4d MD: 0.13 ± 0.03, n = 8, $p > 0.15$) that was not significant. There was, however, an apparently bimodal distribution. Closer inspection revealed that of the two mice in a pair, one would always show some plasticity, whilst the other was unplastic. In Single Arena mice, their ODI remained completely unaltered after 4d MD (control: 0.23 ± 0.02, n = 5, 4d MD: 0.22 ± 0.02, n = 4, $p \sim 1$). In Paired Arena mice, however, quantification confirmed the qualitative impression that, indeed, OD decreased highly significantly after 4d MD from 0.23 ± 0.02 (n = 4) to −0.09 ± 0.03 (n = 9, $p < 0.001$). This shifted ODI was also significantly different from both Paired Cage ($p < 0.001$) and Single Arena mice ($p < 0.001$), showing that the synergistic concurrence of both the Arena and the Paired conditions is necessary to reinstate adult OD plasticity.

We compared the response amplitudes elicited by stimulation of either eye in order to elucidate the mechanism of this plasticity. Whereas the OD shift during the critical period is achieved by weakening of the deprived, contralateral eye, it mainly results from strengthening of the open, ipsilateral eye in adult plasticity (Frenkel et al., 2006; Hofer et al., 2006; Sato and Stryker, 2008). In Paired Arena mice, the response amplitude of the contralateral eye was $(1.91 \pm 0.12)x10^{-4}$ in control mice and dropped significantly ($p < 0.05$, t-test) to $(1.36 \pm 0.11)x10^{-4}$ after 4d MD, whereas the ipsilateral eye's response amplitude did not change significantly ($p = 0.16$), although it increased slightly from $(1.26 \pm 0.1)x10^{-4}$ in control mice to $(1.61 \pm 0.14)x10^{-4}$ after 4d MD. These results suggest that Paired Arena housing reinstated a critical period-like form of OD plasticity in fully adult male mice.

Increased locomotion cannot account for the restoration of ocular dominance plasticity

The opportunity to engage in motor behaviour has been shown to reinstate OD plasticity in adult rats (Baroncelli et al., 2012). Obviously, Arena mice could move more than Cage mice, and it seemed likely that Paired Arena might run around more than Single Arena mice. In order to determine whether this could explain the drastically enhanced OD plasticity in Paired Arena mice, we compared their locomotor behaviour with that of Single Arena mice, using automated tracking software. Paired mice tended to run longer distances within 20 min (90.05 m ± 7.41 m, n = 16) than Single mice (77.75 m ± 3.86 m, n = 6), but this difference was not significant (Fig. 2A, $p > 0.3$, t-test).

To further investigate the issue, we provided Cage mice with a running wheel during the period of MD. If locomotion contributed to visual cortical plasticity, Paired Cage mice would be expected to show a reduced ODI under this condition. This was, however, not the case (Fig. 2B). In both Single (0.21 ± 0.01, n = 4) and Paired mice (0.19 ± 0.06, n = 4), OD remained biased towards the contralateral eye after MD and was not significantly different from control ODIs (pooled Cage Single and Paired control values were used for comparison, $p > 0.3$ both, t-test), from each other ($p > 0.7$, t-test), nor from the respective groups without running wheels (cp. Fig. 1, $p > 0.25$ each, t-test). Like in Paired Cage mice without running wheel, however, ocular dominance plasticity in deprived Paired running wheel mice showed a high variance, which was due to the fact that in each of the two pairs observed, there was one rather plastic (ODIs of 0.03 and 0.16) and one unplastic mouse (0.27 and 0.31).

The social behaviour of paired male mice in the Cage vs. Arena conditions

If locomotion is not the decisive factor: What else can paired mice do in a large arena that they could not do in a standard cage? In order to tackle this question, we performed a behavioural analysis during four five-minute-intervals in the fourth hour of exposure of each day.

The differences in behaviour between the Cage and Arena conditions were surprisingly small. Most notably, agonistic behaviour hardly occurred at all in either condition. A single instance was observed in an Arena pair. Hardly surprisingly, Arena mice moved around more than Cage mice ($F_{1,8} = 5.894$, $p < 0.05$, ANOVA with repeated measures), but their locomotor activity decreased sharply during the first day, from 121 ± 20 s in the first hour (data not shown) to 56 ± 35 s in the fourth hour (Fig. 3A), and declined further to 5 ± 2 s on the fourth day. Cage mice mostly didn't move around at all; the means shown in Fig. 3A result from just two animals on the first and one animal on the second day, out of six, that showed some locomotion. In contrast,

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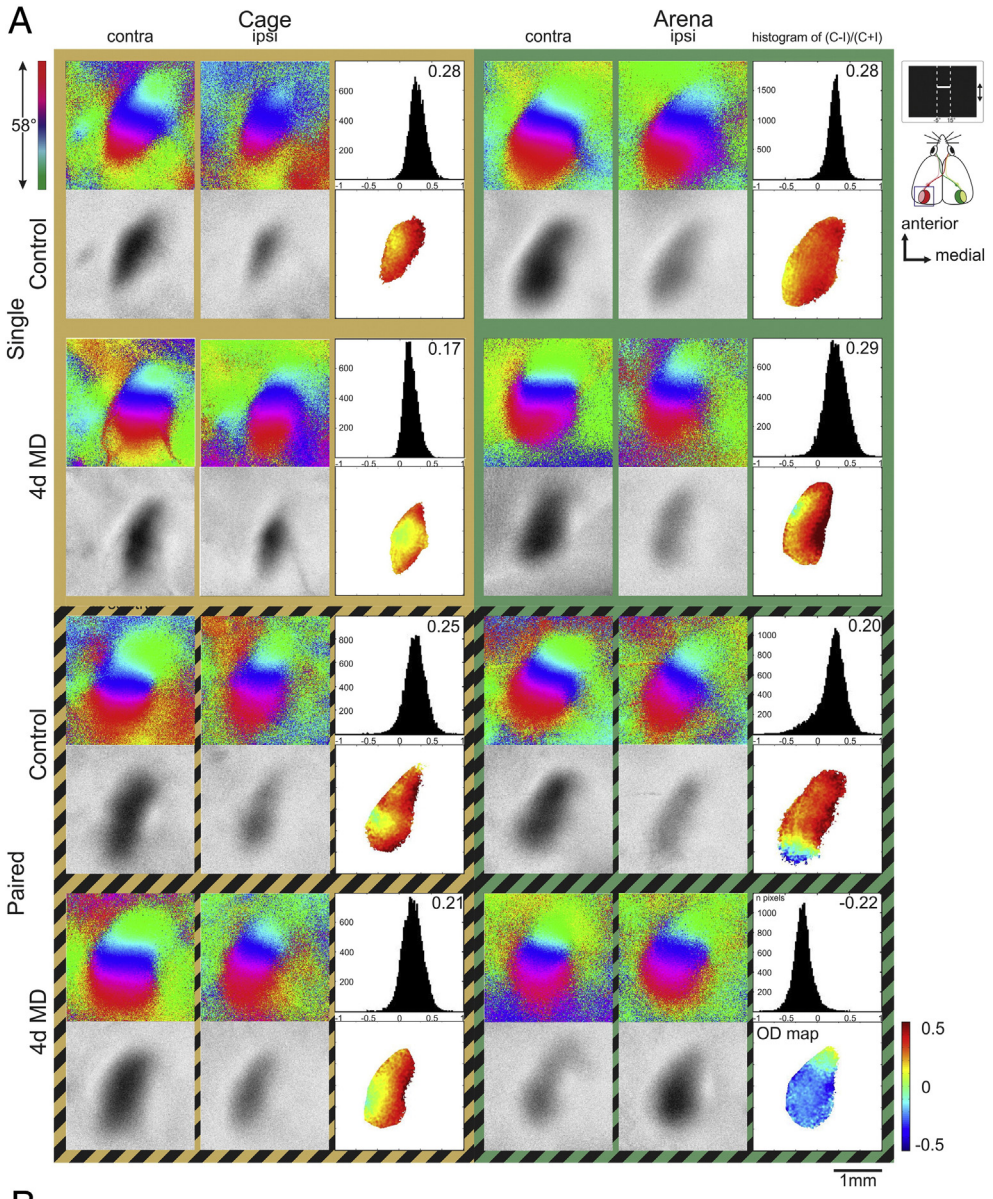

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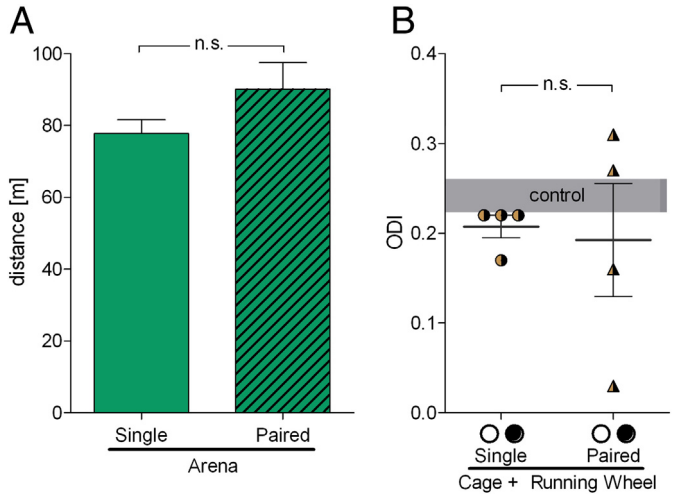

Fig. 2. Locomotor activity has no influence on adult ocular dominance plasticity in mice. (A) Running distance during 20 min was not different between Single and Paired Arena mice. (B) Cage mice provided with a running wheel during monocular deprivation did not show ocular dominance plasticity, irrespective of social condition. The grey control bar shows mean ± s.e.m. of pooled Cage control values (Fig. 1).

Cage mice showed significantly more exploration ($F_{1,8} = 9.98$, $p < 0.05$, Fig. 3B), which likewise declined in both groups over days ($F_{3,24} = 12.18$, $p < 0.001$).

What, then, did the animals do most of the time when they stopped moving around? They sat down together in a corner and huddled together (Fig. 3C). The time spent huddling increased over days ($F_{3,24} = 34.03$, $p < 0.001$) and was not significantly different between groups ($F_{1,8} = 0.205$, $p > 0.5$). This observation was confirmed when we tried to use the tracker programme in order to assess room utilisation (data not shown). Rather then setting up territories, as we had expected, the mice spent an increasing amount of time together in one corner of the arena.

The only unexpected and significant difference in social behaviour that we found between Cage and Arena pairs was a much higher amount of anal sniffing in the Cage mice ($F_{1,8} = 23.104$, $p < 0.001$, Fig. 3D). On the first day, the animals spent on average 33 ± 6 s on this behaviour, but only 1 ± 0.04 s in Arena mice. Although the duration of this behaviour decreased over days in Cage animals, it was still higher on the fourth day in Cage (13 ± 4 s) than Arena (1 ± 0.7 s) mice. Anal sniffing was performed by both mice of a pair to a similar degree without detectable asymmetry; very often, the mouse that showed more of this behaviour on one day would show less on the next.

Social experience reinstates ocular dominance plasticity in adult female mice irrespective of housing environment

While the data reported so far strongly indicates that a certain form of social organization modulates ocular dominance plasticity in male mice, the behavioural differences observed between Paired Cage and Arena mice may not seem sufficiently pronounced to support this conclusion. As an additional approach to test the assumption that social stress influences ocular dominance plasticity, we resorted to female mice, which are less aggressive and territorial than male mice and can be housed together without problems. Thus, the effect of paired housing during MD on ocular dominance plasticity should not depend on the Cage or Arena condition in female mice.

This is precisely what we found (Fig. 4). As in males, individually housed animals showed no shift in ocular dominance after 4d MD in either housing condition, but female both Paired Cage and Paired Arena mice showed a clear reduction in contralateral dominance after MD. Statistical analysis indeed confirmed a strong influence of MD ($F_{1,24} = 19.008$, $p < 0.001$) and social condition ($F_{1,24} = 32.312$, $p < 0.001$), but not of housing ($F_{1,24} = 3.731$, $p > 0.05$). Correspondingly, there was a significant interaction of MD with social condition ($F_{1,24} = 5.631$, $p < 0.05$), indicating that the effect of MD depended on whether the animals were kept alone or in pairs, but no interaction of MD with housing ($F_{1,24} = 1.099$, $p > 0.3$), confirming that the Cage vs. Arena condition did not play a role for ocular dominance plasticity. The interactions housing x social condition ($F_{1,24} = 0.515$, $p > 0.4$) and housing x social condition x MD ($F_{1,24} = 1.343$, $p > 0.25$) were likewise not significant.

Pairwise comparison using Bonferroni-corrected t-tests corroborated that MD did not change ocular dominance in individually housed female mice (Cage controls: 0.27 ± 0.01, n = 4, 4d MD: 0.18 ± 0.03, n = 4, $p > 0.12$; Arena controls: 0.2 ± 0.02, n = 4, 4d MD: 0.2 ± 0.04, n = 4, $p \sim 1$), but did in Paired animals both in the Cage condition (control: 0.19 ± 0.02, n = 4, 4d MD: 0.02 ± 0.04, n = 7, $p < 0.05$) and the Arena condition (control: 0.13 ± 0.02, n = 4; 4d MD: −0.03 ± 0.04, n = 6, $p < 0.05$). Paired 4d MD animals were also significantly different from their respective Single 4d MD groups (Cage: $p < 0.05$; Arena: $p < 0.01$). There was, however, no difference between the deprived Paired groups of the two housing conditions ($p \sim 1$), which contrasts with the significant difference observed in males. Correspondingly, there was a significant difference between the male vs. the female Paired Cage groups ($p < 0.05$), confirming that the females showed higher plasticity under this condition.

In both conditions, female Paired control mice appear to have a lower ODI than Single controls. This is significant in the Cage condition ($p < 0.05$). We cannot offer even a tentative explanation for this apparent effect, but wish to point out that it underlines the importance of always using the appropriate control groups.

Adult ocular dominance plasticity in socially interacting male mice is mediated by serotonin

It has been shown that increased serotonin transmission is able to induce adult OD plasticity (Maya Vetencourt et al., 2008, 2011), and that the plasticity-enhancing effect of an enriched environment can be mediated by serotonin (Baroncelli et al., 2010). We therefore checked whether serotonin transmission is also responsible for the reinstated adult OD plasticity in male Paired Arena mice.

To this end, we used an additional set of animals in which both optical imaging and post-mortem HPLC were performed after 4d MD and Arena housing. As expected, ocular dominance was significantly shifted towards zero in Paired (−0.1 ± 0.06) compared to Single Arena mice (0.22 ± 0.03) in these samples ($p < 0.01$, t-test, Fig. 5A). After having been returned to their respective housing conditions, the animals were sacrificed the following day, and a post-mortem HPLC analysis of

Fig. 1. Adult male mice kept pairwise in a large arena during monocular deprivation show ocular dominance plasticity. (A) Representative retinotopic maps from the left binocular visual cortices of adult male mice kept in a standard cage (Cage, left column, brown background) or open field arena (Arena, right column, green background) are shown. Additionally, the mice were either kept alone (Single, top half, uniform background) or in pairs (Paired, bottom half, hatched background). In each condition, maps from a control mouse (top) and a mouse monocularly deprived for 4d (4d MD, below) are displayed. Within each exemplary case, colour-coded phase maps of absolute retinotopy and grey-scaled amplitude maps of map activity are shown, as produced by stimulation of the contralateral (contra) and ipsilateral (ipsi) eye. The activities elicited by stimulation of both eyes are combined into an ODI. The bottom right panel depicts the ODI map colour-coded according to the bar on the right, the histogram on top demonstrates the pixelwise distribution and the mean ODI for the respective set of maps. Note that in control animals and in 4d MD animals of the Cage conditions and the Single Arena condition, the ODI maps have warm colours signifying contralateral dominance, whereas in the Paired Arena condition, the ODI map is mostly blue, indicating ipsilateral dominance. (B) ODIs of control and monocularly deprived animals of all experimental groups. A positive ODI indicates contra-, a negative ODI ipsilateral dominance. Each symbol represents the ODI of an individual animal, thick horizontal lines show the group mean. Full symbols represent control animals, half symbols MD animals. Cage animals are shown as brown, Arena animals as green symbols. Circles represent Single and triangles Paired animals.

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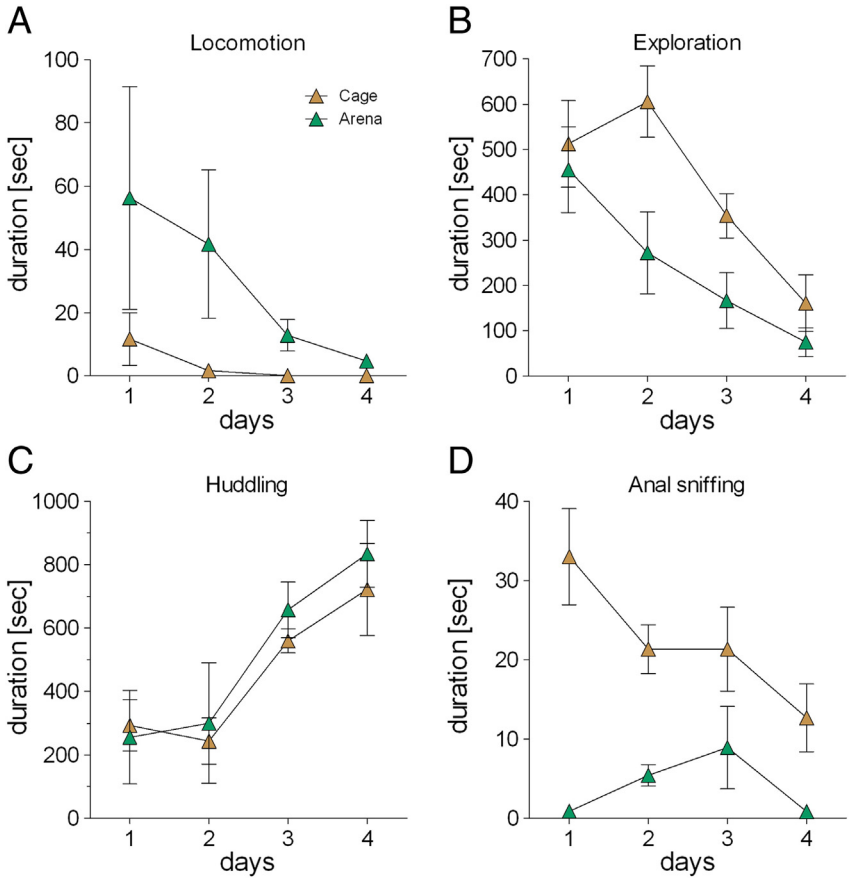

Fig. 3. Social interaction in Cage mice is different from Arena mice. Total durations of several behaviours during four intervals of five minutes within one hour are shown in Cage and Arena pairs of male mice. (A, B) Both locomotion and exploration decreased over days in both Cage and Arena mice. There was significantly (both $p < 0.05$) more locomotion and less exploration in Arena than in Cage mice. (C) In compensation, mice in both conditions spent a daily increasing amount of time huddling together, without a difference between the conditions. (D) Different total durations of anal sniffing ($p < 0.001$) in the two conditions indicate a difference in the social interaction between Cage and Arena mice. Other social behaviours (head sniffing, social grooming) were not significantly different, and there was only a negligible amount of open aggression (data not shown).

the visual cortices was performed (Fig. 5B). While serotonin content did not significantly change (Single: 7.12 ± 2.33 nmol/mg protein, Paired: 5.57 ± 1.13 nmol/mg protein, n = 4 each, $p > 0.5$, t-test), the concentration of its metabolite, 5-hydroxy-indole-acetic acid (5HIAA) was significantly increased in Paired Arena mice (2.92 ± 0.29 nmol/mg protein, compared to 1.45 ± 0.21 in Single Arena mice, $p < 0.01$, t-test), and so was, consequently, the 5HIAA/5HT ratio (turnover), which was 0.24 ± 0.03 in Single and 0.6 ± 0.13 in Paired mice ($p < 0.05$, t-test). Neither 5HIAA content nor 5HT turnover were significantly correlated to ODI on an individual level ($p > 0.1$).

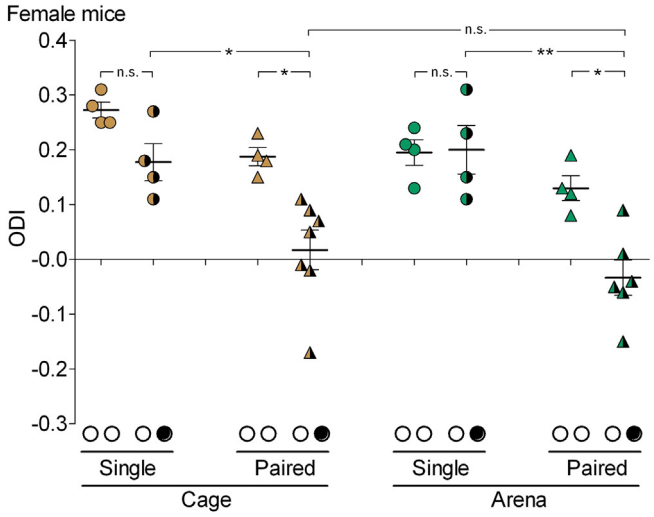

Fig. 4. Social experience induces ocular dominance plasticity in adult female mice, irrespective of housing conditions. Each symbol represents the ODI of an individual animal, thick horizontal lines show the group mean. Full symbols represent control animals, half symbols MD animals. Cage animals are shown as brown, Arena animals as green symbols. Circles represent Single and triangles Paired animals.

While these results indicated that an increased serotonin transmission might be involved in mediating the plasticity-enhancing effect of social experience, they could not prove that it was also necessary. Therefore, we next treated Paired Arena mice with the $5HT_{1A}$ receptor antagonist WAY-100635 or vehicle during the period of MD and social experience (Fig. 5C). Drug treatment by itself had no effect on OD (vehicle: 0.18 ± 0.01, WAY-100635: 0.15 ± 0.03, n = 4 each, $p > 0.4$). After MD, OD shifted highly significantly to 0.03 ± 0.02 ($n = 4$, $p < 0.01$, Bonferroni-corrected t-test) in vehicle-treated animals, but did not change significantly in animals treated with WAY-100635 (0.15 ± 0.03, $n = 4$, $p \sim 1$, Bonferroni-corrected t-test). There was a significant difference between vehicle and drug-treated mice ($p < 0.05$, Bonferroni-corrected t-test), confirming that $5HT_{1A}$ receptor activation is required to mediate the enhanced OD plasticity in Paired Arena mice.

Discussion

Social experience reinstated ocular dominance plasticity after four days of MD in fully adult male mice, but only if the animals were simultaneously living in a large, open environment. This effect was mediated by an increased serotonin transmission. Increased locomotion, in contrast,

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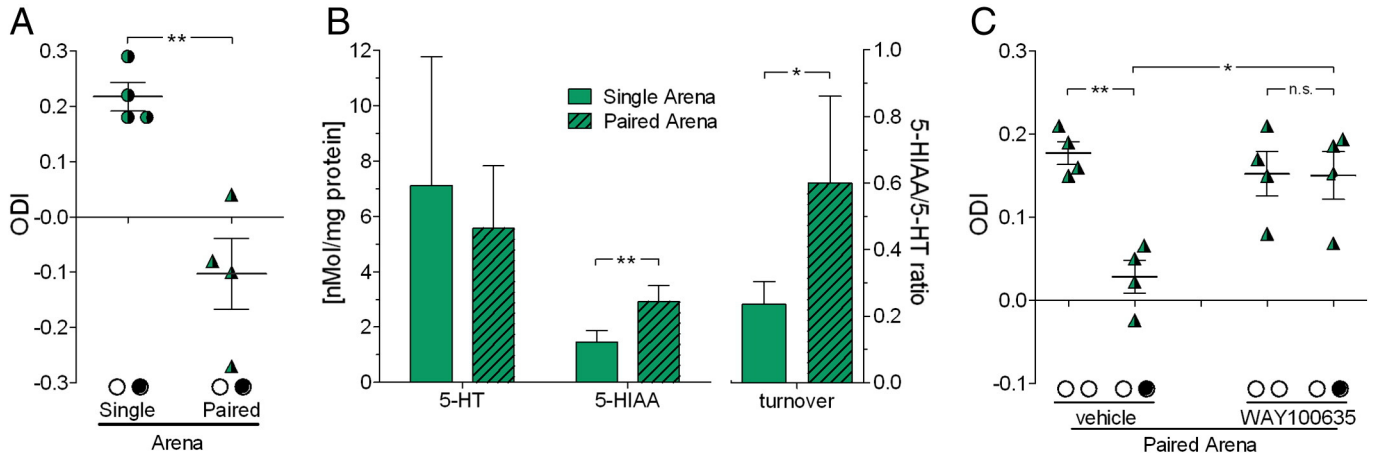

Fig. 5. Adult ocular dominance plasticity induced by social experience depends on $5HT_{1A}$ receptor activation. (A) An additional set of animals was exposed to the Single or Paired Arena conditions during 4d MD. Again, ocular dominance was shifted significantly towards the open, ipsilateral eye in the Paired group. (B) The contents of serotonin (5HT) and its metabolite 5HIAA were determined by HPLC in the visual cortices of the same animals. 5HIAA content and 5HT turnover (5HIAA / 5HT ratio) were significantly increased in Paired mice. (C) Counteracting 5HT at the $5HT_{1A}$ receptor by systemic application of WAY-100635 abolished adult ocular dominance plasticity in Paired Arena mice, whereas full plasticity was observed in vehicle-injected animals.

had no share in this effect and did not, by itself, increase OD plasticity in mice. Other than male mice, females, which are considered more sociable, showed increased plasticity even when pair-housed in a small cage.

In male mice, the sojourn in a large arena enabled OD plasticity only in the presence of another mouse, and in females, only the social, but not the housing condition influenced visual cortical plasticity. These observations exclude some alternative explanations for our findings: Neither novelty nor visual stimulation – recently shown to boost OD plasticity (Matthies et al., 2013) – were different between the Single and Paired conditions. We further verified that the amount of locomotion was not different between male Single and Paired Arena mice, and that wheel running did not increase OD plasticity in Cage mice, thus cancelling out physical activity as another alternative explanation. Moreover, the observation that OD in deprived male Paired, but not Single Cage mice also showed a highly variable, weaker, and not quite significant tendency towards the open eye, underlines the conclusion that social experience, rather than area size per se, is a central factor in regulating visual cortical plasticity in adult mice.

The social environment is of supreme importance to most mammals (Hendrichs, 1978; Makinodan et al., 2012). Forms of social organisation vary between species, but may also change within a species if the population density changes (Sachser, 1986). Mice adopt a territorial organization at low population densities, but switch into a dominance hierarchy if the population density increases (Davis, 1958). Keeping two male mice together in a small cage can possibly be regarded as a crowded condition, which has long been known to impair cortex-dependent learning in rats (Goeckner et al., 1973). Remarkably, paired housing appears to be more stressful for male mice than even living in a group of 16 animals, although basal corticosterone levels correlated with group size for one, four, eight and 16 animals in standard makrolon cages (28 cm × 21 cm) (Brain and Nowell, 1970).

Our results suggest that a much larger space allows male mice to establish a different, less stressful, social organisation. Our behavioural observations do not provide a conclusive picture of the social mechanisms in both conditions, but the significantly lower total duration of anal sniffing observed in Arena pairs indicates that the interaction pattern between the animals was changed in the larger enclosure. Anal or anogenital sniffing is not by itself an agonistic behaviour, but is frequently shown e.g. by a resident towards an intruder (Miczek and O'Donnell, 1978). Its frequent mutual performance in Cage pairs may suggest a higher need for social recognition in order to establish a stable relationship. The bimodal distribution of ocular dominance plasticity observed in monocularly deprived paired male mice in the cage, both with and without a running wheel, suggests that these pairs established a dominance hierarchy which we failed to detect during our behavioural observations. We performed additional experiments which showed that, in the running wheel cage, one mouse of a male pair would always engross access to the wheel, but more generally accepted measures of social dominance need to be applied before this issue can be clarified.

Our data are at odds with result that Baroncelli et al. (2012) obtained in rats. In their study, animals rendered amblyopic by MD during the critical period showed no recovery of OD or visual acuity after reverse suture when exposed to a condition of social stimulation. This condition, however, consisted in doubling both the number of rats per cage (six instead of three in the standard condition) and the cage size, such that the animal density remained high and almost unchanged. Since rats are more sociable than mice (Vestal, 1977), it would be surprising if social experience had less influence on neural function in rats than in mice. As for the conflicting findings concerning the effect of wheel running on OD plasticity, species differences seem to be the most likely explanation.

The brain's pervasive serotonergic innervation is highly responsive to environmental influences (Lehmann et al., 2003; Neddens et al., 2003). Our results confirm that serotonin mediates the effects of environmental – here: social – stimulation on OD plasticity in mice, as shown before in rats (Baroncelli et al., 2010; Maya Vetencourt et al., 2008, 2011). Downstream of neuromodulatory influences, GABA transmission is decreased by serotoninergic activation, thereby raising the excitation-inhibition ratio (Baroncelli et al., 2010; Maya Vetencourt et al., 2008). A recent study has confirmed that rearing mice in an enriched environment (encompassing social, motor and sensory stimulation and increased space) kept the ratio of GABA to AMPA currents in the visual cortex of fully adult mice at low, critical period-like levels (Greifzu et al., 2014). Systemically, serotoninergic transmission in the brain is closely, though not linearly, linked to the peripheral stress response (Linthorst and Reul, 2008), and corticosterone application has recently been shown to induce ocular dominance plasticity in adult rats (Spolidoro et al., 2011). These findings suggest a possible connection between social environment and cortical plasticity, which must, however, be rather complex, since we observed higher plasticity in presumably less stressful conditions (i.e., males paired in the arena, compared to cage; paired cage females compared to males).

In summary, we have shown in the present study that social experience has a strong effect on cortical plasticity in adult mice. This result underscores the necessity to mind social housing conditions in the research on neural plasticity. Since other forms of brain plasticity, like

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e.g. hippocampal cell proliferation and neurogenesis, are influenced by individual differences that are likely of social origin (Freund et al., 2013), and are mainly regulated by serotonin (Klempin et al., 2013), social experience may possibly have a pervasive impact on plasticity everywhere in the brain. Should this be confirmed, it would have implications also for learning and plasticity in human education and rehabilitation.

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