Enhancing visual cortical plasticity in mice by enriching their environment: a combined imaging and behavioural study

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Declaration

Herewith I declare that I wrote this thesis independently and with no other sources and aids than quoted.

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Table of Contents

ABSTRACT

Brain plasticity is important not only for normal brain functions like learning and memory, but is also crucial for recovery after injuries. It has been shown that the environment has a great influence on brain plasticity. Here, I investigate the impact of an enriched environment (EE) on ocular dominance (OD) plasticity of the mouse primary visual cortex (V1), using monocular deprivation (MD) as a model to trigger OD-plasticity and optical imaging of intrinsic signals to monitor it. Additionally, a variety of behavioural tests was used to measure the visual abilities of mice and their alteration after MD. OD-plasticity in V1 is an age-depended phenomenon: it is maximal during the critical period (postnatal day (PD) 21-35), reduced but still present in young adult mice (2-3 months) and absent in fully mature animals (beyond PD110). This age dependence holds true for mice raised in standard cages (SC), however we showed that raising mice in a more complex environment could not only prolong the sensitive phase for OD-plasticity into adulthood but also reinduce OD-plasticity in mice transferred to EE after PD110. Interestingly, the observed OD-plasticity in old EE-mice was similar to that in SC-mice during the critical period, suggesting that EE-housing resulted in a more juvenile brain. Additionally, we found that EE-raising can enable even lifelong OD-plasticity (up to PD900). Using behavioural tests we also showed that EE-raising did not affect the visual abilities of old mice and did not increase the interindividual variability. To test whether OD-plasticity in adult EE-mice is indeed juvenile-like, we tested different age groups of EE-mice after 4 days of MD. We found that 4 days of MD can induce an OD-shift in all the age groups of EE-mice tested, but the OD-shift in young and fully mature EE-mice was similar to adult OD-plasticity observed in around 3 month old SC-mice.

EE-raising provides mice with increased social interactions, physical exercise and cognitive stimulation compared to SC rearing. We asked the question, whether all components are needed or just one of them is already sufficient to prolong OD-plasticity. We tested whether voluntary physical exercise alone prolongs OD-plasticity by raising mice in SCs equipped with a running wheel (RW). RW-raised mice continued to show an OD-plasticity into adulthood, while mice without a RW did not. Moreover, running only for 7 days was sufficient to restored OD-plasticity in adult SC-raised mice. In addition, the OD-shift of RW-mice was mediated by a decrease in deprived eye responses, which was previously seen only in critical period SC-mice or in adult EE-mice.

It was previously shown, that a small lesion in the primary somatosensory cortex (S1) prevented both cortical plasticity and improvement of visual abilities in the adult mouse visual system after MD. However, in adult EE-mice, OD-plasticity was preserved after stroke induction and the improvement of visual abilities was partially preserved. Here, we investigated, whether raising mice in a cage with a RW will preserve OD-plasticity in old animals after a cortical lesion in S1, as well as the therapeutic effect of running after stroke on OD-plasticity. Our data suggest that physical exercise not only preserved but also restored OD-plasticity after a localized cortical stroke.

Additionally, we tested how long the positive effect of EE on OD-plasticity lasts, when mice are transferred to a less stimulating environment. For this purpose mice raised in EE until PD130 were moved to normal SCs and after a short period MD was performed. We found that already after 1 week in a SC, mice did not show OD-plasticity. We tried a pharmacological approach to restore OD-plasticity in those mice by administrating fluoxetine (selective serotonin reuptake inhibitor). However, treatment with fluoxetine did not preserve OD-plasticity. On the other hand, when mice were transferred from EE to a SC with RW, OD-plasticity was preserved.

Furthermore, we investigated the posibility of the effect of EE on OD-plasticity to be transferred to the next generation. To this end, after mating of EE-mice, pregnant dams were transferred to SCs few days before delivery. Offspring was raised exclusively in SCs up to at least PD120. We found, that offspring of EE-parents showed an OD-shift similar to EE-mice while age matched SC-mice did not. Additionally, we tested which parent is responsible for the transmitted effect of EE. For this purpose EE-females were mated with SC-males, or EE-males with SC-females, respectively. Only offspring of EE-mother and SC-father showed an OD-shift after MD. To summarize, the adult offspring of enriched parents still displayed a juvenile OD-plasticity in V1, even if they did not experience any EE and most likely the responsible parent is the mother.

1. Introduction

Neuronal plasticity is the ability of the brain to structurally and functionally reorganize in response to changes in sensory experience. Plasticity is fundamental for the proper development of the neuronal circuits of the brain, for enabling the brain to adapt to its environment, and also for recovery from pathological conditions and brain injuries e.g. stroke. During development, experience of environmental stimulation is continuously interacting with genetic information to shape neuronal circuits and the resulting behaviour.

1.1. Visual system and OD-plasticity

Most of our impressions of the world and our memories of it are based on sight. The human visual system shares a lot of similarities with the mouse visual system. The visual processing begins in the multilayered retina and the information is then transmitted as action potentials in the optic nerve that extends to a midline crossing point, the optic chiasm. Beyond the optic chiasm the nerve fibers from the medial (nasal) part of the retina cross to the other hemisphere of the brain, whereas nerve fibers from the lateral (temporal) part of the retina continue to the hemisphere of the same side. After that the axons from nasal and temporal parts carrying information bound for one hemisphere are forming the optic tract which projects to the lateral geniculate nucleus (LGN) of the thalamus. The thalamic neurons transmit the information through the optic radiation to the primary visual cortex (V1; Figure 1).

The crossing of fibers in the optic chiasm leads nerve fibers from the left part of both eye retinae to project to the left hemisphere and from the right part of retinae to right hemisphere (Dräger and Olsen, 1980). The left part of the retinae is activated by visual stimuli in the right visual field and the right part of the retinae receives information from the left visual field. Thus the visual cortices will process information about the contralateral visual hemifield, with some degree of binocular overlap in the frontal visual field. The spatial arrangement of visual stimuli in the visual field and the resulting stimulation pattern of the retinae preserved throughout the visual pathway: adjacent stimuli in the visual field activate adjacent neurons in V1. The preservation of the spatial arrangement of inputs from retina is referred to as retinotopy and a neuronal map of the visual field as retinotopic map.

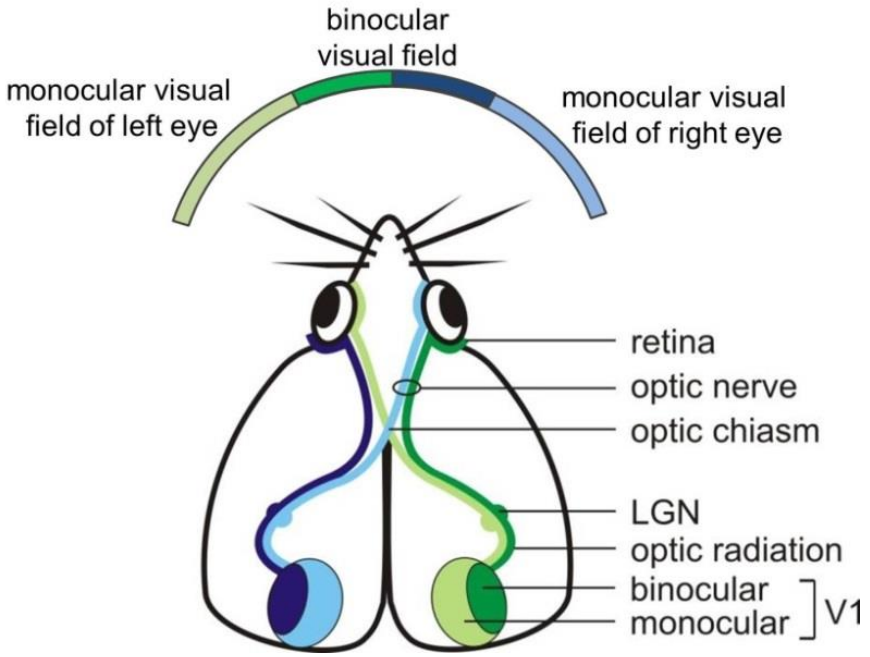


Figure 1: Representation of the mouse visual field in the visual pathway. Left and right visual fields and their respective representations in the visual pathway are illustrated with green and blue colors. The visual information originating from the nasal part of the retina crosses to the other hemisphere in the optic chiasm (light blue and light green for right and the left eye respectively). Whereas visual information from the temporal part of the retina continues within the same hemisphere (dark blue and dark green for the right and left eye respectively). From the retina visual information reaches the lateral geniculate nucleus (LGN) where it is relayed to V1. While the binocular part of V1 receives input from both eyes, the monocular part of V1 receives input from the contralateral eye only. Figure modified from Greifzu et al. (2012).

V1 is divided into two parts: the monocular and the binocular. Neurons of the monocular part are exclusively activated by visual stimulation of the contralateral eye, whereas neurons in the binocular part can be activated by visual stimulation of both eyes (Dräger, 1975). The binocular zone is located at the lateral side of V1 and occupies about one third of it. The frontal part of the visual field is represented in the retina of both eyes and therefore in the binocular zone of V1 (Gordon and Stryker, 1996). The binocular visual cortex of mice shows stronger responses to stimulation of the contralateral eye and weaker responses to ipsilateral eye stimulation (Dräger, 1975; Mangini and Pearlman, 1980; Wagor et al., 1980; Metin et al., 1988). The term contralateral dominance is used to describe this phenomenon. Depriving mice from vision of the contralateral eye causes a change in the dominance so that neurons get activated equally strong by stimulation of each eye (Dräger, 1975; Gordon and Stryker, 1996). This is referred to as ocular dominance (OD) plasticity and is a widely used model for neuronal plasticity.

1.2. OD-plasticity as a model

The study of Hubel and Wiesel (1962), more than 50 years ago laid the foundations for our understanding of brain plasticity. Initially, they discovered that response properties of cat cortical neurons in V1 differ based on their inputs. Neurons in V1 were responding maximal to a stimulus which had a specific orientation and position in the visual field. Moreover, some neurons showed only a response to stimulation of one of the eyes, whereas others responded to stimulation of both eyes. The term ocular dominance is used to describe the balance between the responses to stimulation of the respective eye. Furthermore, Hubel and Wiesel (1962) described that neighboring cells in V1 of cats with similar preferred orientations and similar OD properties were organized in radial columns extending through all the layers of the cortex.

Wiesel and Hubel were the first to perform a more detailed investigation of a critical period at the neuronal level in the visual cortex (Wiesel and Hubel, 1963). Occluding one eye of vision (monocular deprivation, MD) in kittens during a brief period in early postnatal life resulted in a drastically changed OD in their V1. Electrophysiological recordings showed that deprived eye stimulation became less effective in driving cortical cells, whereas the open eye gained influence. Thus, early MD produced an OD-shift towards the open eye (Wiesel and Hubel, 1963). These observations made OD-plasticity a well-established model for neuronal plasticity. Nowadays MD is widely used as an experimental paradigm to study experience-dependent cortical plasticity.

Although Hubel and Wiesel performed their studies in kittens (Hubel and Wiesel, 1962; Wiesel and Hubel, 1963) OD-plasticity has been studied widely also in other species because the changes are dramatic, reproducible, quantifiable and restricted to the cortex. Studies describing OD-plasticity are now available in species like monkeys (Horton and Hocking, 1997), ferrets (Issa et al., 1999) and rodents (Dräger, 1978; Domenici et al., 1992; Maffei et al., 1992; Fagiolini et al., 1994; Gordon and Stryker, 1996; Bartoletti et al., 2002; Lehmann and Löwel, 2008) (for review see Espinosa and Stryker (2012) and Levelt and Hübener (2012)).

Carnivores and primates have a refined visual system including a much larger cortical region for visual processing and orientation, OD, and spatial frequency columns (Issa et al., 2000; Ohki and Reid, 2007; Van Hooser, 2007). The organization of rodent's V1 differs from cats and primates in its functional structural architecture (Ohki et al., 2005). Neurons of higher mammals are organized in radial columns by the neuron's preference for the stimulus orientation (Hubel et al., 1976). In rodents, cells in the visual cortex are spatial distributed with little order (Ohki et al., 2005; Van Hooser, 2007), which is referred to as 'salt-and-pepper' organization (for review see Espinosa and Stryker (2012)).

Despite the differences in the cortical architecture compared to primates, mice are one of the most extensively used organisms in OD-plasticity studies for many reasons. Mouse cortical neurons can be classified into categories similar to those described in higher mammals. They

show selectivity for stimulus parameters such as orientation and spatial frequency similar to other species (Niell and Stryker, 2010). Additionally, mice show a much stronger contralateral dominance in V1 in comparison to higher mammals (Metin et al., 1988). The first plasticity experiments in mice showed with single cell recordings that depriving one eye from vision by MD induces OD-plasticity in V1 (Dräger, 1978). In addition, mice display an OD-plasticity which is age-dependent and a critical period for OD-plasticity (Hubel and Wiesel, 1970; LeVay et al., 1980; Gordon and Stryker, 1996; Lehmann and Löwel, 2008). Finally, a variety of genetic tools are available for mice, which can be used for revealing cellular and molecular mechanisms underlying neuronal plasticity. Therefore, the visual system of mice is a highly suitable model for neuronal plasticity studies.

1.3. Critical period of OD-plasticity in mice

Critical period of OD-plasticity is defined as a sensitive phase of development during which alterations in visual experience can induce cortical plasticity. In mice V1 before the critical period for OD-plasticity, the preferred orientations of the same binocular neuron for stimulation of either eye do not match (Wang et al., 2010). During the critical period stimulus preferences of binocular neurons are gradually refined to match the tuning characteristics of inputs stimulated through the two eyes. Monocular or binocular visual deprivation during the critical period prevented binocular matching, and neurons continued to have different orientation tuning when stimulated through the two eyes (Wang et al., 2010).

Amblyopia, which is a disorder of sight, occurs when the vision in one of the eyes is reduced because the eye and the brain are not working together properly, is the most common impairment of visual function affecting one eye in adult humans (reviewed in Holmes and Clarke (2006)). In animal models, amblyopia can be artificially caused by long-term deprivation of inputs from one eye by MD. This causes a loss of visual acuity in the deprived eye and a pronounced OD-shift towards the open eye, which becomes permanent if left untreated (Singer et al., 1980; Prusky et al., 2006).

1.3.1. Opening of the critical period of OD-plasticity

The timing of the critical period of OD-plasticity strongly depends on the balance of inhibition and excitation of neuronal circuits in the brain. The development of inhibitory circuits in the cortex and the function of a particular set of inhibitory neurons are crucial for opening of the critical period (for review see Hensch (2005)).

First, a minimal level of GABAergic inhibition is necessary for the onset of the critical period. At 1998 Hensch et al., showed that the critical period of OD-plasticity never opened in transgenic mice knock-out for the GABA-synthesizing enzyme GAD65 (glutamic acid decarboxylase 65). Enhancing inhibition by treating these mice with diazepam (positive allosteric $GABA_A$ receptor modulator) reinstated the critical period (Hensch et al., 1998). Likewise, in young wild-type mice (PD15), treatment with diazepam could initiate a

precocious critical period that was similar in duration to the normal critical period (Fagiolini and Hensch, 2000). This finding suggests that a transient increase in GABAergic transmission is sufficient to open the critical period.

Second, GABAergic transmission via α1 subunit of $GABA_A$ receptors is crucial for the opening of the critical period. Diazepam treatment in knock-in mice with insensitive $GABA_A$ receptor subunits, revealed that mutant α2 or α3 $GABA_A$ receptor subunits, but not α1 subunit, could still produce a precocious critical period, as in wild-type mice (Fagiolini et al., 2004). The α1 subunit of $GABA_A$ receptor is enriched in inhibitory synapses formed by fast-spiking parvalbumin (PV) positive interneurons (Klausberger et al., 2002). These findings suggest that inhibitory neurons like the PV basket cells play an important role in opening the critical period for OD-plasticity.

Third, factors involved in the opening of the critical period can also regulate the maturation of inhibitory circuits. Transgenic mice overexpressing the brain-derived neurotropic factor (BDNF) during postnatal development had a precocious critical period and an earlier maturation of inhibitory neurons, suggesting that BDNF is controlling the maturation of GABAergic inhibition (Hanover et al., 1999; Huang et al., 1999). Other growth factors like IGF-1 (insulin-like growth factor) can also accelerate the maturation of circuitry and inhibitory innervations (Tropea et al., 2006; Ciucci et al., 2007). Polysialic acid neural cell adhesion molecule (PSA-NCAM) has also been implicated in the opening of the critical period (Di Cristo et al., 2007).

The opening of the critical period also depends on visual experience. It has been shown that chronic dark rearing from birth, delays the onset of the critical period (Fagiolini et al., 1994). A possible explanation for this observation involves BDNF. Dark-rearing reduces the BDNF levels and delays the critical period (Zafra et al., 1990). Increasing cortical BDNF levels in dark-reared mice resulted in a normal critical period for OD-plasticity (Gianfranceschi et al., 2003). Together these observations suggest that the effects of dark-rearing on plasticity also involve the maturation of the inhibitory circuit.

1.3.2. Closing the critical period for OD-plasticity

Closure of the critical period for OD-plasticity involves several cellular and molecular "brakes". As the animal matures, new structures are formed to downsize the neurite outgrowth, which together with functional changes that regulate the balance between excitation and inhibition, are responsible for the closure of the critical period (for review see Bavelier et al. (2010), Espinosa and Stryker (2012)).

The increase in GABAergic inhibition observed for opening of the critical period is also responsible for the closure of the critical period for OD-plasticity (Huang et al., 1999; Morales et al., 2002; Chattopadhyaya et al., 2004). Manipulations that locally reduced inhibition in adulthood have been found to restore visual plasticity (He et al., 2007; Sugiyama et al., 2008; Harauzov et al., 2010)). Additionally, depletion of the endogenous prototoxin

Lynx, which reduces cholinergic transmission, during adulthood also enhanced OD-plasticity in mice (Morishita et al., 2010). Furthermore, chronic treatment with the serotonin reuptake inhibitor fluoxetine restored OD-plasticity in adult rats by resetting excitation/inhibition balance (Maya-Vetencourt et al., 2008).

In addition to neurochemical regulations, the closure of the critical period may in part be under the control of structural factors responsible for the remodeling of the extracellular matrix. A major component of the complex network of the extracellular matrix are the chrondroitin-sulfate proteoglycans (CSPGs), which form tight perineuronal nets (PNNs) around the basket-type GABAergic cells (Pizzorusso et al., 2006; Carulli et al., 2010) (for review see Galtrey and Fawcett (2007)). The maturation of PNNs in adulthood has been proposed to inhibit the remodeling of neuronal connections, which in turn prevents OD-plasticity (Carulli et al., 2010). Degradation of CSPGs by the enzymatic activity of chondroitinase ABC reactivates OD-plasticity and recovery from long-term MD (Pizzorusso et al., 2002; Pizzorusso et al., 2006). In support of the involvement of PNNs in the closure of the critical period are studies, which showed that the formation of PNNs in the visual cortex correlates with the end of the critical period (Pizzorusso et al., 2002) (for reviews see Berardi et al. (2005), Hensch (2005)). In addition, mice lacking the cartilage link protein 1 (Crtl1), which triggers the neuronal production of PNNs, show diminished PNNs and retain juvenile levels of OD-plasticity during adulthood (Carulli et al., 2010).

Another example of the importance of the extracellular matrix in the OD-plasticity has been pointed out by studies, which showed that disruption of the function of proteins taking part in degradation of the extracellular matrix resulted in impairments of OD-plasticity. In particular, studies from both juvenile and adult mice showed that inhibition of matrix metalloproteinases (MMPs), which are involved in the degradation of the extracellular matrix, reduced OD-plasticity in juvenile rats (Spolidoro et al., 2012) and completely abolished it in adult mice (Pielecka-Fortuna et al., 2015a). Likewise, tissue plasminogen activator (tPA), which activates MMPs, was found to be increased during MD in mice and is essential for OD-plasticity to occur (Mataga et al., 2002). Additionally, mutant mice lacking the extracellular matrix protein reelin, were found to maintain OD-plasticity into the late adulthood, regardless of highly disorganized cortical layers (Pielecka-Fortuna et al., 2014).

Other more widely distributed structural factors can limit OD-plasticity, such as myelin related proteins. Myelination in the central nervous system increases as the critical period closes (McGee et al., 2005). Mutant mice for receptors of myelin-associated proteins like Nogo-66 receptor (NgR) and immunoglobulin-like receptor B (PirB) showed disrupted myelination and enhanced OD-plasticity in adulthood (McGee et al., 2005; Syken et al., 2006; Atwal et al., 2008).

It was shown recently that the maturation of silent glutamatergic synapses onto principal neurons play an important role in the duration of the critical period for OD-plasticity of mice (Huang et al., 2015). Specifically, postsynaptic density protein-95 (PSD-95) is essential for the experience-dependent maturation of silent synapses, and its absence resulted in a

prolonged period of OD-plasticity. Furthermore, silent synapse-based OD-plasticity was found to be independent of the inhibitory tone, suggesting an alternative mechanism controlling the duration of critical period (Huang et al., 2015).

1.4. OD-plasticity through age

Although, numerous studies in various species described dramatic changes in MD-induced OD-plasticity during the critical period, OD-plasticity is not limited to this particular period. Studies in cats and rats showed that OD-plasticity decreases slowly after the critical period, however can persist during adolescence (Daw et al., 1992; Guire et al., 1999). Likewise, OD-plasticity in mouse V1 is not lost by the end of the critical period, but instead declines progressively until the limit of 110 days of age, when measured with optical imaging of intrinsic signals (Lehmann and Löwel, 2008). It is important to mention that the characteristics of OD-plasticity are also changing with maturation, thus OD-plasticity during the critical period is distinctive from the adult type of OD-plasticity (Sato and Stryker, 2008).

MD for a short period of time during the critical period causes a dramatic shift in OD towards the open eye (Gordon and Stryker, 1996) and occurs between PD21 and PD35 with a peak of sensitivity for MD at PD28. During that time OD-plasticity in V1 can be easily induced after only 4 days of MD (Frenkel and Bear; Gordon and Stryker, 1996; Hofer et al., 2006a; Lehmann and Löwel, 2008; Sato and Stryker, 2008). The critical period in mice is characterized by three consecutive stages which are mechanistically different (for review see Espinosa and Stryker (2012)). The first stage refers to the initial rapid reduction of responses to the deprived eye inputs taking place 2 to 3 days after MD (Sato and Stryker, 2008). The second stage is characterized by an increase in open eye responses after longer periods of MD (Kaneko et al., 2008; Sato and Stryker, 2008). During the third stage of critical period reopening of the deprived eye leads to the restoration of the normal visual responses (Kaneko et al., 2008). Additionally, MD during the critical period also produces a strong OD-shift in the ipsilateral hemisphere (Sato and Stryker, 2008; Figure 2A).

In young-adult mice (PD60-90) OD-plasticity is quantitatively and qualitatively different from critical period OD-plasticity. After the critical period a longer deprivation period of 7 days is required to induce an observable OD-shift (Sawtell et al., 2003; Lehmann and Löwel, 2008; Sato and Stryker, 2008). Even after 7 days of MD the OD-shift is less than the one observed after 4 days MD in critical period mice. One of the characteristic features of OD-plasticity in adult mice is that the OD-shift is predominantly mediated by elevation of open eye responses in V1 (Sawtell et al., 2003; Hofer et al., 2006a; Sato and Stryker, 2008) (Figure 2B). Additionally, ipsilateral eye deprivation does not lead to OD-plasticity (Sato and Stryker, 2008). Adult OD-plasticity appears to be less permanent than critical period OD-plasticity, with recovery after long-term MD and restoration of normal vision taking half longer (Prusky and Douglas, 2003). Beyond the age limit of 110 days OD-plasticity in mice is fully absent as measured by optical imaging of intrinsic signals (Lehmann and Löwel, 2008).

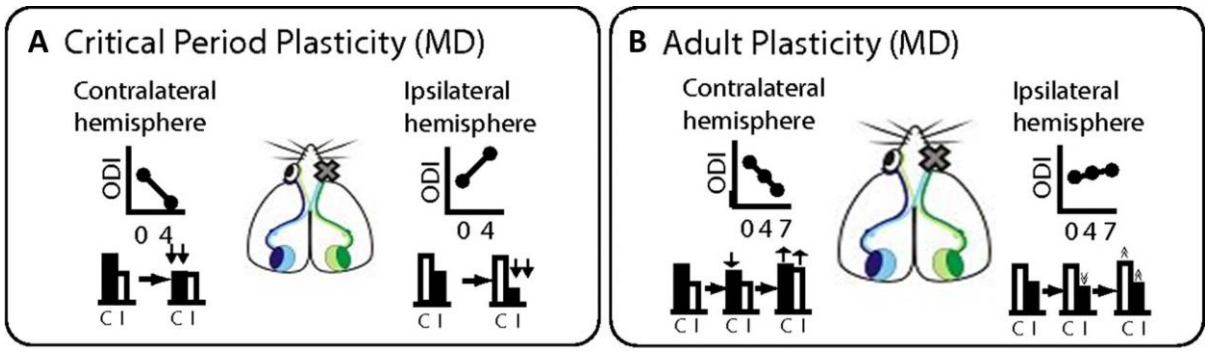


Figure 2: Comparison of the effects of monocular deprivation on OD-plasticity in juvenile and adult mice. **A**. During the critical period, just 4 days of MD caused a strong OD-shift towards the open eye in both hemispheres. The OD-shift is primarily mediated by decrease in closed eye responses in V1. **B.** In adult mice 7 days of MD are required to induce an OD-shift in the hemisphere contralateral to the deprived eye, whereas there is only a small change in OD of the ipsilateral hemisphere. In this case the observed effect is mediated by increase in open eye responses in V1. C refers to contralateral and I to ipsilateral eye. Figure modified from Sato and Stryker (2008).

1.5. Restoring OD-plasticity in adult mice

Restoration of OD-plasticity in older ages is of a particular interest not only for keeping the brain in a more juvenile state and therefore more plastic, facilitating learning and memory , but also has great potential for therapeutic rehabilitation and recovery from injury in the adult brain. Several pharmacological and environmental manipulations have been proposed over the years to prolong or restore OD-plasticity in adult animals by reducing local inhibition (for review see Bavelier et al. (2010), Spolidoro et al. (2009)). Some of the more common manipulations that successfully promoted adult OD-plasticity are described below.

Pharmacological approaches:

As mentioned earlier, increased inhibition is necessary for both opening and closure of the critical period for OD-plasticity (Fagiolini and Hensch, 2000). It should be possible then, to restore OD-plasticity in the mature visual cortex by reducing the levels of inhibition. Indeed, reduction of intracortical inhibition in adult rats by infusion of either $GABA_A$ agonist picrotoxin (PTX) or GABA synthesis inhibitor 3-mercaptopropionic acid (3-MPA) into V1 restored OD-plasticity in response to MD (Berardi et al., 2005).

Another pharmacological approach to restore OD-plasticity involved chronic treatment with fluoxetine (Maya-Vetencourt et al., 2008). Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) widely prescribed for treatment of depression. SSRIs are known to increase the extracellular serotonin and/or noradrenalin levels although the relationship between acute increases in these neurotransmitters and the clinical antidepressant effect, developing with a time delay, remains unclear (Nestler, 1998; Castren, 2005). Maya-Vetencourt et al. (2008)

showed that treatment with fluoxetine effectively restored OD-plasticity in adult rats' visual system using two classical models of plasticity: the OD-shift after MD and the recovery from amblyopia. They observed a strong OD-shift in fluoxetine treated rats, mediated by a reduction of V1 activation elicited by stimulation of the deprived eye, as typically observed in the juvenile cortex. Moreover, fluoxetine-treated rats recovered fully from amblyopia after long-term MD (Maya-Vetencourt et al., 2008). The OD-shift induced by MD in fluoxetine-treated rats was completely abolished when diazepam was applied, suggesting that the effect of fluoxetine on OD-plasticity is associated with a reduction of cortical inhibition (Maya-Vetencourt et al., 2008).

Epigenetic modifications have been recently implicated in the regulation of OD-plasticity in the adult visual cortex (for a review see Fagiolini et al. (2009)). During the critical period visual experience activates histone acetylation in the visual cortex but this capacity is downregulated in adult animals (Putignano et al., 2007). Pharmacological treatment during adulthood with a histone deacetylase inhibitor (trichostatin) enhanced OD-plasticity in adult mice (Putignano et al., 2007) and treatment with other histone deacetylases inhibitors (valproic acid, sodium butyrate) also promoted recovery of visual acuity in adult rats after long-term MD (Silingardi et al., 2010).

Non-pharmacological approaches:

Several manipulations to alter the animal's stimulating environment have been tested for the possibility to restore OD-plasticity in the adult brain. One of these manipulations was a previous MD during the critical period. In mice, which underwent an MD during the critical period, then were allowed to recover to normal vision and received a second MD during adulthood for only 3 days, the second MD caused a significant OD-shift (Hofer et al., 2006b). Thus, previous experience of a particular stimulation – here the first MD during the critical period – can promote OD-plasticity in adult mice.

OD-plasticity was also shown to be dependent on temporal coherence of visual stimuli. In a recent study, Matthies et al. (2013) showed that stimulation with moving square wave gratings for 6 hours per day during the MD period induced OD-plasticity in adult mice. Interestingly only 4 days of MD were sufficient to induce an OD-shift with the characteristics of juvenile plasticity: in particular reduction of deprived eye responses in V1 (Matthies et al., 2013).

Furthermore, light deprivation resulted in OD-plasticity in old animals. Specifically, extended dark housing of adult rats could restore a juvenile OD-plasticity, which was induced by only 3 days of MD and was driven by a decrease in response to the deprived input (He et al., 2006). In addition, short-term dark exposure in adult mice also restored OD-plasticity in both adult and aging mice (Stodieck et al., 2014).

Another example and one of the most interesting non-pharmacological approaches used to restore OD-plasticity in the adult brain is the enrichment of the housing environment.

Exposing or raising animals in a more stimulating environment compared to the restricted standard cage (SC) housing promoted OD-plasticity in adulthood (Baroncelli et al., 2010b; Greifzu et al., 2014). The general effect of environmental enrichment on OD-plasticity and potential mechanism will be further discussed in the following section.

In figure 3 a summary of the most successful approaches proposed to prolong OD-plasticity in animals after the critical period is presented.

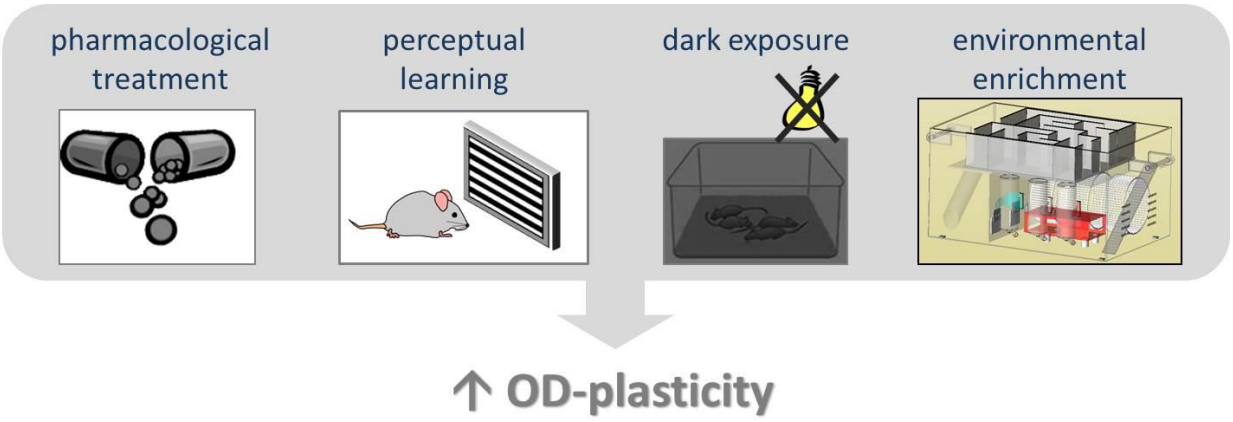


Figure 3: Restoring OD-plasticity in adult mice. Several manipulations have been proposed to prolong OD-plasticity in adult animals including pharmacological treatments like fluoxetine administration, and non-pharmacological approaches that alter animal's stimulatory environment like perceptual learning, dark exposure and environmental enrichment.

1.6. Enriched environment

The enriched environment (EE) was first proposed as an experimental paradigm by Hebb (1947), when he reported that rats that he took home as pets showed behavioural improvements over those that had been left in laboratory cages. In the early 1960s, the work from Hubel and Wiesel (1963) showed the importance of experience in development of cortical circuits and soon after the studies from Rosenzweig et al. (1962) proposing the EE as a testable scientific concept, pointing out the influence of the environment on brain development. It is now clear that during development experience is continuously interacting with genetic information to shape neuronal circuits and behaviour, thus the final phenotype is the combination of the genetic information and the environmental experience.

Enriched environment is classically defined as "a combination of complex inanimate and social stimulation" (Rosenzweig et al., 1962). Compared to the simple SC where the animals are housed in small groups (up to 4 animals) or even alone with only nesting material, food, and water, EE cages are larger with the mice housed in bigger groups (up to 16 animals) and

equipped with a variety of stimulating objects (e.g. running wheels, maze, tunnels, nesting material and stairs; Figure 4). Thus, EE cages provide animals with optimal conditions for social interactions, more physical exercise, enhanced exploration and cognitive stimulation as they are bigger with novel objects and/or regularly changed mazes (for review Sale et al. (2009)).

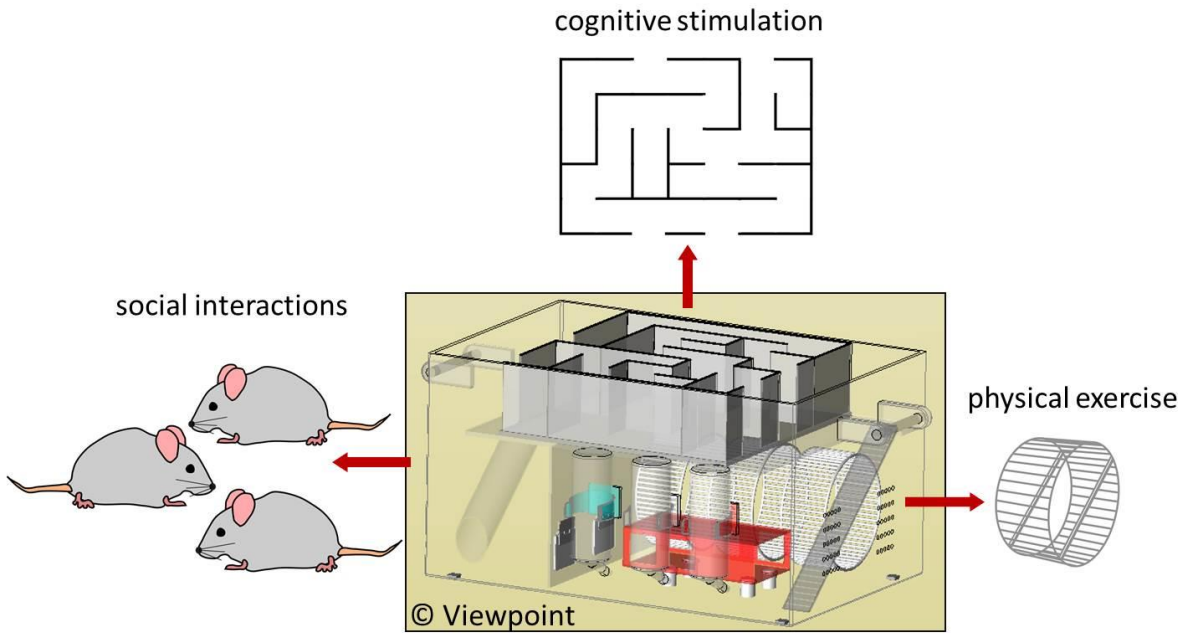


Figure 4: Enriched environment cage. The larger EE cage provides more space for exploration to the mice. Mice are housed in bigger groups for more social interactions. Regularly changed mazes enhance cognitive stimulation and running wheels provide free access to physical exercise.

1.6.1. General effects of EE in wild-type rodents

Over the years, it has become clear that EE can exert powerful effects on the brain, ranging from anatomical and molecular to behavioural consequences (for review see van Praag et al. (2000)). First EE studies showed differences on an anatomical level: EE-housing increased the cortical thickness and weight (Diamond et al., 1964; Beaulieu and Colonnier, 1987). Subsequently, more studies showed that EE increased the size of the cell soma and nucleus, dendritic branching and length of cortical neurons (Holloway Jr, 1966; Greenough et al., 1973; Kozorovitskiy et al., 2005) and also synaptic size and number (Diamond et al., 1964; Mollgaard et al., 1971; Turner and Greenough, 1985). Furthermore, EE increased hippocampal neurogenesis (Kempermann et al., 1997) as well as reduced apoptotic cell death (Young et al., 1999).

Moreover, EE induces alterations at the molecular level, changing the expression of several genes involved in synaptic function and cellular plasticity (Rampon and Tsien, 2000) (Figure 5). Elevated levels of neurotrophins have been found after EE, such as BDNF (Falkenberg et

al., 1992), nerve growth factor (NGF) (Mohammed et al., 1993; Pham et al., 1999), glial-cell-derived neurotrophic factor (GDNF) (Young et al., 1999) and IGF (Carro et al., 2000). Several of these factors have been suggested to play role in learning and synaptic plasticity (Fischer et al., 1987; Kang and Schuman, 1995; Figurov et al., 1996). Enrichment also increased the expression of synaptic proteins, such as the presynaptic vesicle protein synaptophysin (Frick and Fernandez, 2003; Lambert et al., 2005) and postsynaptic density-95 protein (PSD-95) (Nithianantharajah et al., 2004). Additionally, EE increased histone acetylation levels in the hippocampus and neocortex (Fischer et al., 2007).

Enriched environment also affects several neurotransmitter systems in the brain. Increased levels of acetylcholinesterase activity after enrichment were reported, indicating an effect of EE on the cholinergic system (Rosenzweig and Bennett, 1996). The serotoninergic system is also affected by enrichment, as EE animals showed enhanced expression of the serotonin1A receptor gene (Rasmuson et al., 1998). Furthermore, the activity of opioid systems is enhanced after enrichment (Sforzo et al., 1986). All of these neurotransmitters are known to influence learning and synaptic plasticity in the adult brain (for review see Gu (2002)).

Together with cellular and molecular changes EE is able to modify behaviour in various tasks involving complex cognitive functions (Renner and Rosenzweig, 1987). EE-mice performed better in the Morris-water-maze, the novel object recognition task and fear-conditioning task suggesting an enhanced learning and memory function after enrichment (for a review see Rampon and Tsien (2000)). Moreover, the typically cognitive decline observed in aging animals is reduced after EE (Bennett et al., 2006).

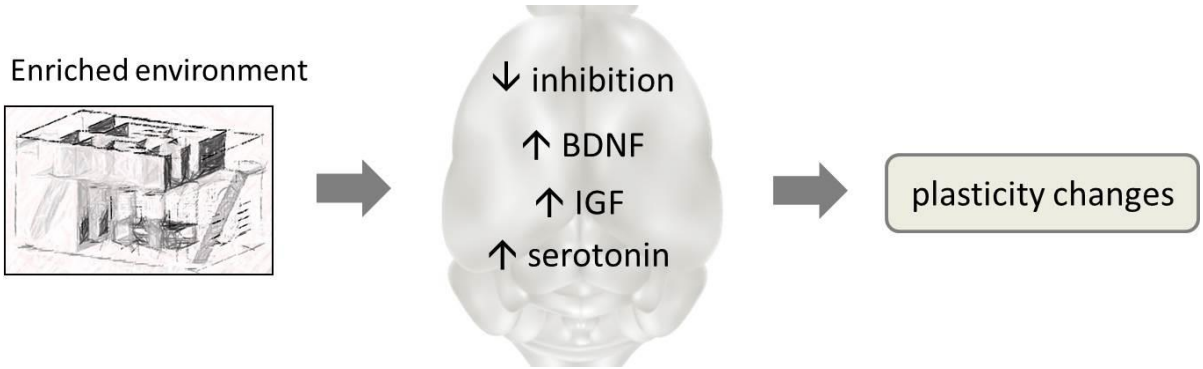


Figure 5: EE induces plasticity changes in the brain by altering the expression of several molecular factors. Reduced levels of inhibition, elevated levels of neurotrophins like BDNF and IGF, and increased levels of the neurotransmitter serotonin have been reported after EE-housing, resulting in behavioural changes and brain plasticity.

1.6.2. Enriched environment effects in visual system and on OD-plasticity

The visual system has been used as a model to study the effects of environment, revealing previously unknown effects of EE on the development and plasticity of neural circuits.

Additionally, EE has been a helpful tool to identify molecular factors implicated in visual cortex plasticity.

During development, EE has been shown to accelerate the visual system development (Prusky et al., 2000a; Cancedda et al., 2004; Sale et al., 2004) and advance the eye opening (Cancedda et al., 2004). There are several changes in molecular factors potentially responsible for this effect. Animals reared from birth in EE had higher levels of BDNF in their visual cortex (Cancedda et al., 2004; Sale et al., 2004), increased expression of the GABA biosynthetic enzymes GAD65 and GAD67 (Cancedda et al., 2004; Ciucci et al., 2007), increased levels of IGF-I (Ciucci et al., 2007; Maya-Vetencourt and Origlia, 2012) and accelerated CRE/CREB (cAMP response element-binding protein)-mediated gene expression (Cancedda et al., 2004).

EE has already been tested as a non-invasive approach to reactivate OD-plasticity in rodents (for review see Sale et al. (2014)). In adult rats, 3 weeks of EE-housing promoted OD-plasticity after 1 week of MD compared to SC-housed rats (Baroncelli et al., 2010b). Visually evoked potentials recordings revealed that OD-plasticity in EE rats was similar to critical period mechanism with decrease in responses through the deprived eye in V1 (Baroncelli et al., 2010b). Similar experiments performed in aging rats (22 to 24 months) showed that, 3 weeks of EE-housing are sufficient to reactivate OD-plasticity (Scali et al., 2012). Moreover, adult amblyopic rats transferred to an EE for 3 weeks underwent full recovery of visual function in contrast to SC-mice (Sale et al., 2007; Baroncelli et al., 2010b). In these studies, recovery of plasticity was accompanied by increased expression of BDNF, decreased density of PNNs and a reduction in GABA levels (Sale et al., 2007; Baroncelli et al., 2010b; Baroncelli et al., 2012; Scali et al., 2012). The reduction of inhibition by EE is crucial to rescue OD-plasticity as demonstrated by complete lack of OD-plasticity after diazepam treatment (Sale et al., 2007; Baroncelli et al., 2010a). We recently showed that EE extended the sensitive phase for OD-plasticity into adulthood and restored OD-plasticity in old SC-raised mice (Greifzu et al., 2014). Additionally, we found reduced local inhibition in V1 suggesting that EE probably keeps the adult brain in a more juvenile state by preserving low levels of inhibition into adulthood (Greifzu et al., 2014).

1.6.3. Transgenerational effect of EE

During the first two weeks of life, rodents stay in the nest, totally depending on the mother, which is the most important source of sensory experience (Liu et al., 2000). EE during the first days of life was shown to affect the visual-system development of pups caused by changes of the maternal behaviour (Cancedda et al., 2004). Quantitative analysis of maternal care in EE showed that EE-pups receive higher levels of maternal care, continuous physical contact and higher levels of licking compared to SC-reared pups (Sale et al., 2004). It has been proposed that higher maternal care in EE-pups affects their brain development. In support of this hypothesis is the observation that variations in maternal care increase BDNF levels in the offspring (Liu et al., 2000). In a recent study Guzzetta et al. (2009) were able to reproduce the EE-dependent acceleration of visual development in rat pups born in SCs by

mimicking maternal behaviour. Specifically, during the first ten days of pup's life, they applied a tactile stimulation (massage), a procedure previously shown to compensate for the negative effects of maternal deprivation (Schanberg and Field, 1987). Additionally they observed increased IGF-I levels in PD18 pups and when blocked, prevented the effects on visual system development (Guzzetta et al., 2009). Furthermore, the level of maternal care is also influencing the response to stress phenotype in adulthood (Meaney, 2001).

Interestingly, the licking-grooming behaviour is heritable: the offspring of high and low frequent licking-grooming mothers become high and low frequent licking-grooming mothers respectively (for review see Arai and Feig (2011)). For example, high levels of maternal care resulted in elevated levels of serotonin in the pup's hippocampus, leading to increased expression of the transcription factor GFI-A. This stimulates posttranslational modifications like DNA hypomethylation and histone acetylation, leading to increased expression of glucocorticoid receptor (GR), which is related to reduced stress levels. These epigenetic modifications were preserved into adulthood and determined the level of maternal care, thus the phenotype was preserved across generations (Weaver et al., 2004).

Early studies showed that exposure of pregnant rats to an enriched environment enhanced not only their ability to find their way in a maze, but also the ability of their future offspring to do the same (Kiyono et al., 1985). Also, exposure of the mother to EE even before pregnancy resulted in an enhanced learning ability that was transmitted to her offspring (Dell and Rose, 1987). Similar results were obtained when offspring of EE-mothers were raised from birth by non-EE foster mothers, suggesting that the effect of EE was transmitted to the offspring before birth, presumably *in utero*.

Studies from Arai et al. (2009) showed that 4-week old offspring of EE-mice displayed enhanced synaptic plasticity in the hippocampus similar to their parents, even if they themselves never experienced EE. Using knock out mice with defective long-term potentiation (LTP) and fear conditioning, they showed that this phenotype was reversed in offspring of EE-mice. The effect was maintained even when the offspring were raised with non-EE foster mothers, suggesting that the effects of EE on synaptic plasticity to the next generation were transmitted during embryogenesis (Arai et al., 2009).

1.6.4. Physical exercise and plasticity

Enriched environment is a complex combination of social stimulation, learning and physical activity. Among the various EE components, physical activity seems to be one of the most crucial, with several studies exploring its capability to mimic a more complex EE and its benefits for the brain (for review see van Praag et al. (1999)). The different components of EE were already studied with respect to their role in recovery from amblyopia in rats (Baroncelli et al., 2012): physical exercise and visual enrichment promoted the recovery from amblyopia, whereas social interaction had no effect.

Physical exercise improves cognition and might delay age-related memory decline in humans (Cotman et al., 2007; Hillman et al., 2008). Physical exercise was also shown to protect against brain damage and to promote recovery from injuries (Griesbach et al., 2008). Increased cell proliferation, higher neuron survival and elevated levels of BDNF in the hippocampus have been reported for mice with access to a running wheel (Kobilo et al., 2011; Mustroph et al., 2012). Many molecular factors that changed after EE are also found to be changed after physical exercise for example increased levels of BDNF (Berchtold et al., 2005), NGF (Neeper et al., 1996), and IGF (Carro et al., 2000) were found in the brain after running (for review see Vivar et al. (2013)). Moreover physical activity influences several neurotransmitter systems in the brain such as the glutamatergic (Kitamura et al., 2003; Farmer et al., 2004; Lou et al., 2008) and the GABAergic system (Molteni et al., 2002).

Although many studies showed the positive effect of physical exercise in the brain, only recently the effect of running on visual cortex neuronal activity and plasticity has been addressed. Studies from Stryker's group showed that locomotion increases pyramidal cell firing in V1 (Niell and Stryker, 2010) and that the enhancement of visual responses induced by locomotion is sufficient to promote recovery of visual function after long term MD (Kaneko and Stryker, 2014). Additionally, they showed that the activity of a specific class of V1 interneurons that express the vasoactive intestinal protein (VIP) is directly modulated by locomotion (Fu et al., 2014). Specifically, using two-photon calcium fluorescence imaging to monitor the activity of genetically labeled VIP neurons, they found a strong correlation between the calcium signals in VIP neurons and bouts of running. Direct activation using optogenetic tools of VIP cells was sufficient to increase visual responses of neighboring neurons, mimicking the effects of locomotion while ablation of VIP neurons blocked the effects of locomotion. They proposed a possible neural circuit underlying these effects, where the specific recruitment of VIP cells by locomotion directly modulates V1 activity through a disinhibitory mechanism. Since VIP neurons in V1 are known to provide a major source of inhibition to somatostatin (SST) expressing interneurons (Pfeffer et al., 2013), suppression of SST interneurons resulted in decreased inhibition onto pyramidal cells, which in turn generated an increase in V1 responsivity (Fu et al., 2014; Lee et al., 2014).

1.6.5. EE and recovery from stroke

Since EE has several positive effects on brain and behaviour, the benefits of EE have been investigated in animal models for diseases like Alzheimer, Parkinson and Down syndrome but also after brain injury, including stroke (for a review see Nithianantharajah and Hannan (2006)). Previous studies on stroke showed that stroke not only destroys the affected brain areas, but also disturbs the surrounding areas (Buchkremer-Ratzmann et al., 1996) (for review see Andrews (1991)). It is important to mention that recovery after stroke requires cortical plasticity, which results in the rewiring of the brain by modulating the strength of synaptic connections and neuronal properties (Seitz et al., 2004) (for review see Murphy and Corbett (2009)). However, an *in vivo* study provided evidence for diminished plasticity in neighboring

areas of a cortical lesion. After a focal photothrombotic stroke next to the barrel cortex, no experience-dependent rearrangement of neurons occurred (Jablonka et al., 2007). Additionally, studies from our laboratory showed that after a photothrombotic lesion in the primary somatosensory cortex (S1) or secondary motor cortex (M2) visual cortex OD-plasticity and the increase of visual abilities usually occurring after MD were diminished in young mice, while anti-inflammatory treatment successfully restored increased visual abilities after MD but not OD-plasticity (Greifzu et al., 2011; Pielecka-Fortuna et al., 2015b).

Enriched environment has been also proven beneficial for the recovery after stroke. Experiments in rodent models of stroke showed that EE promoted motor function recovery (Ohlsson and Johansson, 1995; Johansson, 1996; Biernaskie et al., 2004; Wurm et al., 2007) and reduced deficits in learning and memory (Dahlqvist et al., 2004; Rönnbäck et al., 2005; Wurm et al., 2007). Moreover the lost OD-plasticity in V1 after a lesion in S1 was preserved, when the mice were raised in EE (Greifzu et al., 2014). While the benefits of EE after stroke have been investigated, there is no study investigating the effects of physical exercise on OD-plasticity lost after stroke.

1.7. Enriched environment from animals to humans

Learning in humans is a result of practice and can be achieved even in adulthood, but there is no doubt that children learn faster and more effectively. Reactivation of plasticity in adults would be a significant clinical advance for rehabilitation after cortical damage, but also for promoting learning and cognition. Non-pharmacological treatments to promote plasticity, like EE, seem to be a better choice for clinical applications because they minimize the side effects. EE has proven to be beneficial for animals in restoration of cortical plasticity but how to apply the animal EE paradigm to humans is still debated.

One proposed way of enrichment in humans is the aerobic exercise. The positive effects of aerobic exercise are known in the field of aging: individuals, who normally exercise, perform better in various tasks as dual-task performance, executive attention or distractor rejection, compared to those who do not (for reviews see Hillman et al. (2008), Kramer and Erickson (2007)). Additionally, aerobic exercise leads to neuroanatomical and neurophysiological changes in older humans, including increased gray matter volume in the prefrontal and temporal areas and reduced brain tissue loss (Colcombe and Kramer, 2003). Whether aerobic exercise can enhance brain plasticity in healthy, young humans needs to be determined.

Another approach of enrichment in humans is by playing videogames, which combines various EE components, such as visual attention and enhanced sensory stimulation (Green and Bavelier, 2012). Fast-paced, action-packed games have already been documented to have potent positive impact on an array of skills, including perception, visual-motor coordination, spatial cognition, attention and decision making (Dorval and Pepin, 1986; Li et al., 2009; Green and Bavelier, 2012). In a recent study, amblyopic adults were asked to play an off-the-shelf action video-game with their fellow eye patched. This resulted in an improvement of a

wide range of fundamental visual functions, including visual acuity, positional acuity and visual attention (Li et al., 2011). Interestingly, improvement in amblyopic vision was also observed after playing non-action videogames such as SimCity which are not efficient in boosting normal vision (Li et al., 2009). These plastic modifications have been shown to be long lasting, with beneficial effects lasting from six months to two years (Li et al., 2009). Like in EE, the factors responsible for inducing brain plasticity within the action game experience remain to be determined.

1.8. Scope of the thesis

The positive effect of EE on adult plasticity has been the subject of studies for the past few years, but there are still open questions as for the mechanisms of how EE can promote plasticity. The aim of this study is to investigate more in detail the effects of EE on adult OD-plasticity of mice with the ultimate goal to clarify the following questions:

a) Can EE prolong the sensitive phase of OD-plasticity into adulthood and until when do the mice show OD-plasticity when raised in EE?

b) Is the observed OD-shift in adult EE-mice similar to critical period SC-mice?

c) Is running, as one component of EE, sufficient to enable OD-plasticity in adult mice? Can running also preserve OD-plasticity after stroke?

d) How long do the mice show OD-plasticity if they do not experience an EE anymore? Can we preserve OD-plasticity in mice transferred from EE to SCs?

e) Can the positive effect of EE on OD-plasticity be passed to the next generation of non-EE-mice? Which parent is responsible for conducting the effect?

2. Materials and Methods

2.1. Animals

Male and female C57BL/6J mice were obtained from the mouse colony of the central animal facility of the University Medical Center, Göttingen, Germany, and housed in an animal room with a 12-h light/dark cycle with food and water available ad libitum. All experimental procedures were approved by the local government: Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit. For every experiment the age range of the mice used is given as postnatal days (PD) on the day of optical imaging experiment.

2.2. Design of the study

2.2.1. OD-plasticity can be induced after 4 days of MD in EE-mice

One of the questions, that I focus my thesis on, was the effect of 4 days of monocular deprivation (MD) on OD-plasticity in three different age groups of mice born and raised in enriched environment (EE). To address that question, in total 33 female mice of three different age groups born and raised in EE were used. The first group consisted of 10 mice in the critical period (EE_cp; PD27-33), the second group of 13 mice between PD80 and PD101 (EE-young) and the third group of 10 mice older than PD110 days (EE_old; PD121-183). Detailed description of the groups is presented in Table 1.

All animals were checked daily during MD period in the optomotor setup to measure spatial frequency and contrast sensitivity thresholds of the optomotor reflex. On the 4th day, optical imaging of intrinsic signals was performed to assess the ocular dominance index (ODI; Figure 6). As controls matching age mice without MD were also measured daily in optomotry and optical imaging was performed.

Table 1: Description of EE mice of different ages used for this study. The group name, if the mice had MD, the age range and the total number of animals in each group are listed.

Group name	Monocular Deprivation (MD)	Age (days)	Number of mice
EE_cp	no MD	27-34	4
EE_cp_MD	MD	27-33	6
EE_young	no MD	82-101	7
EE_young_MD	MD	80-99	6
EE_old	no MD	127-183	5
EE_old_MD	MD	121-177	5

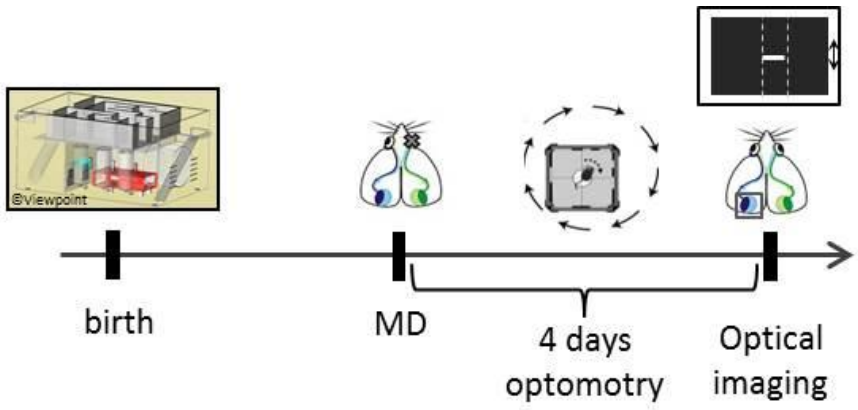


Figure 6: Experimental design to test the effect of 4 days MD in 3 different age groups of EE mice. All mice were born in EE cages and when they were at the proper age MD was performed. During the following 4 days mice were tested daily in the optomotor setup and at the end optical imaging was performed.

2.2.2. The sensitive phase of OD-plasticity can be prolonged in mice with running wheels

To investigate whether running alone has a beneficial effect on OD-plasticity of adult mice I used two different experimental conditions: mice housed from birth in standard cages (SCs) with a running wheel (RW) and mice exposed to RW for only 7 days during the MD period. As control group mice born and raised in SCs were used.

For the first case (RW group) a total number of 25 male and female mice with age range PD138-242 were used (Table 2). The mice were born and raised in a group of 3 to 5 animals in SC equipped with a RW. When mice were at least 130 days old 8 of them received 7 days of MD and 7 mice were used for the no MD group. In both groups daily optometry was performed for a period of 7 days, to measure spatial frequency and contrast sensitivity thresholds of the optomotor reflex, followed by optical imaging of intrinsic signals to calculate the ODI (Figure 7A). As control group, mice born in exactly same size SC but without RW were used (n=10; PD137-200). Again these mice had MD/noMD (n=5/5) for 7 days, optomotry measurements during MD and at the end optical imaging was performed. Part of the optomotry measurements and imaging analysis was performed with the help of Franziska Haack as a part of her bachelor thesis.

For the second case (7 days running) the total number of 12 male and female mice with age range PD141-217 was used (Table 2). Here, all mice were born and raised in SCs until they were at least 130 days old and transferred in a SC with RW soon after 6 mice underwent MD (PD141-214) and 6 had no MD (PD143-217). All mice were tested in optomotry daily for 7 days, and on the 7th day optical imaging of intrinsic signals was performed (Figure 7B).

Table 2: Groups used for the RW study. The group name, the housing conditions, the MD, the age range of the animals in each group and the total number of animals in each group are listed.

Group name	Housing (RW/SC)	Monocular Deprivation (MD)	Age (days)	Number of mice
RW_MD	RW	MD	138-242	8
RW	RW	no MD	140-240	7
SC_MD	SC	MD	145-195	5
SC	SC	no MD	137-200	5
7dRW_MD	From SC to RW	MD	141-214	6
7dRW	From SC to RW	no MD	143-217	6

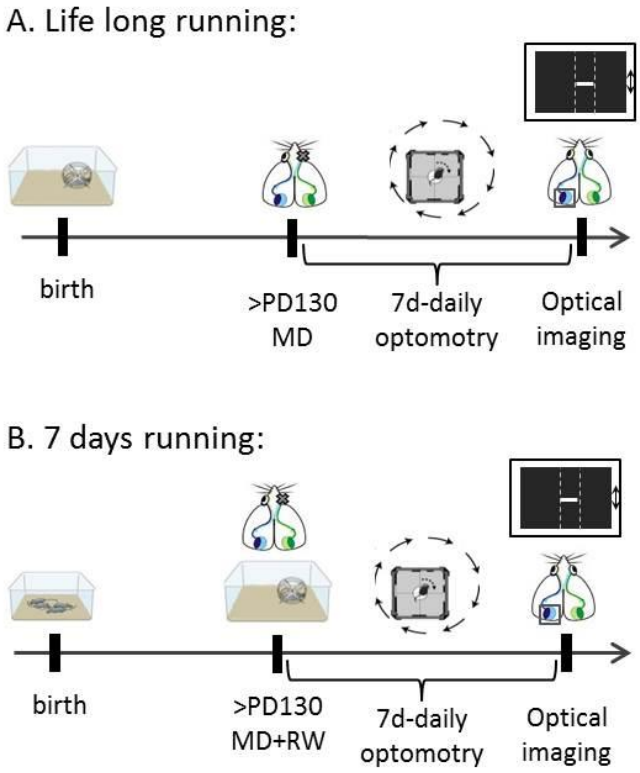


Figure 7: Experimental design to test the effect of running on OD-plasticity. A. Mice born in SCs with a RW received MD when above PD130. During 7 days of MD mice were tested daily in the optomotor setup. On the 7th day optical imaging was performed. **B.** Mice born and raised in SCs until at least PD130, transferred to a cage with a RW after MD. After 7 days of daily optometry optical imaging was performed.

2.2.3. Voluntary running preserved OD-plasticity after an induction of a cortical lesion

In order to test the effect of physical exercise on preserving OD-plasticity after stroke, mice were raised in a slightly bigger SC equipped with a RW. When mice where above 110 days old a localized cortical lesion was induced using photothrombosis (PT) (Watson et al., 1985). Few hours after PT surgery (2-3 h), some of the mice received MD and some did not. Mice were then transferred back in their home cages. Optomotry measurements were done daily for the next 7 days before optical imaging on the last day (Figure 8A). As control groups we used sham-operated mice with or without MD. A total number of 17 male and female mice between PD149 and PD222 were used for this study (Table 3).

In addition, the therapeutic effect of a short exposure to RWs after PT was tested. In this study 18 males and females mice born and raised in SCs were used (Age range: PD119-258; table 3). PT or sham operation was performed after which mice were immediately transferred in a cage with a RW. After one week of recovery from stroke, MD was performed in some of the mice. All the mice were tested in the optomotor setup for 7 days before optical imaging (Figure 8B). As part for her bachelor thesis, Janika-Marrie Hüppe performed some of the optomotry measurements and imaging analysis.

Table 3: Description of the groups used for RW stroke study. The group name, the treatment (PT or sham surgery), the MD, the age range and the total number of animals in each group are listed.

Group name	Treatment (PT/sham)	Monocular Deprivation (MD)	Age (days)	Number of mice
RW_PT_MD	PT	MD	149-204	5
RW_PT	PT	no MD	174-222	5
RW_sham_MD	Sham	MD	156-218	4
RW_sham	Sham	no MD	180-215	3
14dRW_PT_MD	PT	MD	119-213	7
14dRW_PT	PT	no MD	124-258	5
14dRW_sham_MD	Sham	MD	120-197	3
14dRW_sham	Sham	no MD	119-162	3

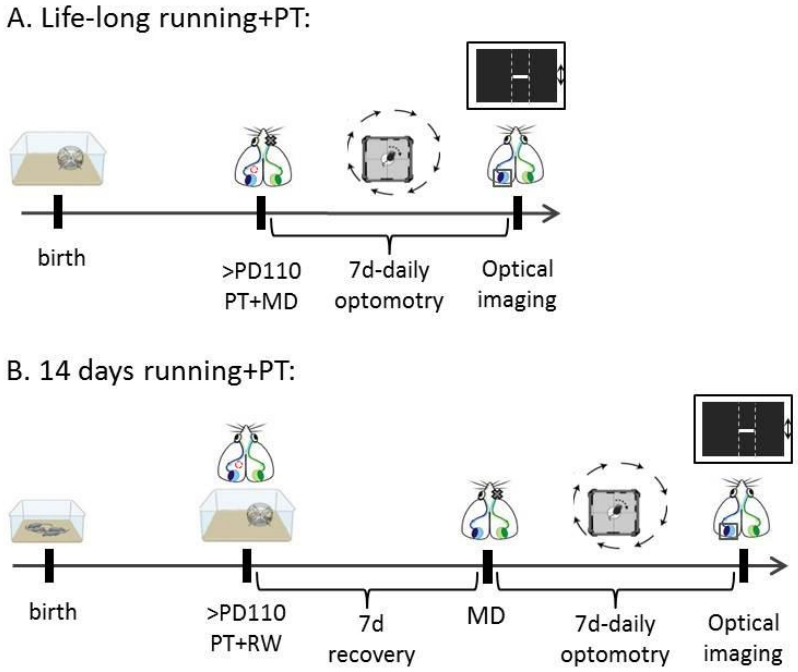


Figure 8: Experimental design to test therapeutic potential of physical exercise on lost OD-plasticity after stroke. A. A lesion was induced using PT in adult mice (>PD110) born in SCs with a RW. Shortly after, mice received an MD and were transferred back in their home cages. After 7 days with daily optomotry measurements, optical imaging was performed. **B.** Mice were born and raised in SCs until at least PD110 and transferred in a cage with a RW immediately after PT. After 7 days of recovery mice received an MD followed by 7 days of daily optomotry measurements. On the last day optical imaging was performed.

2.2.4. How long OD-plasticity can last after transferring EE-mice to SCs

While mice in EE showed life-long OD-plasticity, it is not clear whether changing their living environment by transferring them to SCs will result in loss of OD-plasticity. To clarify that, 8 mice raised in EE where transferred alone to a SCs after PD110 (Table 4). After a short period in SC (from 1 day to 1 week) mice received MD for 7 days. During the MD period all mice were measured daily in the optomotor setup and optical imaging was performed on the 7th day (Figure 9A).

The OD-plasticity was absent in these mice already after 1 week in SC. To restore OD-plasticity I treated the animals with fluoxetine, a selective serotonin reuptake inhibitor (Fluoxetine hydrochloride, Tocris bioscience), administrated through the drinking water. To this end, female mice raised in EE were transferred in SCs after PD110, in groups of 3 to 5 animals per SC cage and fluoxetine was given to them through the drinking water for a period of 3 weeks. On the last week mice underwent MD, then were measured daily in the optomotor setup during the MD period and on the 7th day optical imaging of intrinsic signals was performed (Figure 9B). In order to reach an average daily intake of 10 mg/kg fluoxetine per mouse, the concentration of the drug in the drinking water was calculated based on the

average daily drinking amount of mice (5ml, Bechmanov et al., 2002) and the average mouse weight (25g, JAX® Mice, Clinical & Research Services). The concentration of fluoxetine in the drinking water was 0.05 mg/ml per day. The solution was prepared fresh every day and the average consumption was measured. Dripping-free bottles (Bioscape GmbH, Castrop-Rauxel) were used for that propose. As control group EE-mice from the same litter transferred in SCs drinking only water (without fluoxetine) were used. A total number of 14 female mice born in EE were used in this study (Table 4). Part of the optomotry measurements were performed by Jonas Brettschneider as a part of his bachelor thesis.

Furthermore, I tried to restore OD-plasticity in mice transferred from EE to SC using a RW. For this, 3 adult female mice (PD211-298) raised in EE were transferred in a SC with a RW for 3 weeks (Table 4). MD was performed after 2 weeks for 7 days. Optical imaging was performed after these 3 weeks. During the MD period mice were tested daily in optomotor setup (Figure 9C).

Table 4: Description of groups used for this study. The group name, the treatment (no, fluoxetine or RW), whether MD was performed in that group, the age range of the animals as well as the total number of animals in each group are listed.

Group name	Treatment	Monocular Deprivation (MD)	Age (days)	Number of mice
EEtoSC_1day	-	MD	164-278	4
EEtoSC_1week	-	MD	162-281	4
EEtoSC_fluox_MD	Fluoxetine	MD	278-283	3
EEtoSC_fluox	Fluoxetine	no MD	260-284	4
EEtoSC_water_MD	-	MD	266-269	3
EEtoSC_water	-	no MD	266-274	4
EEtoRW_MD	RW	MD	211-298	3

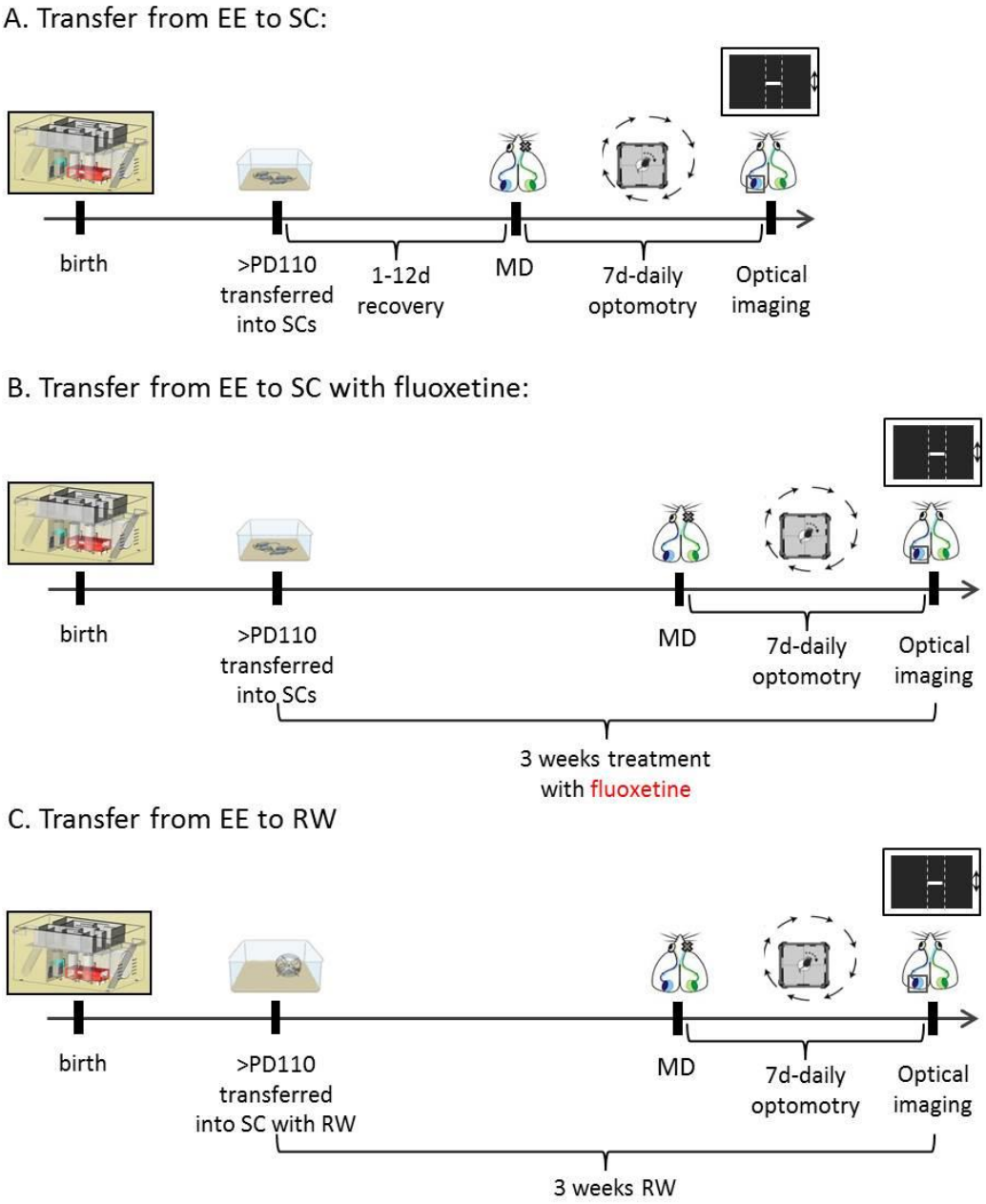


Figure 9: Experimental design to test whether OD-plasticity is lost after transferring EE mice to SCs and if it can be restored by fluoxetine or running. A. Mice born and raised until at least PD110 in EE were transferred to SCs alone and after 1 or 12 days MD was performed. For the next 7 days mice were measured in optomotor setup daily and on the 7th day optical imaging was performed. **B.** Mice born and raised in EE until PD110 were transferred to SCs and were treated with fluoxetine through the drinking water for 3 weeks. On the last week mice received an MD, followed by 7 days of daily optomotry and then optical imaging. **C.** Adult mice (>PD110) born and raised in EE were transferred to a SC with a RW for a period of 3 weeks. After 2 weeks MD was performed and afterwards (last week) mice were tested in optomotor setup daily for 7 days before optical imaging on the last day.

2.2.5. OD-plasticity can be passed on to the next generation

To answer the question if the positive effect of EE on OD-plasticity can be transmitted from EE-parents to the next generation of non-enriched offspring, mating of EE-mice took place in EE cages and pregnant females were transferred to SCs 7-5 days before delivery. Pups were born and raised in SCs until at least PD110 and then OD-plasticity was examined. To this end, MD (n=7) or noMD (n=5) adult non-enriched animals from EE-parents were checked daily in optomotry for a period of 7 days with optical imaging taking place on the last day (Figure 10A).

Furthermore, to investigate from which parent the effect on OD-plasticity is passed on to the next generation, mating between EE-males and SC-females as well as between SC-males and EE-females were done. The mating happened always in the cage of the female mice and pregnant dams were transferred to SCs few days before delivery (7-5 days). All offspring were born and raised in SCs and same procedure was followed to measure OD: when they were above PD110 some of the mice received MD, daily optometry and finally optical imaging (Figure 10B and C). In total 31 male and female mice were used for this study. Detailed group description is presented in the table 5.

Table 5: Description of the groups used to test whether OD-plasticity can be transmitted to the next generation of non-enriched mice. The group name, which parent was enriched, whether MD was performed in that group, the age range of the animals as well as the total number of animals in each group are listed.

Group name	EE Parent	Monocular Deprivation (MD)	Age (days)	Number of mice
EEparents_MD	father & mother	MD	130-261	7
EEparents	father & mother	no MD	131-205	4
EEfather_MD	father	MD	132-194	5
EEfather	father	no MD	127-188	6
EEmother_MD	mother	MD	143-177	4
EEmother	mother	no MD	142-171	5

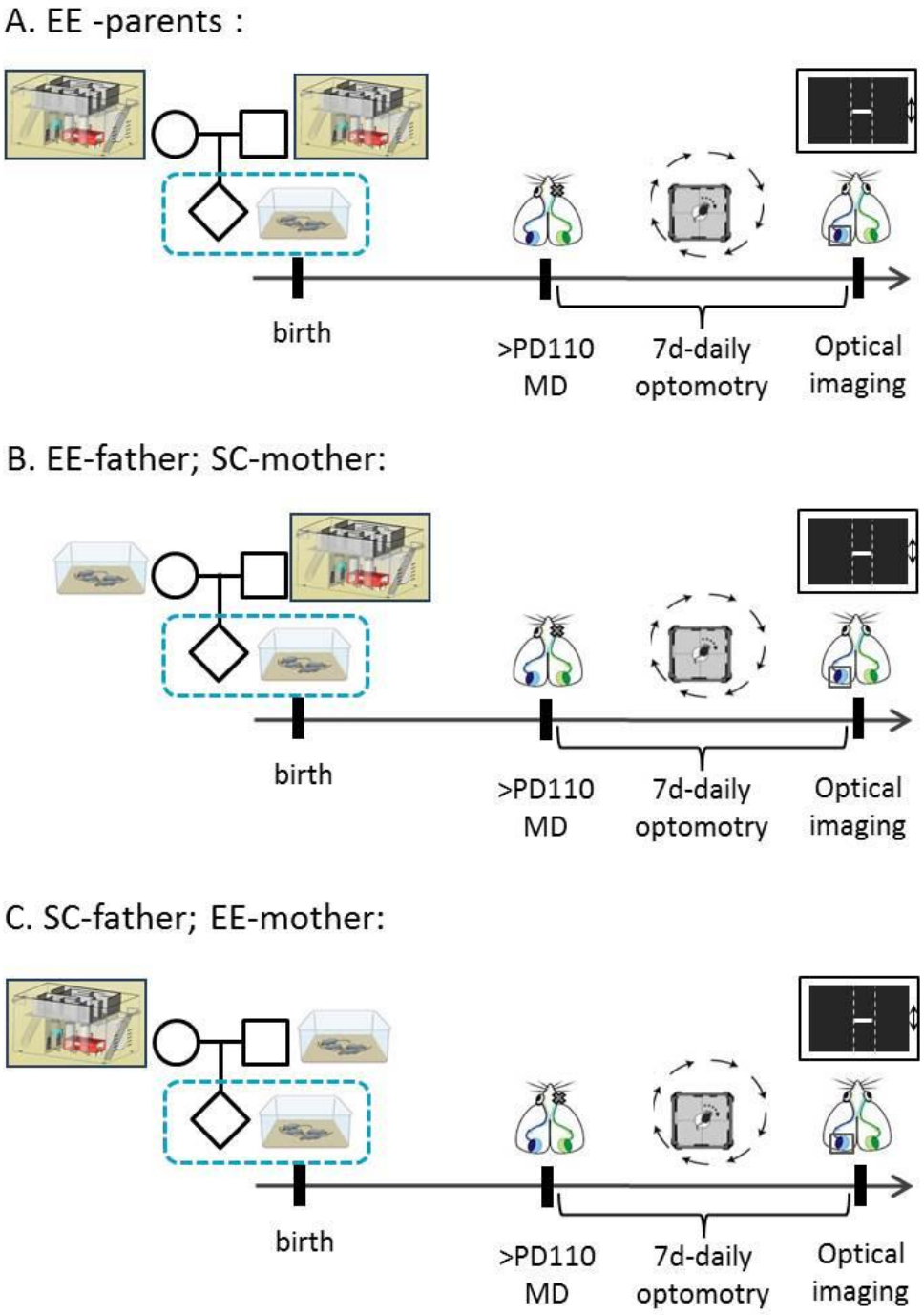


Figure 10: Experimental design to test if OD-plasticity can be transferred from EE-mice to the next generation of non-enriched pups. A. Mating of male and female enriched mice. Offspring were born and raised in SC. When offspring were above 110 days old, MD was performed. For the next 7 days mice were checked daily in optomotry. On the 7th day after MD optical imaging took place. **B.** Mating between EE-male and SC-female took place in SC. Pups were born in SC and tested for OD-plasticity when they were above PD110. To this end, mice received an MD, followed by 7 days optomotry measurements and at the end optical imaging. **C.** Mating of SC-male and EE-female took place in EE cage. Pregnant females were transferred to SC before delivery, where offspring were born and raised. Adult offspring (>PD110) were tested in optomotor setup daily during MD and after 7 days optical imaging was performed.

2.3. Housing conditions

2.3.1. Enriched environment cages

For enriched environment experiments pregnant females were put into commercially available EE cages (Marlau, Viewpoint, Frances) one week before delivery (5–7 days). Pups were separated from their mothers and placed in separate female and male groups at PD30.

The EE cages (56 × 37 × 32 cm [L×W×H]) are about nine times larger than standard cages (SC; 26 × 20 × 14 cm [L×W×H]), with two floors linked by a ladder for going up and a tube for sliding down. On the lower compartment is the "living area" with three running wheels for physical exercise, a red tunnel to protect the animals from light, and the "food area" where the mice can find food. In order to move from the "living area" to the "food area", mice have to go to the upper compartment using the ladder, pass through the maze and slide down. They can return to the "living area" through a revolving door which opens only in one direction, thus they are forced to move through the maze again in order to get food. The maze was changed three times per week, and there were in total 12 different configurations. Additionally, mice in EE had more social interactions as they were housed in bigger groups with up to 16 mice per cage compare to 3 to 4 mice per SC (Figure 11).

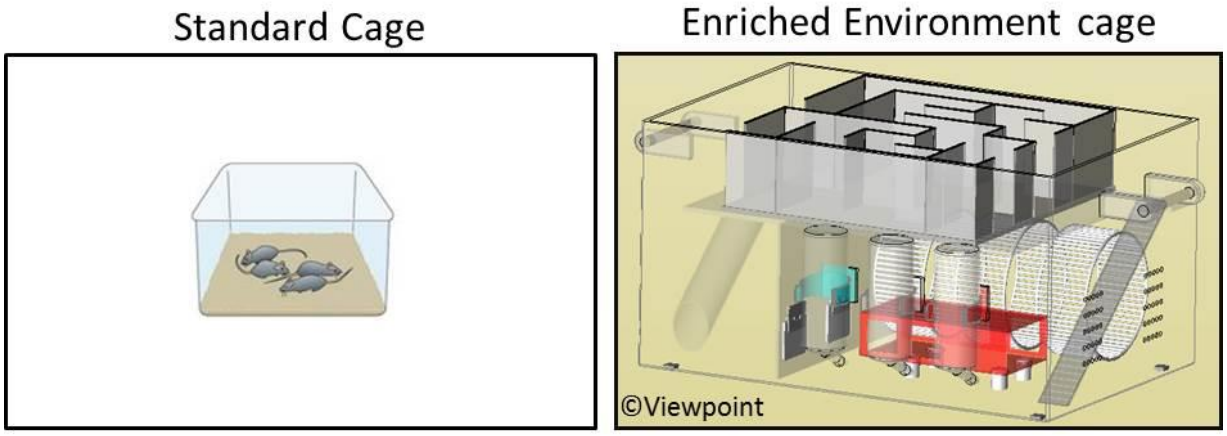


Figure 11: Enriched environment housing provides more social interaction, complexity, physical exercise and novelty. Compared to the restricted standard cage, on the left side (Figure from van Praag et al. (2000)), the EE cage on the right side (Marlau, Viewpoint, Frances) is bigger, with more compartments and more possibilities for voluntary physical exercise (running wheels), cognitive stimulation (regularly changed maze that the mice have to cross to get to the food compartment) and social interaction (larger number of mice housed together).

2.3.2. Running wheel cages

For experiments with mice in cages equipped with a running wheel (RW) pregnant females were put into slightly larger than normal SCs (43 × 27 × 19 cm [L×W×H]; normal SCs: 26 × 20 × 14 cm [L×W×H]) either with or without a RW 6-11 days before delivery. Offspring were separated into female and male groups at PD28 without changing the housing conditions. Male/female with/without RW-mice were housed as groups of 3 to 5 mice per cage. The number of RW-turns was counted daily using a counter bind on the wheel and the average RW-turns per animal/per day was calculated.

2.4. Monocular Deprivation

In order to induce OD-plasticity the right eye of the mice was deprived for 7 days according to published protocols (Gordon and Stryker, 1996; Greifzu et al., 2014). In detail, mice were initially box anesthetized with 2% isoflurane in O_2:N_2O (1:1), and placed on a heated pad to keep the body temperature stable at 37°C. Then the anesthesia was reduced to 1% isoflurane in O_2:N_2O (1:1), the eyelids were trimmed, an antibiotic gel (Gentamycin gel, active agent: gentamicin sulfat, 0.3%) was applied on the eye to prevent inflammation and the lids were closed with two sutures using 7-0 silk (Ethicon, Norderstedt). Finally, the analgesic Rimadyl (5µg/g mouse; active agent: carprofen) was injected intraperitoneally (i.p.) and the mice were returned to their home cages. Animals were checked daily to make sure that the eyes remained closed. In case of an open MD eye mice were excluded from further experiments.

2.5. Behavioural tasks to measure visual abilities

2.5.1. Optomotry

In order to quantify the spatial vision of the mice both the spatial frequency threshold ("visual acuity") and the contrast threshold ("contrast sensitivity") of the optomotor reflex all mice were measured using the virtual reality optomotor system developed by Prusky et al. (2004). This method bases on mice's optokinetic reflex, thus mice do not have to be trained. It had been previously described that the optomotor reflex is mediated by subcortical pathways (Giolli et al., 2006) and the visual capabilities measured by optomotry measurements mainly reflect the properties of the retinal ganglion cells that project to these subcortical structures (Douglas et al., 2005). After MD mice measured daily in the optomotor setup showed an enhancement of spatial vision through the open eye that is cortex-dependent (Prusky et al., 2006).

The optomotor testing apparatus consists of four computer monitors (each 33.5 x 26.5 cm [WxH]) arranged in a square with the screens facing each other, forming a box where the stimulus is presented. The mouse is placed in the middle of the box on a round elevated platform (13 cm high) with diameter of 5.3 cm and is able to move freely. Mirrors are placed

at the bottom as well as the top of the box therefore the animal in the middle gets the impression of sitting in an endless cylinder. A camera (FireWire iSight; Apple Computer Corp., Mountain View, Canada) is mounted above the platform on the lid of the box and recordings are transmitted to an external monitor, so that the experimenter can observe the animal's behaviour (Figure 12).

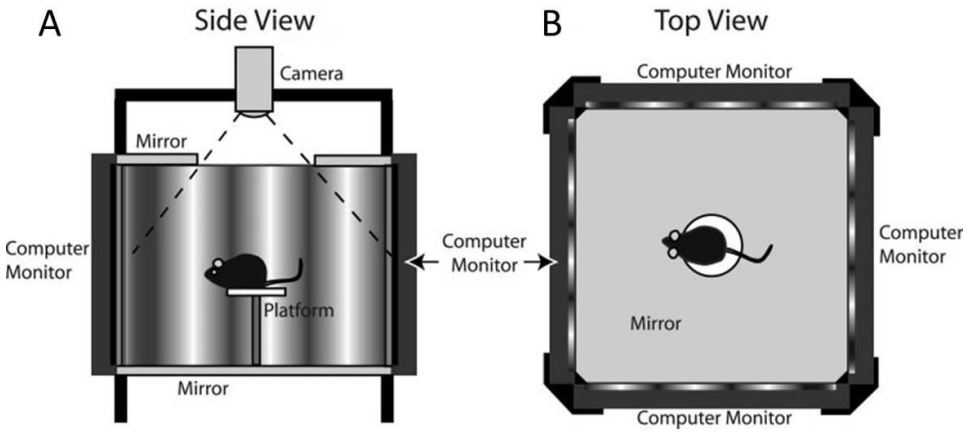


Figure 12: Schematic representation of the optomotor testing apparatus. A. Side view. The mouse can freely move on a platform positioned in the middle of an arena created by four computer screens. Sine wave gratings on the screens are extended vertically with mirrors on ceiling and floor. A video camera is placed above to monitor the mouse responses. **B.** Top view. The mouse is surrounded by 360° of gratings. Figure modified from Prusky et al., 2004.

Using the computer program OptoMotry (Version 1.4.0; CerebralMechanics, Lethbride, Alberta, Canada) the virtual cylinder, composed of a vertical sine wave grating, is projected on the screens. The virtual cylinder can rotate clockwise or counterclockwise. The center of the rotating virtual cylinder was always positioned between the eyes of the mouse, to make sure, that the walls of the virtual cylinder had always the same distance to the eyes of the mouse (Figure 13). Parameters like spatial frequency, contrast and speed of the sine wave grating can be varied by the experimenter. In case the mouse can detect the stimulus, it is reflexively tracking the grating by moving the head in the rotation's direction. Since only rotating gratings in the temporal-to-nasal direction provoke tracking (Douglas et al., 2005), it is possible to measure thresholds for both eyes separately.

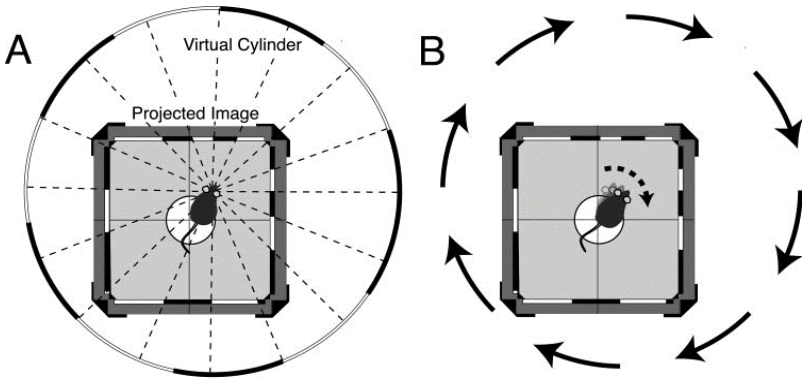


Figure 13: Simulating a three-dimensional cylinder and reflexive response. A. Centered at the animal's head, a 3-dimensional coordinate cylinder is projected on the screens. **B.** When a stimulus is presented which the mouse can detect, it is tracking it with reflexive head and neck movements. Figure from Prusky et al., 2004.

Spatial frequency at full contrast and contrast at six different spatial frequencies [0.031, 0.064, 0.092, 0.103, 0.192 and 0.272 cycles/degree (cyc/deg)] were varied by the experimenter until the threshold of tracking was determined. Contrast sensitivity measured was converted into Michelson contrasts according to the following equation:

$$contrast\ sensitivity = \frac{100}{determined\ contrast\ [\%] \times 0.997}\ .$$

The factor 0.997 resulted from following equation: $0.997 = \frac{luminance\ black - luminance\ white}{luminance\ black + luminance\ white}$.

2.5.2. Visual water task

As a second method used to assess visual abilities in mice the visual water task, a visual discrimination task based on reinforcement learning (Prusky et al., 2000b; Prusky and Douglas, 2003) was used. For this study, both SC and EE mice were trained and tested in the visual water task (VWT).

Animals are initially trained to distinguish a low spatial frequency grating (0.086 cyc/deg) from isoiluminant grey (Figure 14B) and then their ability to recognize higher spatial frequencies is tested. The apparatus consists of a trapezoidal shaped pool with two monitors placed side by side at one end (Figure 14A). A midline divider is extended from the wide end into the pool, creating a maze with a stem and two arms. An escape platform that is invisible to the animals is placed below the monitor, where the grating is projected. The position of the grating and the platform is alternated in a pseudorandom sequence over the training and test trials. When 90% accuracy is achieved 3 times (training phase), the discrimination threshold is determined by increasing the spatial frequency of the grating until performance falls below

70% accuracy (testing phase). The highest spatial frequency at which 70% accuracy is achieved is taken as the maximum visual acuity.

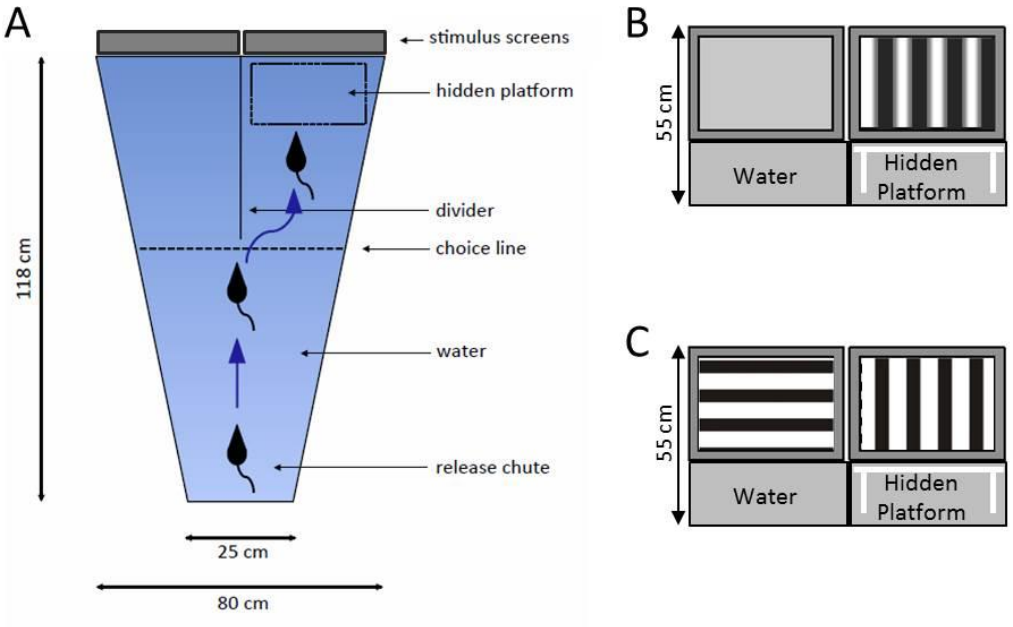


Figure 14: Schematic representation of the visual water task. A. View from the top illustrating the important components of visual water task including the pool, the platform, the midline divider, and the two monitors. The pool is filled with water. From the release chute, animals learn to swim on the side of the pool on which the grating is projected to find the hidden platform to escape from the water. **B.** To measure visual acuity mice were trained to distinguish between isoilluminant grey and vertical gratings. **C.** To test the orientation discrimination a different conformation of stimuli was used. Here the mice were trained to distinguish between vertical and horizontal square gratings. The angle difference of the two stimuli is reduced gradually until the mice cannot discriminate.

In addition, orientation discrimination was measured also by using the VWT. Here, different visual stimuli were used (Figure 14C). First, mice were trained to distinguish between horizontal and vertical square wave gratings of a low spatial frequency (0.086 cyc/deg, training phase) on each stimulus monitor respectively. Once 90% accuracy was achieved, the test phase was started. In order to test the orientation discrimination ability of each mouse the orientation difference of the two gratings was stepwise (in steps of 5°) reduced until the performance of the mice to decide for the correct side/monitor fell below 70% accuracy. The smallest orientation difference at which 70% accuracy was achieved was taken as the minimum discrimination threshold.

2.6. Photothrombosis

Photothrombosis (PT) as a model to induce a cortical stroke developed by Watson et al. (1985) was used (as described in Greifzu et al. (2011)) to study the effect of a cortical lesion on OD-plasticity of adult mice with running wheels. All PT inductions were performed by Dr Justyna Pielecka-Fortuna.

PT is known as a minimally invasive technique to induce a local cortical lesion and was induced in the left primary somatosensory cortex (S1). Mice received an intravenous tail vein injection of the photosensitive dye Rose Bengal (Sigma, Germany) and the brain region where the lesion should be localized was illuminated through the intact skull with a focused cold light source (Figure 15). The illumination causes a photochemical reaction in the blood vessels in the brain that leads to endothelial damage which induces platelet aggregation and leads to an ischemic infarct with cell death in the illuminated area (Watson et al., 1985). Due to specific determined stereotaxic coordinates the position of the lesion is quite precise and at every mouse easy to reproduce. Besides, the lesion can be restricted to the cortex so that subcortical brain regions are not affected (Domann et al., 1993).

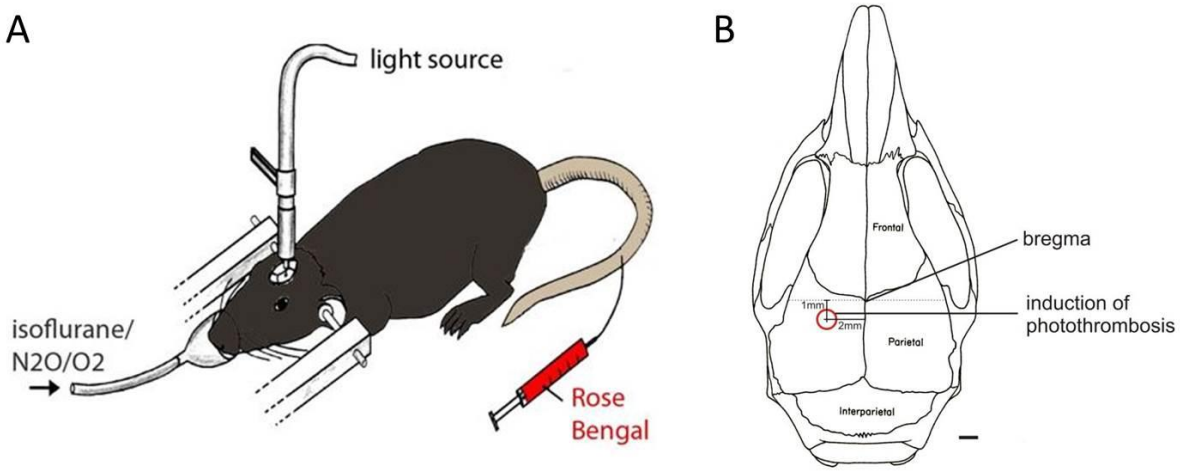


Figure 15: Lesion induction using the photothrombosis technique. A. Photothrombotic stroke induction by injecting Rose Bengal into the tail vein. Figure modified from Witte and Stoll (1997). **B.** The brain region (2mm lateral, 1mm posterior to the bregma) which was illuminated with a cold light source is marked with a red cycle. Scale bar: 1 mm. Figure modified from Cook (1965).

For our experiments, the photothrombotic lesion was always positioned in the left S1, at least 1 mm anterior to the anterior border of V1. For the surgical preparations, mice were initially box anesthetized with 2% isoflurane in a mixture of 1:1 N_2O:O_2. During the surgery, anesthesia was maintained at 0.8-1% isoflurane through an inhalation mask and the mice were placed in a stereotaxic frame while the body temperature was kept at 37°C using a heating pad with a feedback mechanism (FHC, Bowdoinham, Maine, USA) and a rectal thermometer to monitor the temperature. To protect the animal's eyes from light as well as the drying of the cornea during the procedure, they were covered with eye gel (Lac-Ophtal MP, Winzer Pharma

GmbH, Berlin) and aluminum foil. The skin above the skull was disinfected with 70% ethanol and local anesthetic (Xylocaine gel; AstraZeneca GmbH, Wedel) was applied on the skin before incised. 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. Afterwards, 0.1 ml Rose Bengal (Sigma; 10 mg/ml in 0.9% NaCl) was injected intravenously. After 5 minutes of waiting, the illumination with 12000-14000 lux was started for 15 min. Then, the skin was sutured, antiseptic Braunovidon (B. Braun Melsungen AG, Melsungen) gel was applied, and the animals returned to their cages to recover.

2.7. Optical imaging of intrinsic signals

To visualize and calculate visual cortex activity and acquire the ocular dominance index (ODI) and therefore the OD-plasticity, the *in vivo* technique of optical imaging of intrinsic signals (Blasdel and Salama, 1986; Grinvald et al., 1986; Kalatsky and Stryker, 2003) was used. The main component of the intrinsic signal derives from the high difference in reflectance of active and inactive areas at an illumination of 610 nm wavelength. The metabolic activity of neurons changes in response to visual stimulation, especially the oxygen consumption in active neurons is much higher than in inactivate ones. At 610 nm, deoxyhemoglobin absorbs more light and therefore reflects less than oxyhemoglobin (Figure 16). Thus, active brain areas accumulate a higher concentration of deoxyhemoglobin than surrounding areas and appear darker when illuminated with this red light. This change in reflection, which correlates with the neuronal activity, can be measured in the visual cortex.

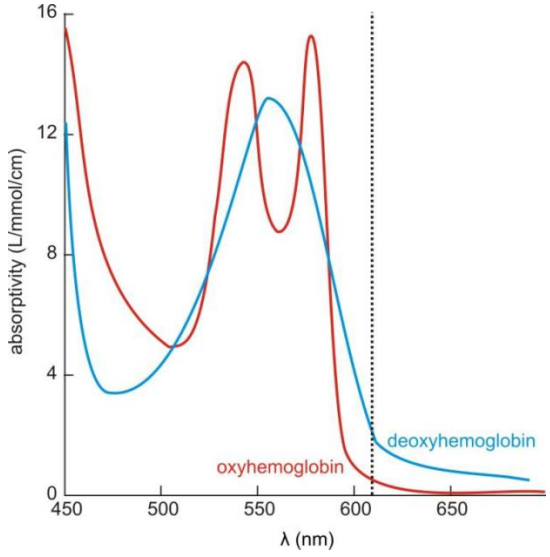


Figure 16: Absorption spectra of deoxy- and oxyhemoglobin. At the wavelength of 610 nm, which is used for optical imaging, deoxyhemoglobin (blue line) has a higher absorption and therefore lower reflectance than oxyhemoglobin (red line) which is almost zero. Figure modified from Hallum et al. (2006).

2.7.1. Surgical procedure

Surgical preparations for optical imaging were performed as published before (Kalatsky and Stryker, 2003; Greifzu et al., 2014). The mice were box-anesthetized with 2% halothane in a mixture of 1:1 O_2:N_2O and injected with atropine (0.3 mg/mouse; subcutaneously; Franz Köhler Chemie, Bensheim) to expand the pupils and inhibit saliva production, dexamethasone (0.2 mg/mouse; subcutaneously; Ratiopharm, Ulm) to reduce cerebral edema, and chlorprothixene (0.2 mg/mouse intramuscularly; Sigma-Aldrich, St. Louis, USA) to supplement the anesthesia. To prevent dehydration during the experiment 0.2 ml of 0.9% saline was injected subcutaneously. During surgery, anesthesia was maintained at 0.8% halothane in 1:1 N_2O:O_2 using an inhalation mask. The mice were placed in a stereotaxic frame and fixed with metal ear bars in each auditory canal providing horizontal stabilization, while a mouth holder provides vertical stabilization. The animals' body temperature was maintained at 37°C using a rectal thermo probe with feedback mechanism to a heating pad (FHC, Bowdoinham, Maine, USA). Heart rate was monitored throughout the experiment. In case of MD, the stitches were removed and the eye was reopened. Throughout the surgical procedure the animals' eyes were covered with silicon oil (Carl Roth, Germany) and aluminum foil to keep the cornea of the eyes moist and for dark adaptation. The skin above the visual cortex was disinfected with 70% ethanol and an anesthetic gel (Xylocaine, AstraZeneca GmbH, Wedel) was applied on the location where the skin was incised to expose the visual cortex. The exposed area was covered by agarose (2.5% in 0.9% NaCl) and a glass coverslip was placed on top to create a suitable optical surface and prevent the drying of the skull.

2.7.2. Data acquisition

Mouse visual cortical responses were recorded through the skull using the "Fourier"-imaging method developed by Kalatsky and Stryker (2003) and optimized for the assessment of OD-plasticity (Cang et al., 2005b) (Figure 17). V1 was illuminated with green light (550±10 nm) to visualize the surface vascular pattern with a CCD-camera (coupled charged device: Dalsa 1M30, Waterloo, Canada) using a 135 x 50 mm or 50 x 50 mm (for 1 or 2 hemisphere recordings respectively) tandem lens configuration (Nikon, Inc., Melville, NY). After acquisition of a surface image of blood vessels, the camera was focused 600 μm below the pial surface to make sure to record at least from cortical layer I-IV. The illumination light was then changed to red (610±10 nm) and an additional red filter was interposed between camera and objective to reduce the effect of light scatter. Frames were acquired at a rate of 30 Hz, temporally binned to 7.5 Hz, and stored as 512 x 512 pixel images after spatial binning of the camera image.

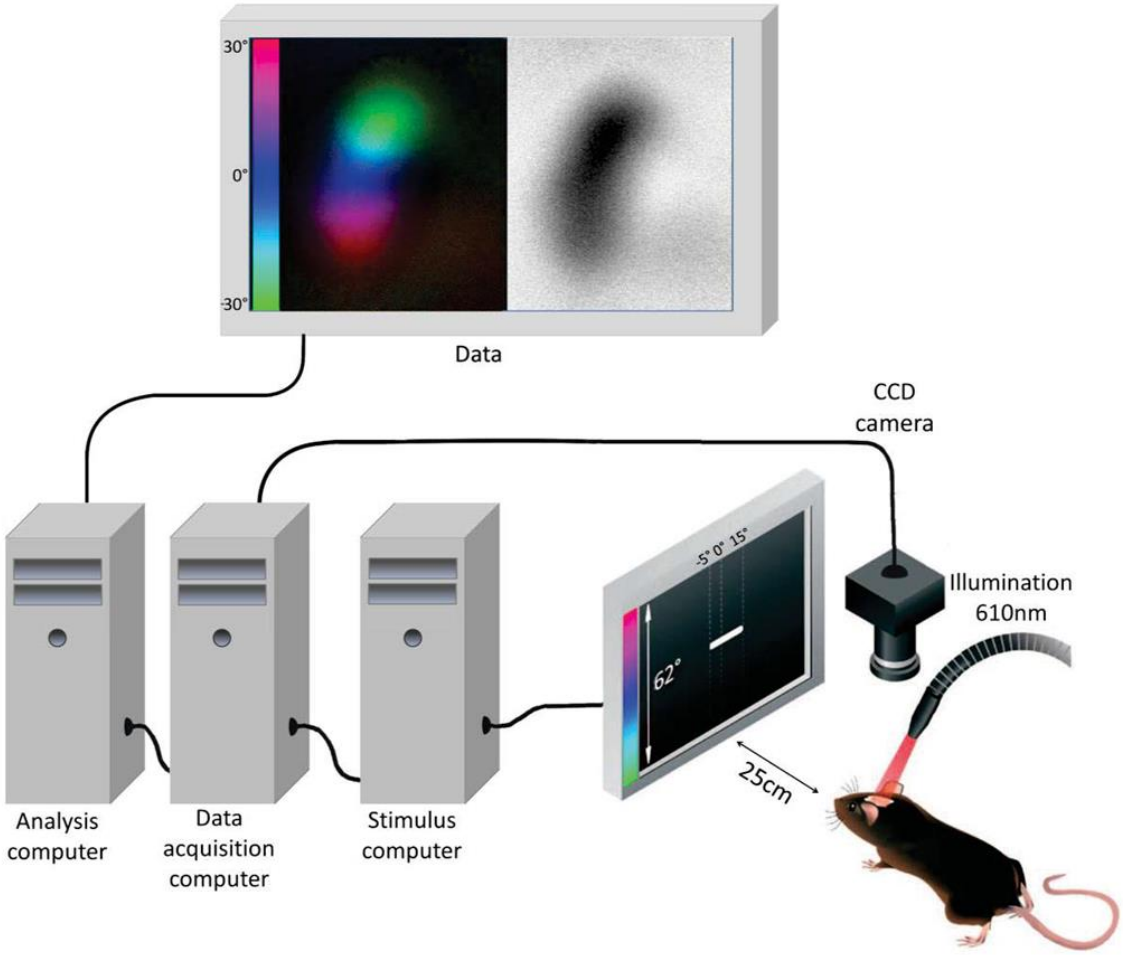


Figure 17: Experimental setup of optical imaging of intrinsic signals. The imaging system consists of a flat-screen monitor showing a moving bar as a stimulus generated by the stimulus computer, a CCD-camera, which acquires cortical responses after illumination of the visual cortex of 610 nm. The data send from the Data acquisition computer to the Analysis computer where they are extracted by Fourier analysis. Figure modified from Greifzu et al. (2012).

2.7.3. Visual stimulation

Visual stimuli were presented on a high refresh rate monitor (Hitachi, ACCUVUE, HM-4921-D, 21") positioned 25 cm in front of the mouse. Stimuli consisted of white drifting bars (2° wide) on black background generated by the program ContStim (CONTinousSTIMulation; VK Imaging, Houston, Texas), with a spatial frequency of 1 cyc/ 80°, at a temporal frequency of 0.125 Hz. The stimuli were presented in two opponent moving directions (e.g. 90°↑ and 270°↓) to correct the hemodynamic delay which is due to the delay in the change in the absorption of the blood. For determining ODI, the monitor was placed in front of the mouse with the monitor's center axis aligned with the mouse's nose. Vertically moving bars (90°↑and 270°↓) were restricted to stimulate either only the binocular visual field of the left V1 (−5° to +15° azimuth; Figure 18A) or the binocular visual field of the right V1 (−15° to +5° azimuth), and animals were stimulated through either the left or right eye in alternation

for 5 minutes. 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).

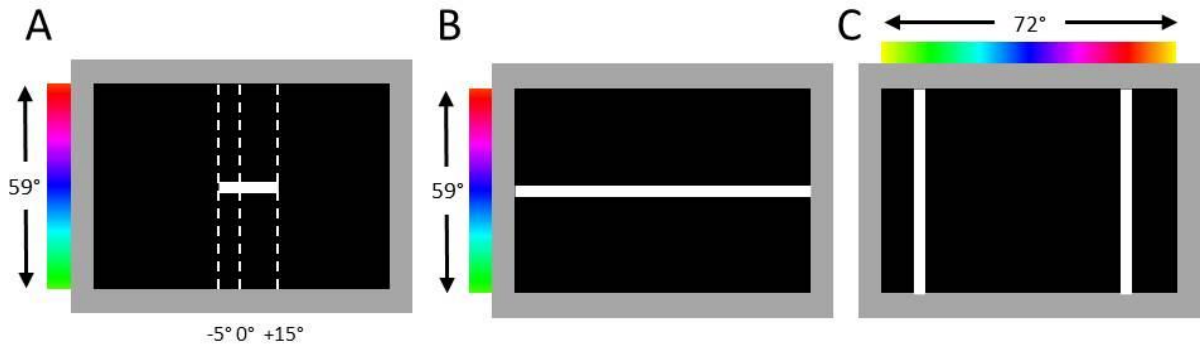


Figure 18: Visual stimuli. A. Visual stimuli for ODI measurements. To compare the cortical activity after the separate visual stimulation of the two eyes the vertical moving bar was restricted to 20° width so just the binocular zone of the visual cortex was stimulated. **B.** Visual stimuli to record maximum response and map quality of elevation maps **C.** Visual stimuli to record maximum response and map quality of azimuth maps.

To calculate the map quality the monitor was placed in the right visual field of the animal at a distance of 25 cm to optimally stimulate the right eye (contralateral to the recorded hemisphere), while the left eye remained covered. In this case the drifting bars were shown across the full screen (78° azimuth and 59° elevation, respectively). Vertical (90°↑ and 270°↓; elevation orientation) or horizontal drifting (0°→and 180° ←; azimuth orientation) bars were presented to the mouse (Figure 18B and C).

2.7.4. Data analysis

Visual cortical maps were recorded from the acquired frames by Fourier analysis to extract the signal at the stimulation frequency using custom software ("iman" (IMageANalysis; VK Imaging, Houston, Texas), "mapans" (MAP ANalysis Single; VK Imaging, Houston, Texas)). The phase component of the signal is used to calculate the retinotopy. The phase map color-codes the activated area in the visual cortex by referring to a position of the stimulus bar on the monitor at that moment (Figure 19A). The amplitude component of the optical signal represented the intensity of neuronal activation (expressed as fractional change in reflectance $x10^{-4}$; Figure 19B) and was used to calculate ODI. The polar map combines the information of both retinotopy and activity maps (Figure 19C).

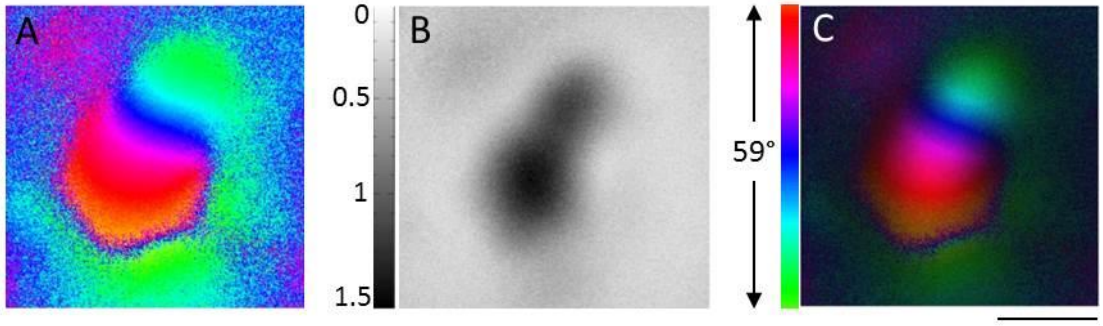


Figure 19: Examples of cortical maps acquired by optical imaging after right eye stimulation. A. Retinotopic phase map acquired by optical imaging is illustrated. The color code is based on the position of the bar on the screen. Neighbored colors in the color code of the bar position are also neighbored in the retinotopic phase map. **B.** Activity map of the same experiment. Darker areas correspond to stronger activation. **C.** Illustration of a polar map of the same mouse. The polar map combines the retinotopic map with the activity map. Higher activity is encoded by lighter areas. Scale bar: 1 mm.

To calculate the ODI, the ipsilateral eye magnitude map was first smoothed to reduce pixel shot noise by low pass filtering using a uniform kernel of 5 x 5 pixels and then thresholded at 30% of peak response amplitude to eliminate the background noise. The value of the pixel of the strongest response was then determined as the maximum response magnitude. Afterwards, the ratio of contralateral and ipsilateral responses in V1 was calculated. For every pixel in this region the ODI was computed as (C−I)/(C+I), with C and I representing the response magnitudes of each pixel to visual stimulation of the contralateral and ipsilateral eye, respectively. The ODI can reach values between -1 to +1, thereby -1 is representing a complete ipsilateral and +1 a complete contralateral dominated activation of V1. Additionally, the ODIs for every pixel in the map were color-coded in a 2-dimensional map of the OD scores (OD-map; Figure 20). Here, cold blue colors represent negative values (ipsilateral eye dominance) and warm red colors represent positive values (contralateral eye dominance). ODI values of every pixel are also plotted as a histogram (Figure 20). To compute an average ODI for each animal at least 3 maps per animal were averaged using an averaging program (MATLAB, Version: 7.12.0, The MathWorks, Natick, USA).

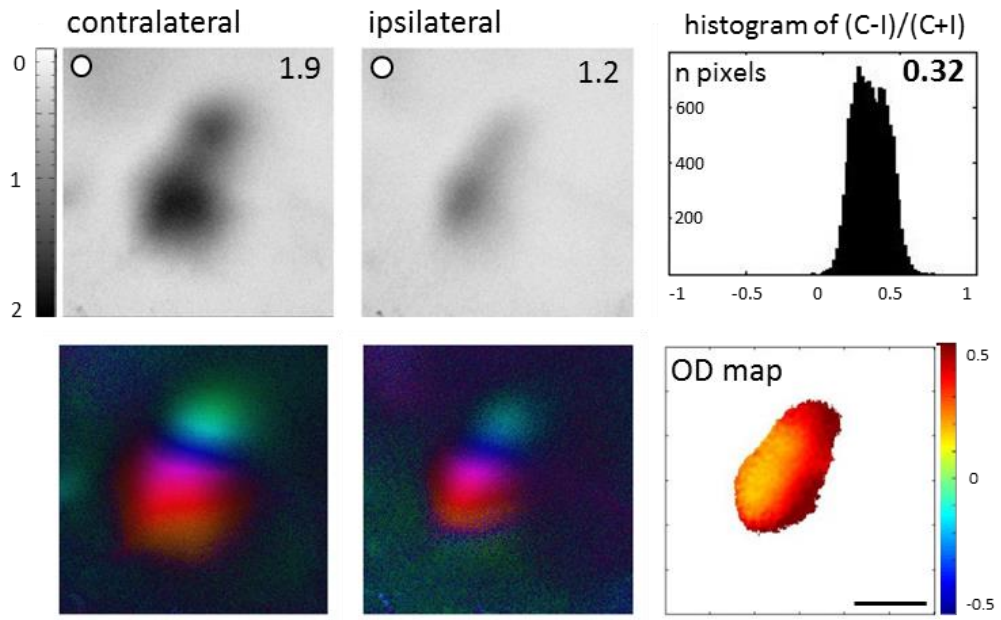


Figure 20: Example of cortical maps acquired with optical imaging and quantification of the ocular dominance index. The maximum response maps (grey scaled) of the left visual cortex after visual stimulation (with a 20° horizontal bar) of the contralateral and the ipsilateral eye, respectively with the activity value on the top right corner are illustrated. Additionally the color coded polar maps after each eye stimulation are presented. On the top right corner is the histogram of the OD-scores and their distribution in number of pixel. The calculated ODI for this example as an average of all single OD-scores is shown on the right top corner of the histogram. At last the OD-map with the calculation of OD-scores for every pixel is illustrated. The pixels of the OD-map are color-coded with warm colors to represent a contralateral dominance in the binocular part of V1. Scale bar: 1 mm.

To determine monocular V1-activation and the quality of the retinotopic maps, we used the calculation introduced by Cang et al. (2005a). For quantification, the most responsive 20,000 pixels in V1 for both azimuth and elevation maps were selected. For every pixel, the difference between its phase and the mean phase of its 24 surrounding pixels was calculated. The standard deviation of the position difference was used as an index of the quality of the retinotopic maps. Lower values indicate lower map scatter and thus higher map quality and vice versa (Figure 21).

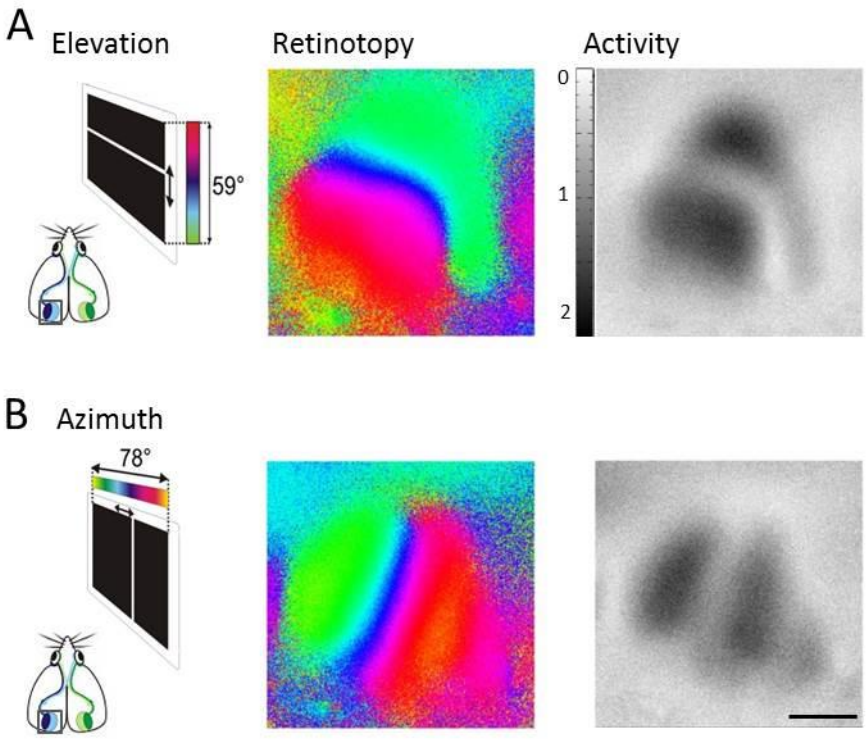


Figure 21: Example of optically imaged maps used for calculation of map quality. A. Examples of retinotopic and activity maps after elevation stimulation (full length bar moving horizontally) from the left V1. **B.** Examples from the same animal of retinotopic and activity maps after azimuth stimulation (full length bar moving vertically). For the calculation of the standard deviation as indicator of map quality the 20,000 most responsive pixels from the activity map are selected and a phase difference based on the retinotopic map was calculated. Scale bar: 1 mm.

2.8. Lesion analysis

2.8.1. Perfusion and preparation of the tissue

After optical imaging, mice with PT were deeply anesthetized with 30% chloral hydrate (0.2 ml, intraperitoneal injection). The abdomen was opened and a cannula was put into the left ventricle and the right atrium was cut. Mice were perfused transcardially using a perfusion pump (MC-MS CA8/6, Ismatec; pump output of 25 ml/min) with 1% heparin in 0.9% NaCl for 2 minutes followed by 4% paraformaldehyde (PFA, pH 7.4) for 3 minutes. The brains were removed, postfixed in 4% PFA (pH 7.4) at 4°C overnight and then transferred to cryoprotectant solution (10% sucrose, 20% glycerol). The brains were frozen in methylbutane at -40°C and stored at -80°C. Before slicing, a lateral-ventral cut was positioned on the right side of the brain over the whole anterior to posterior extend of the brain in order to distinguish between right and left hemisphere after cutting and staining. Coronal brain sections were sliced using Leica SM 2010R sledge microtome at 40 µm. Sections were collected in antifreeze solution (30% ethylene glycol, 15% glucose in phosphate buffer (PB)) and stored at -20°C before further analysis.

2.8.2. Nissl staining

To analyze the size and the position of the lesion in EE-mice, brain sections were mounted on microscope slides and Nissl-stained. First sections were dehydrated in an ascending ethanol series: 70%, 80%, 96% and 100% ethanol for 2 minutes each. Then they were rehydrated by shortly dipping them into bidest water and stained in cresyl violet (0.5% in ddH_20) for 2 to 4 minutes. Afterwards the sections were transferred in a series of ethanol: 70%, 80%, 96% and 2 times 100% for 2 minutes in each for destaining and dehydration. Finally, sections were transferred to Roti-Histol (Roti®-Histol, Roth) and coverslip using Roti®-Histokitt (Roth).

2.8.3. Immunostaining with GFAP

In the study examining the therapeutic effect of the physical exercise after stroke a different method was performed to characterize the exact size and position of the cortical lesions. Here immunostaining with an antibody against Glial Fibrillary Acidic Protein (Rabbit polyclonal Antibody to GFAP; Immunological Sciences) was performed (Lai et al., 2014). Initially the sections were transferred to 0.1M PB solution and sorted from anterior to posterior direction. Then every third section was picked and transferred in a well plate (free floating) containing 2 ml of 0.1M PB keeping the order of the sections. Sections were washed for 10 minutes with 0.1M PB at room temperature and incubated for 10 minutes with 0.1M PB-Triton-X-100 (0.3%), followed by 30 minutes blocking in 10% normal donkey serum in PB-Triton-X-100 (0.3%) at room temperature. The sections were incubated with the primary polyclonal rabbit-anti-GFAP antibody (Immunological Science) 1:1000 diluted in 0.1M PB-Triton-X-100 (0.3%) over night at 4°C. The following day the sections were washed 3 times for 5 minutes with 0.1M PB at room temperature. Incubation with the secondary Cy3-goat-anti-rabbit antibody (Jackson ImmunoResearchInc.) was for 2 hours at room temperature in dark (1:1000 diluted in PB-Triton-X-100 (0.3 %)) followed by 3 washes for 5 minutes with 0.1M PB in room temperature. Afterwards the sections were transferred on a microscope slide based on order and dried for 30 minutes. The dried sections were mounted with Fluoromount-G with 4′6-diamidin-2-phenylindol (DAPI; Jackson ImmunoResearchInc.) and a glass cover slip was placed on top. After drying overnight at 4°C slides were stored in a light tight box at 20°C.

2.8.4. Measurements of the lesions

To determine the size and location of the cortical PT-lesions, every 3rd of the stained either with Nissl (for the enriched environment study) or GFAP (for running wheel study) brain sections were analyzed under the microscope with 2.5x objective (Axioskop, Carl Zeiss). We focused on the areas of lesions and measured parameters such as depth and length using AxioVision (40 4.8.2.0.).

2.9. Immunohistochemistry with PV-WFA

The number of perineuronal nets (PNNs) and of PV positive interneurons was counted in mice raised in SC or EE cages. PNNs were labeled cytochemically by N-acetylgalactosamine-binding (in the glycosaminoglycan chains of the chondroitinsulfat-proteoglycanes) by *Wisteria floribunda* agglutinin (WFA; Lectin from Wisteria Floribunda, Sigma), an established marker for PNNs (Hartig et al., 1992). PV antibody (monoclonal anti Parvalbumin, Immunological Science) was used for the staining of the PV cells.

Coronal brain sections of 40 µm thickness that included V1 (approximately 2.70 mm to 3.88 mm posterior to bregma) were stained. Free floating sections were initially washed with 0.1 M PB for 10 minutes and incubated for 30 minutes in a blocking solution (10% donkey serum, 0.3% Triton X-100 in 0.1M PB, pH 7.4) at room temperature. Afterwards, the brain sections were washed with 0.1M PB. Subsequently, sections were incubated overnight at 4 C with mouse anti-PV (Immunological Science, 1:500) and biotin-conjugated lectin WFA (Sigma, 1:1000) in 0.1M PB including 0.3% Triton X-100. After washing the sections again in 0.1M PB antibodies were revealed with Cy2-conjugeated donkey anti-mouse (Biotium, 1:200) and Cy3-conjugated streptavidin (Jackson ImmunoResearch, 1:1000) in 0.1M PB with 0.3% Triton X-100 (2 hours incubation at room temperature, light protected). Sections were washed again in 0.1M PB before they were transferred on a microscope slide and dried for 30 minutes. Finally, the dried sections were mounted with Fluoromount-G with 4′.6-diamidin-2-phenylindol (DAPI) and covered with a glass cover slip. After drying overnight at 4 C slides were stored in a light tight box at 4 C. The analysis of the stained sections and the counting of the cells were performed later by Dr. Franziska Greifzu.

2.10. Statistical analysis

Statistical analyses were done using Microsoft Excel 2010 and Graphs were prepared with GraphPad Prism (version 5.04). 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” and “contrast sensitivity” were analyzed by ANOVA with repeated measurements and Bonferroni correction. Correlation analysis was done using Pearson-correlation test. The levels of significance were set as *$p<0.05$; **$p<0.01$; ***$p<0.001$. Data are represented as means±SEM.

2.11. Used chemicals, antibodies and solutions

A list of the used chemicals, drugs and substances as well as the recipes for all the solutions used in this study can be found in this chapter.

2.11.1. Chemicals, drugs, antibodies and substances

Chemical	Company	Order number/PZN*
0.9 % NaCl	Braun	PZN: 2946431
2-Methylbutan	Roth	3927.1
Acetic Acid	Roth	3738.1
Agarose	Biomol	1280
Atropine	Franz Köhler	PZN: 1821288
Biotin-conjugated lectin *Wisteria Floribunda* agglutinin (WFA)	Sigma	L1516
Braunovidon gel	Braun	PZN: 2336939
Chloral hydrate	Sigma	15307
Chlorprothixene	Sigma	C1671
Corneregel	Dr Gerhard Mann GmbH	PZN:1224641
Cresyl violet	Merk	5235
Cy2 Donkey Anti-Mouse	Biotium	CF 488A, 20014
Cy3 Donkey Anti-Rabbit	Jackson ImmunoResearch	711-165-152
Cy3-Streptavidin	Jackson ImmunoResearch	016-160-084
Dexamethasone	Ratiopharm	PZN: 7720996
Ethanol (99.8 %)	Roth	K928.1
Ethylene glycol	Fluka	03750
Fluoromount+DAPI	Southern Biotech	0100-20
Fluoxetine hydrochloride	Tocris Bioscience	0927
Gentamycin gel	Medphano	PZN: 6877307
Glucose	Roth	6780.1
Glycerol	Roth	3783.1
GFAP-Rabbit polyclonal	Immunological Science	AB-10682

antibody		
Halothane	Sigma	B4388
Heparin	Rotexmedica	PZN: 3862340
Isoflurane	Abbott	PZN: 4831850
Na_2HPO_4	Roth	P030.2
Na_2HPO_4 x $2H_2O$	Roth	4984.1
NaCl	Roth	3957.1
NaH_2PO_4 x H_2O	Roth	T879.2
Normal Donkey Serum	Jackson ImmunoResearch	017-000-121
Parvalbumin anti mouse	Immunological Science	MAB-1-233
PFA	Roth	0335.3
Rimadyl (Carprofen)	Pfizer	PZN: 0110208
Rose bengal	Sigma	PZN: 0110208
Roti®-Histol	Roth	6640.0
Roti®-Histokitt	Roth	6638.1
Sucrose	Roth	4621.1
Silicon oil	Roth	4060.1
Triton X-100	Sigma	X100

*PZN = pharmaceutical identification number

2.11.2. Solutions

Solution	Recipe
Agarose (2.5 %)	5 g Agarose in 200 ml 0.9 % NaCl
Anti-Freeze solution (30 % ethylene glycol, 15 % glucose in PB)	300 ml Ethylenglycol 500 ml 0.1 M Phosphate buffer (PB), pH 7.4 150 g Glucose Fill up to 1000 ml with aqua bidest.
Chloral hydrate (30 %)	30 g Chloral hydrate in 100 ml aqua bidest.

Chlorprothixene	4 mg Chlorprothixene in 1 ml aqua bidest.
Cryoprotection (10 % sucrose, 20 % glycerol & 0.02 % sodium azide)	10 g D(+)-Saccharose 20 ml Glycerol 0.02 g Sodium azide Fill up to 100 ml with 0.1 M PB
Heparin (1 %)	53.33 ml Heparin (25.000 I.E.) 9 g NaCl Fill up to 1000 ml with aqua bidest.
PB (Phosphate buffer, 0.1 M)	3.75 g NaH2PO4 x 2H2O 9.75 g Na2HPO4 Fill up to 1000 ml with aqua bidest.
PFA (4%, pH 7.4)	40 g Paraformaldehyde (PFA) 300 ml aqua bidest. heat to ~ 60 °C until suspension is clear (if not, add six to eight drops of 10M NaOH) filter solution, adjust to pH 7.4 Fill up to 1000 ml with aqua bidest.
Rose bengal	100 mg Rose bengal 10 ml 0.9 % NaCl
Sucrose (30%)	30 g Sucrose Fill up to 100 ml with 0.1 M PB
Saline (NaCl, 0.9 %, pH 7.0)	0.9 g NaCl Fill up to 1000 ml with aqua bidest

3. Results

3.1. EE extends ocular dominance plasticity into adulthood and protects from stroke-induced impairments of plasticity (Greifzu et al., 2014)

Ocular dominance (OD) plasticity is an age depended phenomenon: it is maximal during the critical period, reduced but still present in young (3 months old) and absent in adult mice beyond postnatal day (PD) 110 when mice are raised in standard cages (SC; for review see Espinosa and Stryker (2012)). We described recently that raising mice in an enriched environment (EE) promotes mice OD-plasticity in older age (Greifzu et al., 2014). Specifically, we found that old EE-mice (>PD110) showed a strong OD-shift after monocular deprivation (MD) which was mediated by decreased deprived eye responses in V1, a type of OD-plasticity described previously only in juvenile SC-mice. Additionally, EE not only promoted plasticity in adult mice but also restored already lost OD-plasticity in adult SC-mice transferred to EE and preserved OD-plasticity after a localized cortical stroke. Furthermore, in collaboration with Dr. Schlüter's laboratory, we described that local inhibition was significantly reduced in adult EE-mice V1 and the GABA/AMPA ratio was similar to juvenile SC-mice, using in vitro electrophysiology. These observations were confirmed by *in vivo* analyses showing that diazepam treatment significantly reduced the OD-shift of EE-mice after MD. We proposed that the effect of EE was mediated most likely by preserving low juvenile levels of inhibition into adulthood, which potentially promoted adaptive changes in cortical circuits. As part of this study I performed experiments to reveal any possible alterations in the number of parvalbumin (PV) positive cells as well as of perineuronal nets (PNNs) which were found to be reduced in EE-rats (Sale et al., 2007; Baroncelli et al., 2010b). Additionally, I performed histological staining to calculate the size and the position of the cortical lesion after a localized cortical stroke. In the paragraph below, I describe thoroughly my contribution to the Greifzu et al. (2014) study.

3.1.1. Number of parvalbumin-positive interneurons and PNNs was similar in EE- and SC-mice

Inhibitory PV-interneurons are thought to have an important role for OD-plasticity in the adult brain (for reviews see Bavelier et al., 2010; Baroncelli et al., 2011). Moreover, the degradation of PNNs was shown to play a role in the enhancement of adult OD-plasticity (Pizzorusso et al., 2002) and reduced PNNs density was reported after EE-housing (Sale et al., 2007). To examine whether a change in the number of PV-interneurons or PNNs could contribute to the prolonged sensitive phase for OD-plasticity in EE-mice, I used immunofluorescence staining. Precisely, triple immunofluorescence staining was performed for PV (Cy2 labeling), PNNs (with WFA, Cy3 labeling) and DAPI (to visualize all cell nuclei and identify the cortical layers). The number of all labeled cells of layers II-VI in V1 was then counted and compared between SC- and EE-mice by Dr. Franziska Greifzu.

Initially, PV-labeled cells were found throughout the analyzed layers II-VI in V1 of SC- (PD235) and EE-mice (PD220). The overview of PV-positive cells in all layers as well as a higher magnification already suggests that the numbers of PV-positive cells were not different between SC- and EE-mice. Then the number of PNNs was calculated after WFA staining. The stained pictures already gave the impression of an unchanged number of WFA-positive PNNs after EE-raising. At a higher magnification the staining revealed lattice-like structures around the cells, often around PV-positive cells (Figure 22).

Quantification of the number of PV-positive cells and WFA-positive PNNs, by Dr. Franziska Greifzu, confirmed that there are no differences between SC- and EE-mice. The number of PV-positive cells in SC-mice were 5683.39±562.95 cells/mm^3 (n=4) and not significantly different from the EE-mice with 6396.47±277.76 cells/mm^3 (n =4 mice; $p>0.05$, t-test). Similarly, for the number of WFA-positive PNNs were not different in SC-mice (6167.34±726.69 PNNs/mm^3, n=4) and EE-mice (6408.04±130.80 PNNs/mm^3, n=4; $p>0.05$, t-test). In conclusion, the number of PV-positive cells and WFA-positive PNNs was not altered in EE-mice compared to SC-mice.

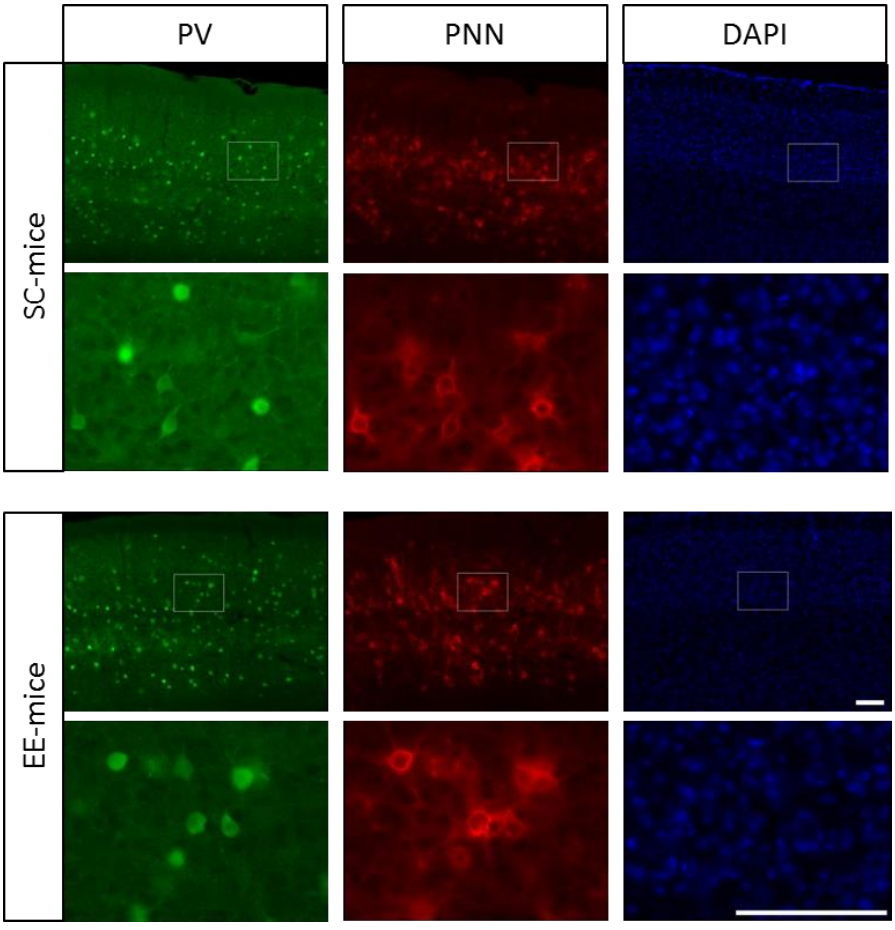


Figure 22: Number of PV-positive and WFA-positive PNNs in V1 was not different between mice raised in SC or EE. Pictures of the fluorescent triple staining of PV-positive cells (green), WFA-positive PNNs (red) and cell nuclei (blue) are presented. On top a representative example of a SC-mouse and a magnified picture of the region marked with a white square are illustrated. On the

bottom is a representative example of an EE-mouse also with a magnified region marked with a white square. Scale bars: 100 µm.

3.1.2. Localization and size of the photothrombotic lesions

To investigate whether EE could also prevent the loss of OD-plasticity in mice after stroke the photothrombotic (PT) method was used to induce a small cortical lesion in the left primary somatosensory cortex (S1). After imaging experiments the animals were perfused and brains were collected. I sliced the brain in 40 µm thick coronal sections and performed Nissl staining to describe the exact location and size of the PT-lesions. I found that the PT-lesions were localized in the left S1 at 0.6±0.19 mm anterior from the anterior border of V1, at 1.2±0.25 mm lateral to the midline and 1.6±0.19 mm posterior to the Bregma. The average size of the lesions was 0.6±0.11 mm in the mediolateral and 0.8±0.09 mm in the anterioposterior directions (Figure 23).

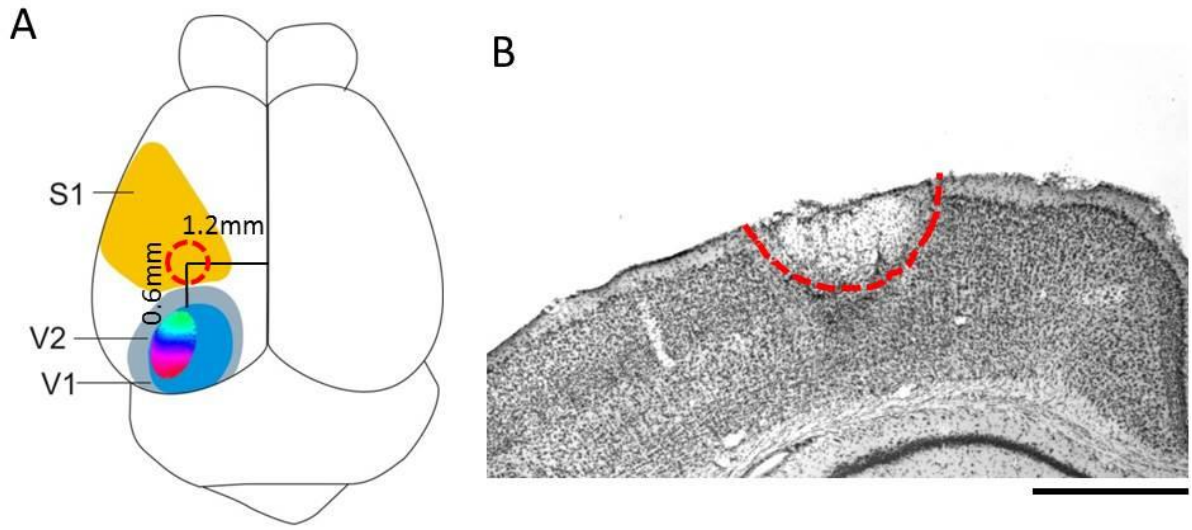


Figure 23: Localization of PT-lesion. A. Schematic representation of the lesion location in the left primary somatosensory cortex (S1). With yellow the S1 is illustrated, with grey the secondary visual cortex (V2) and blue the primary visual cortex (V1). A retinotopic map of the binocular zone in V1 is also displayed in the binocular part of V1. The positon of the lesion is marked with a red circle and the distance from midline and anterior border of V1 are illustrated. **B**. Representative example of a PT-lesion after Nissl staining of a section of an EE-mouse brain. The red line marks the lesion site. Scale bar: 100 µm.

3.2. Environmental enrichment preserved lifelong OD-plasticity, but did not improve visual abilities

As we described previously for EE-mice the OD-plasticity was preserved in adulthood (Greifzu et al., 2014). The oldest mouse tested in this study was 196 days. We then investigated whether OD-plasticity can be prolonged into even older age (>PD400) and whether long-term EE modifies visual abilities of the old mice. We found that EE-raised mice display OD-plasticity until PD809, thus presumably lifelong. Furthermore, mice raised in SCs until PD110 and then transferred to EE displayed OD-plasticity until PD922 (oldest mouse tested). Moreover, the visual abilities of very old SC- and EE-mice were tested using two different behavioural tests: the optomotor setup (Prusky et al., 2004) and the visual water task (Prusky et al., 2000b). We found no differences between old SC- and EE-mice in: (i) the spatial frequency thresholds of the optomotor reflex, (ii) their experience-enabled increase after MD, (iii) the visual acuity and the orientation discrimination and (iv) the learning time for the visual water task. Taken together, EE-raising preserved a lifelong OD-plasticity but did not affect basic visual performance. This study is submitted for publication and I contributed equally by performing the behavioural tests which are thoroughly describe in the following paragraph.

3.2.1. Basic visual abilities were similar in old SC- and EE-mice

The visual acuity of both SC-mice (PD690) and EE-mice (PD687) was determined using two different behavioural vision tests: the virtual reality optomotor setup (Prusky et al., 2004) and the visual water task (VWT) (Prusky et al., 2000b). The optomotor system was used to measure the spatial frequency threshold ("visual acuity") of the optomotor reflex, mediated by subcortical circuitry, while the VWT is a cortex-dependent paradigm of visual discrimination learning (Prusky et al., 2006). In both tests, visual performance of old mice of both groups (SC and EE) was statistically not significant. In addition the learning speed for the VWT was similar in both SC and EE old mice.

In the optomotor setup the spatial frequency threshold that elicited an optomotor response was 0.36±0.002 cyc/deg for SC-mice (n=5) and 0.36±0.004 cyc/deg for EE-mice (n=4) and was not significantly different (p=0.735, t-test; Figure 24A).

Similarly, in the visual water task visual acuity for SC-old mice was 0.51±0.02 cyc/deg (n=4) and not significantly different from the EE-old mice (0.48±0.01 cyc/deg; n=4; p=0.325, t-test; Figure 24B). It is evident that the visual acuity values measured with the VWT were higher than those from the optomotor setup for both groups (SC/ EE: p=0.003/ 0.0004, t-test). This observation was in line with previously described measurements (Douglas et al., 2005). Summarizing, the EE housing had no effect on the spatial frequency threshold of old mice.

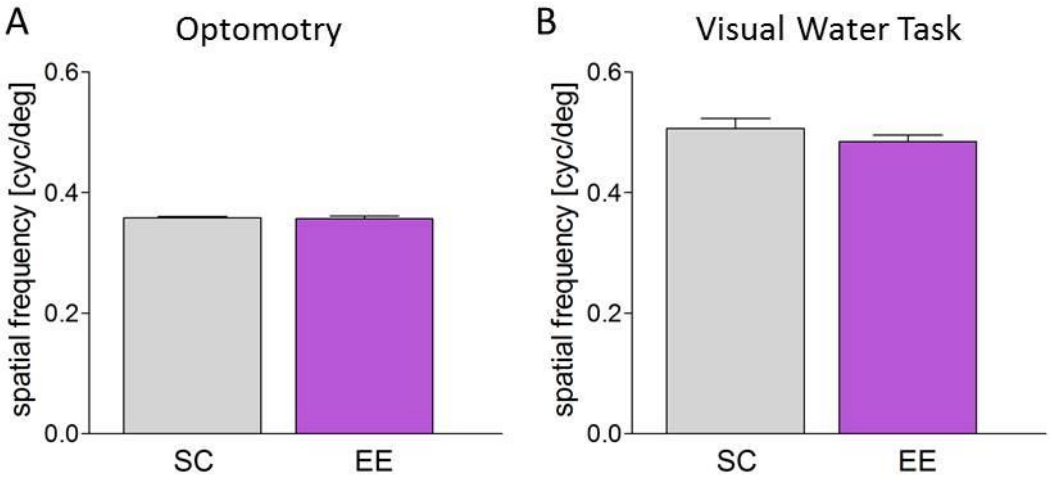


Figure 24: Visual abilities of old SC-and EE-mice. A. Mean spatial frequency threshold ("visual acuity") of the optomotor reflex (in cycles/degree) of old SC- (grey) and EE-mice (purple) tested with the optomotor setup. The two tested groups showed similar "visual acuity" values. **B**. Mean visual acuity values for the same groups of mice measured in the visual water task. No differences were observed between the groups also for this behavioural test.

3.2.2. Orientation discrimination and learning the visual water task were similar in EE- and SC-raised mice

In order to test visual abilities of old SC (PD690) and EE-mice (PD687) in a more elaborate perceptual task the orientation discrimination of the mice was measured using the visual water task (Prusky et al., 2000b; Pielecka-Fortuna et al., 2014). Before the testing phase the mice had to learn the task by swimming towards the rewarded orientation grating, where the escape platform was located. All the mice learned how to perform the task independent from the housing conditions (SC or EE) and there was no difference in the learning curves of animals. Specifically, old SC-mice completed the training phase within 20±3 training blocks (n=5) corresponding to 9 days of training whereas old EE-mice within 15±1 training blocks, equal to 7 days of training (n=5; p=0.210, t-test; Figure 25).

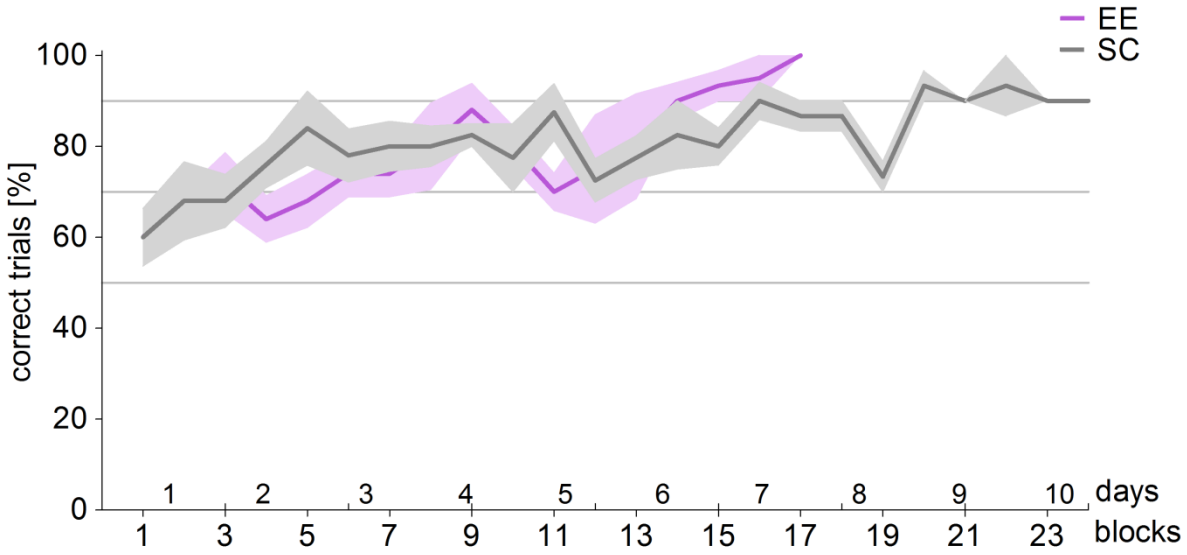


Figure 25: Learning curve of the visual water task. The number of correct trials plotted against the blocks and the respective days of training is illustrated. SC (grey) and EE (purple) mice had on average no differences in their learning curve. In SC group, 2 mice needed more blocks to learn the task but at the end the differences between SC-and EE-mice were not significant.

After the mice learned to perform the task we continued with the testing phase, where the orientation discrimination threshold of individual animals was identified by gradually decreasing the orientation difference of the rewarded with respect to a distractor grating. Again we did not find any significant difference between the two groups. The SC-mice could make the correct choice on at least 23.7±4.4° (n=5) of orientation difference, and the EE-mice at 17.9±2.5° (n=5) orientation difference (Figure 26). Comparing the values of the two groups no significant difference was found in orientation discrimination (p=0.286, t-test).

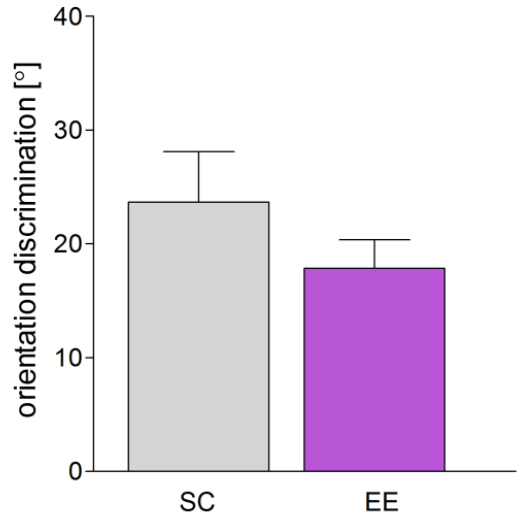


Figure 26: Orientation discrimination of SC- and EE-mice. The average orientation difference in degree that the mice needed to make the correct choice is plotted for the SC (gray) and EE (purple) mice. The difference between the groups is not significant.

3.3. Four days of MD are enough to induce OD-plasticity in EE-mice

OD-plasticity is age-dependent in mice raised in SCs: it is maximal during the critical period (PD25-40), reduced in young adults (PD90) and absent in mice older than PD110 (for review see Espinosa and Stryker (2012)). The mechanism mediating the OD-shift differs between the ages: during the critical period, 4 days of MD are sufficient to induce an OD-shift which is mediated by reductions of deprived eye responses in V1 (Sato and Stryker, 2008). In contrast, in 2-3-month-old mice, OD-shifts need 7 days of MD and are mediated by increases of the open eye responses in V1 (Levelt and Hübener, 2012). We described before that raising mice in EE prolonged the sensitive phase of OD-plasticity in mice older than PD110 (Greifzu et al., 2014). The OD-shifts were mediated by reductions in deprived eye responses like in critical period SC-mice (juvenile-like OD-plasticity). If the OD-plasticity mechanism in adult EE-mice is indeed like in juvenile SC-mice then 4 days of MD will be enough to induce an OD-shift. Here, I investigated whether 4 days of MD are sufficient to induce an OD-shift in EE-mice of 3 different age groups (critical period: PD27-34, young: PD80-101 and adult: PD121-183). All groups used for this study are described in detail in material and method section (part 2.2.1). For this study all the mice were tested using the optomotor setup and the optical imaging of intrinsic signals. We observed that 4 days of MD can induce an OD-shift in all the age groups of EE-mice tested. Critical period mice showed an OD-shift mediated by reduced closed eye responses in V1 like in age-matched SC-mice. This was not the case for the other two age groups (young and old). In young and old EE-mice the OD-shift was mainly mediated by increased open eye responses in V1 resembling the adult type of OD-plasticity observed in around 3 months old SC-mice.

3.3.1. Basic visual abilities and enhanced optomotor reflex after MD did not change with age in EE-mice

Initially the “visual acuity” and “contrast sensitivity” thresholds were determined in the three different age groups of EE-mice, using the optomotor setup. The baseline visual acuity threshold of EE-mice in critical period (EE_cp) was 0.38±0.01 cyc/deg (n=10; PD27-34), of young EE-mice (EE_young) was 0.38±0.001 cyc/deg (n=13; PD80-101) and of EE-mice >PD120 (EE_old) was 0.38±0.001 cyc/deg (n=10; PD121-183). No significant differences observed between the three tested groups ($p>0.05$, ANOVA; Figure 27). The baseline visual acuity values were similar to those previously published for SC raised C57BL/6J mice (Prusky et al., 2004; Lehmann and Löwel, 2008) and old EE-mice (Greifzu et al., 2014).

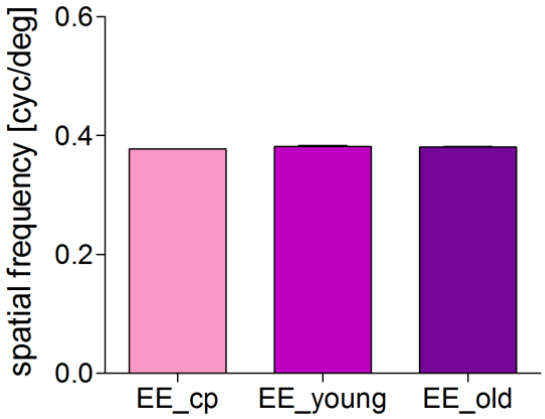


Figure 27: Baseline "visual acuity" in EE-mice of three different age groups. The highest spatial frequency that elicited a response in the optomotor setup was similar between EE_cp (pink), EE_young (purple) and EE_old (violet) mice.

The baseline contrast sensitivity thresholds were also determined for the three mice groups at six different frequencies (0.031, 0.064, 0.092, 0.103, 1.192 and 0.272 cyc/deg; Table 6). There were no significant differences between the baseline values of all the groups tested ($p>0.05$ for every spatial frequency, ANOVA). The measured values were also comparable to those described before for EE-mice above PD120 days old (Greifzu et al., 2014). All together our observations suggest that baseline visual abilities are similar in all age groups of EE-mice.

Table 6: Baseline contrast sensitivity values of the three different age groups of EE-mice. For the six different spatial frequencies tested the average contrast sensitivity for each group is listed as mean±SEM.

Spatial frequency (cyc/deg)	EE_cp (n=10)	EE_young (n=13)	EE_old (n=10)
0.031	3.7±0.10	3.6±0.20	3.9±0.10
0.064	13.4±0.30	11.5±0.20	11.8±0.10
0.092	12.2±0.10	11.3±0.20	11.3±0.10
0.103	11.0±1.70	11.0±0.30	10.6±0.30
0.192	7.0±1.60	7.5±0.20	7.8±0.20
0.272	4.1±1.02	3.6±0.01	3.8±0.001

To measure the experience-induced improvements in spatial frequency and contrast sensitivity thresholds of the optomotor reflex mice underwent MD and were measured daily during the MD period in the optomotor setup. All three age groups of EE-mice showed a significant increase in spatial frequency and contrast sensitivity thresholds after 4 days of MD (Figure 28). Specifically, "visual acuity" increased by 11.2±0.3 % in EE_cp mice from 0.38±0.001 cyc/deg without MD (n=4; Figure 28A, Figure 29A) to 0.40±0.007 cyc/deg after MD (n=6; $p<0.001$, Bonferroni-adjusted t-test; Figure 29B), by 8.6±0.01% in EE_young mice from 0.38±0.001 cyc/deg (n=7; Figure 29C) without MD to 0.40±0.006 cyc/deg with MD (n=5; $p<0.001$, Bonferroni-adjusted t-test; Figure 29D) and by 12.7±0.4 % in EE_old mice from 0.38±0.001 cyc/deg (n=4; Figure 29E) without MD to 0.41±0.008 cyc/deg with MD

(n=5; p<0.001, Bonferroni-adjusted t-test; Figure 29F). The increase in "visual acuity" was indistinguishable between all tested groups (p>0.05, ANOVA; Figure 28). Mice without MD from all age groups did not show improvement in "visual acuity" over the 4 days (p>0.05, compared to day 0, Bonferroni-adjusted t-test).

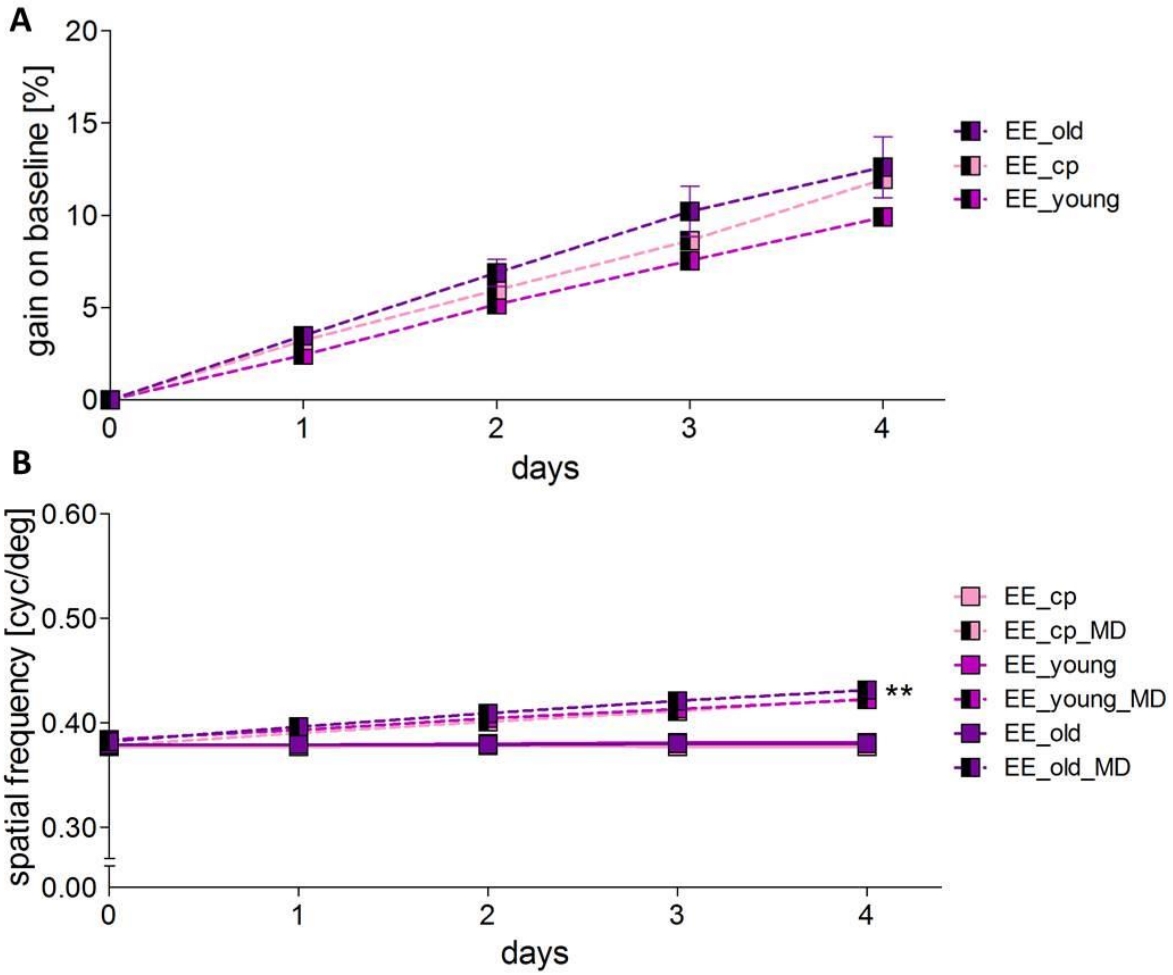


Figure 28: Improvement of "visual acuity" over 4 days of MD in EE-mice. A. Gain on baseline is plotted as percent over days of MD for all three age groups of EE-mice after MD. No significant differences were observed between the groups over MD days. **B.** "Visual acuity" values in cyc/deg are plotted against days, for mice without (boxes) and with MD (half-filled boxes) for all age groups. After 4 days of MD "visual acuity" improved significantly for all groups compared to mice without MD. Mice with MD did not show any differences over days.

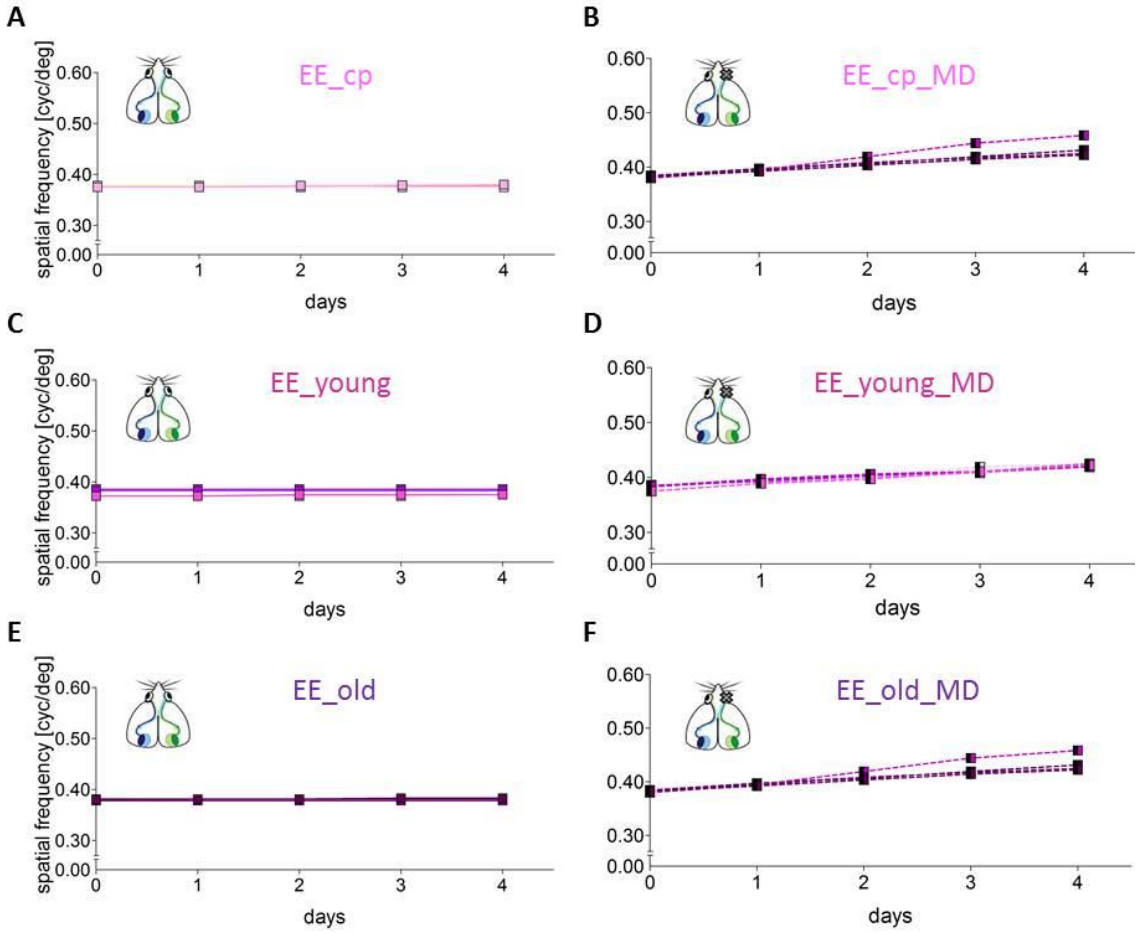


Figure 29: Spatial frequency thresholds of individual EE-mice over the 4 days of noMD/MD period. The spatial frequency thresholds (in cycles/degree) are presented for every age group: **A** and **B** EE-mice in critical period, **C** and **D** young EE-mice and **E** and **F** old EE-mice**.** Each line represents one animal. The "visual acuity" of control (**A**, **C** and **E**) without MD remained stable over the 4 days. EE-mice from every age group showed a significant increase in visual acuity thresholds of the open eye after 4 days of MD (**B, D** and **F**).

The contrast sensitivity thresholds of the optomotor reflex of the open eye were also increased significantly over the 4 days MD period in all MD groups (at 0.031, 0.064, 0.092, 0.103, 0.192 and 0.272 cyc/deg: $p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$ and $p>0.05$, compared to day 0 for all groups, ANOVA; Table 7, Figure 30, Figure 31B, D and F). As expected, all mice without MD did not change in contrast sensitivity threshold over days (Figure 30; Figure 31A, C and E).The measured contrast sensitivity thresholds were not different between the groups on the 4th day after MD ($p>0.05$ for every frequency, 2-way ANOVA). To conclude neither basic visual abilities nor the experience-enabled increase of "visual acuity" and contrast sensitivity thresholds after MD were changed during aging in EE-mice.

Table 7: Optomotry measured contrast sensitivity improvements on the 4th day after MD for the three different age groups. Values of contrast sensitivity for every spatial frequency measured on day 4 after MD for all groups as mean±SEM.

Spatial frequency (cyc/deg)	EE_cp (n=6)	EE_young (n=6)	EE_old (n=5)
0.031	4.3±0.001	4.0±0.001	4.7±0.1
0.064	20.9±0.3	16.6±0.5	19.8±1.2
0.092	18.0±0.4	15.2±0.7	17.9±1.0
0.103	16.7±0.2	15.2±0.4	16.7±0.8
0.192	9.8±0.1	9.9±0.4	11.3±0.7
0.272	4.1±0.001	4.1±0.001	4.5±0.2

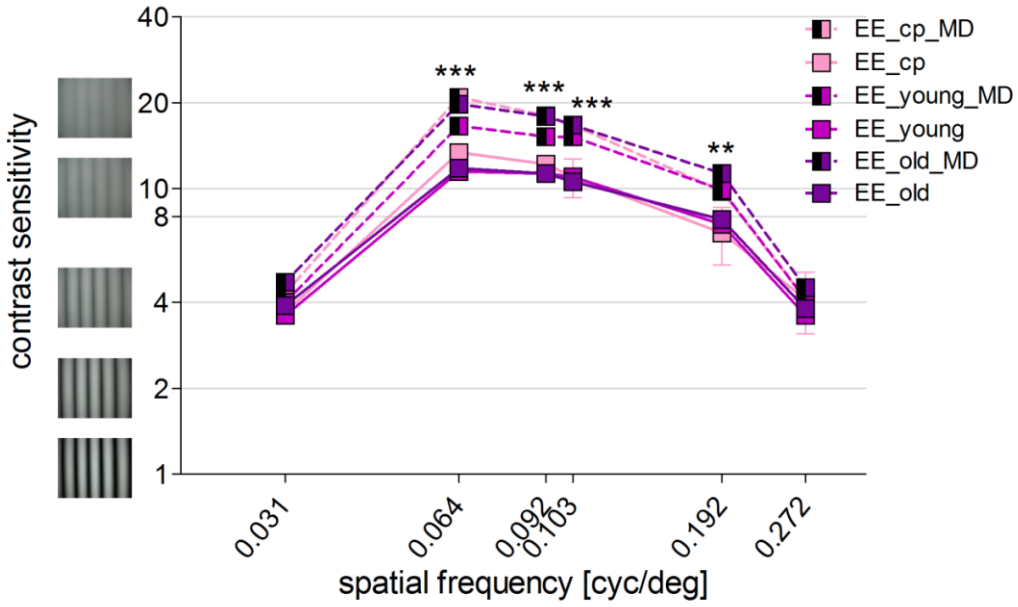


Figure 30: Contrast sensitivity improvements on the 4th day of MD. All mice groups after 4 days of MD (half-filled boxes) improved significantly in 4 out of 6 frequencies tested compared to mice without MD (filled boxes) on the 4th day.

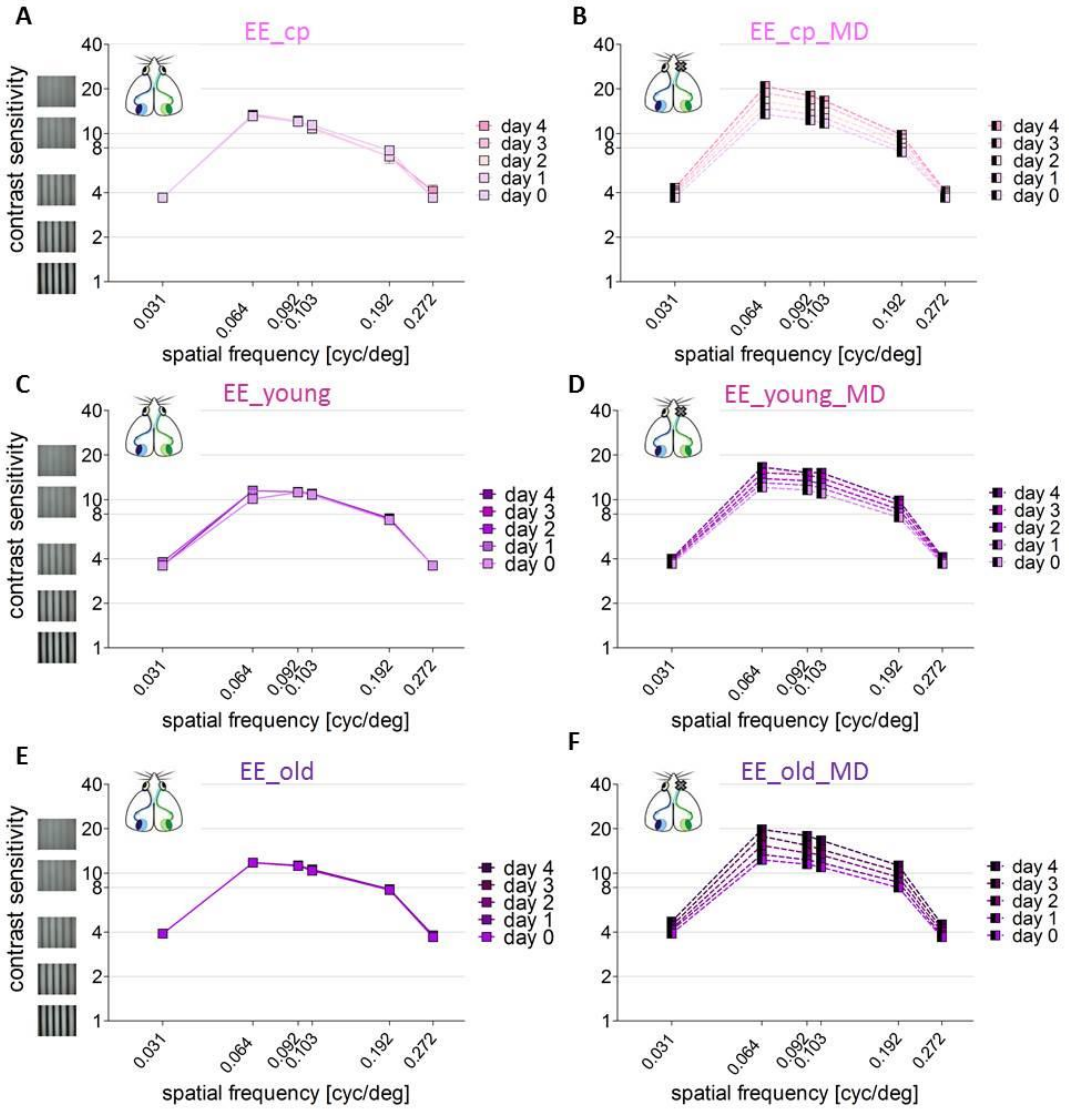


Figure 31: Mean contrast sensitivity thresholds of the optomotor reflex at the 6 spatial frequencies tested over 4 days. Baseline values of contrast sensitivity thresholds referred as day 0. **A.** In EE critical period mice without MD contrast sensitivity remained stable over 4 days. **B.** After 4 days of MD in EE critical period mice the contrast sensitivity of the open eye increased significantly. **C**. Young EE-mice without MD did not improve over days while MD mice of the same age group did (**D**). **E.** Similarly, old EE-mice without MD had the same contrast sensitivity values over days. **F.** Old EE-mice with MD showed a significant improvement over days.

3.3.2. An OD-shift observed after 4 days of MD in EE-mice

The ODI was determined for each mouse of all three age groups of EE-mice using optical imaging of intrinsic signals, by measuring V1-activation after stimulation of each eye. All mice without MD showed a contralateral dominance irrespective of age. V1-activation in the binocular zone of V1 was stronger after contralateral eye stimulation compared to the one after ipsilateral eye stimulation, the calculated average ODIs were positive, and warm colors

prevailed the 2-dimensional OD-maps (Figure 32A, C and E). Contrary to the mice without MD, an OD-shift was observed after 4 days of MD in all the three age groups. In this case, the activity patch in V1 after contralateral eye stimulation was equally strong to the one after ipsilateral eye stimulation (in some cases V1-activation after ipsilateral eye stimulation was even stronger than the contralateral, example in Figure 32B), ODI values were closer to zero, colder colors predominated in the OD-map and the ODI-histogram was shifted to the left (Figure 32B, D and F).

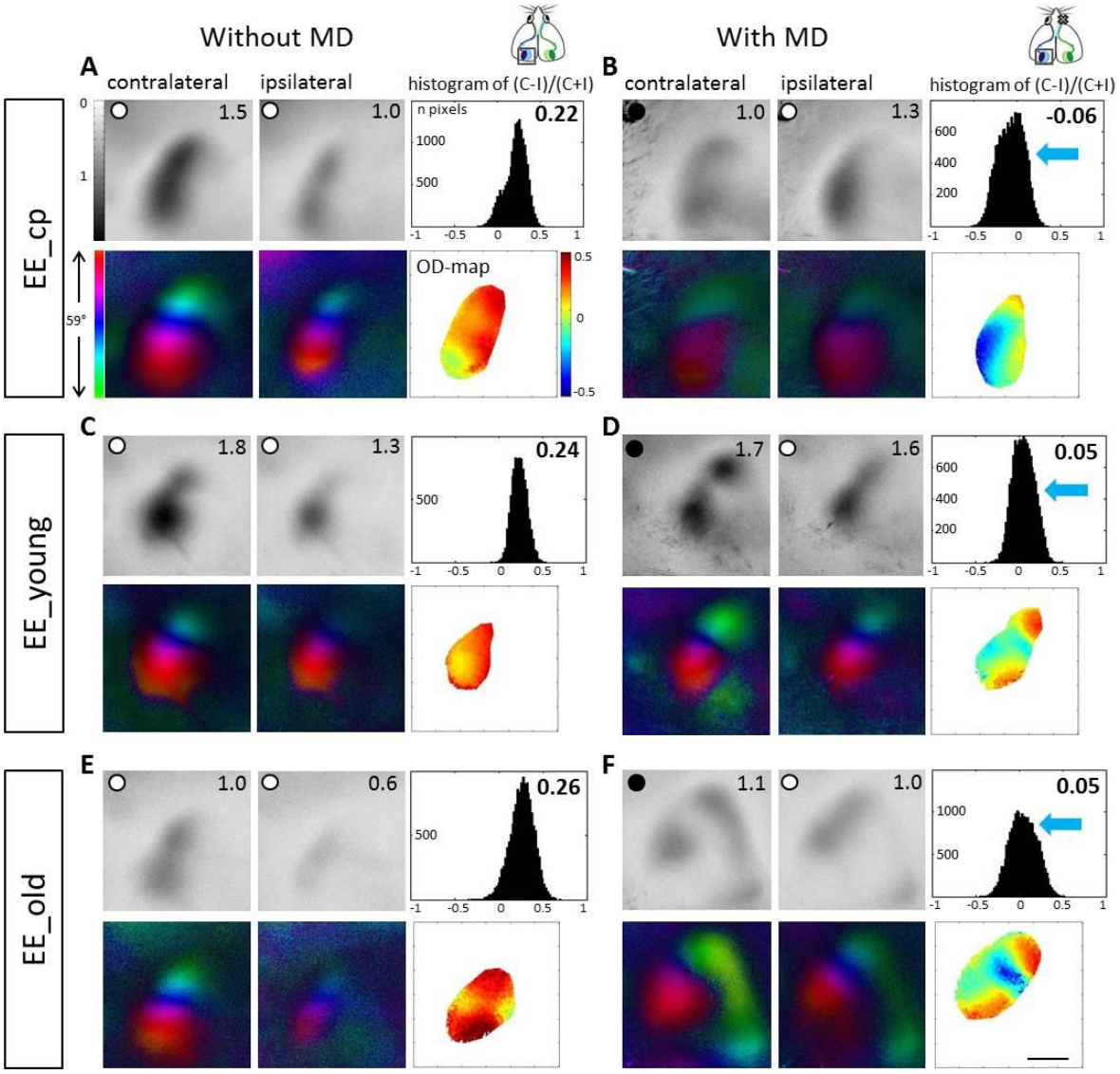


Figure 32: The OD changed after 4 days of MD in all age groups of EE-mice. Representative examples of optically recorded activity maps after contralateral and ipsilateral eye stimulation in the binocular region of mouse V1 are presented for critical period EE-mice (PD27-34 **A** and **B**), young EE-mice (PD80-101; **C** and **D**) and old EE-mice (PD121-183; **E** and **F**), without MD (**A**, **C** and **E**) and after 4 days of MD (**B**, **D** and **F**). Grayscale-coded response magnitude maps with the activity value on top right corner, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. 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 a contralateral dominance (**A**, **C** and

E). Four days of MD, induced a strong OD-shift towards the open eye in all three age groups of EE-mice (**B**, **D** and **F**): after MD, the contra- and ipsilateral eye activated V1 about equally strong, colder colors appeared in the OD-map, and the histogram of OD-scores shifted to the left (blue arrows). Scale bar: 1 mm.

After quantification of V1-activation of all recorded maps and calculation of ODIs, all age groups showed a significant OD-shift after MD (Figure 33A). Specifically, EE-mice in critical period without MD (PD27-34) had an ODI of 0.19±0.03 (n=4), which decreased significantly to -0.06±0.03 after 4 days of MD (n=6, PD27-33, p=0.0005, Bonferroni-adjusted t-test). Young EE-mice showed also a significant decrease in the ODI from 0.26±0.01 in mice without MD (n=7, PD82-101) to 0.04±0.03 in mice after MD (n=6, PD80-99; p<0.0001, Bonferroni-adjusted t-test). Similarly, the old EE-mice without MD had an ODI of 0.29±0.03 (n=5; PD127-183), while old EE-mice with MD had an ODI of 0.08±0.02 (n=5, PD121-177). The difference between these groups was also significant (p=0.004, Bonferroni-adjusted, t-test).

As expected, EE-mice during the critical period showed an OD-shift mediated by decreased V1-responses after deprived eye stimulation (contralateral) while the activities after open eye (ipsilateral) stimulation remained unchanged, as in age matched SC mice. In detail, V1-activation after contralateral eye stimulation decreased from 1.62±0.26 without MD to 1.06±0.09 after MD (p=0.041, t-test) whereas ipsilateral eye responses before MD were 1.16±0.23 and after MD 1.27±0.13 and thus not significantly different (p=0.675, t-test). In contrast, 4 days of MD in the other two age groups resulted in an OD-shift that derived from an increase in open eye responses (ipsilateral) in the binocular part of V1. Specifically the V1-activation after contralateral eye stimulation was 1.62±0.09 in EE-young before MD and did not change significantly after MD (1.53±0.15; p=0.589, t-test), while the ipsilateral eye responses in V1 increased from 0.95±0.08 without MD to 1.44±0.12 after MD (p=0.006, t-test). Similarly for the old EE-mice, contralateral eye responses remained unchained (without/with MD: 1.60±0.05/1.57±0.16, p=0.841, t-test) but the V1-activation after stimulation of the ipsilateral eye was significantly elevated after MD (without/with MD: 0.92±0.02/1.26±0.11; p=0.026, t-test; Figure 33B). Taken together our data suggest that 4 days of MD are enough to induce an OD-shift in EE-mice but the observed OD-shift differs from what was previously described in EE-mice after 7 days of MD, as it is mediated by increased open eye responses in V1.

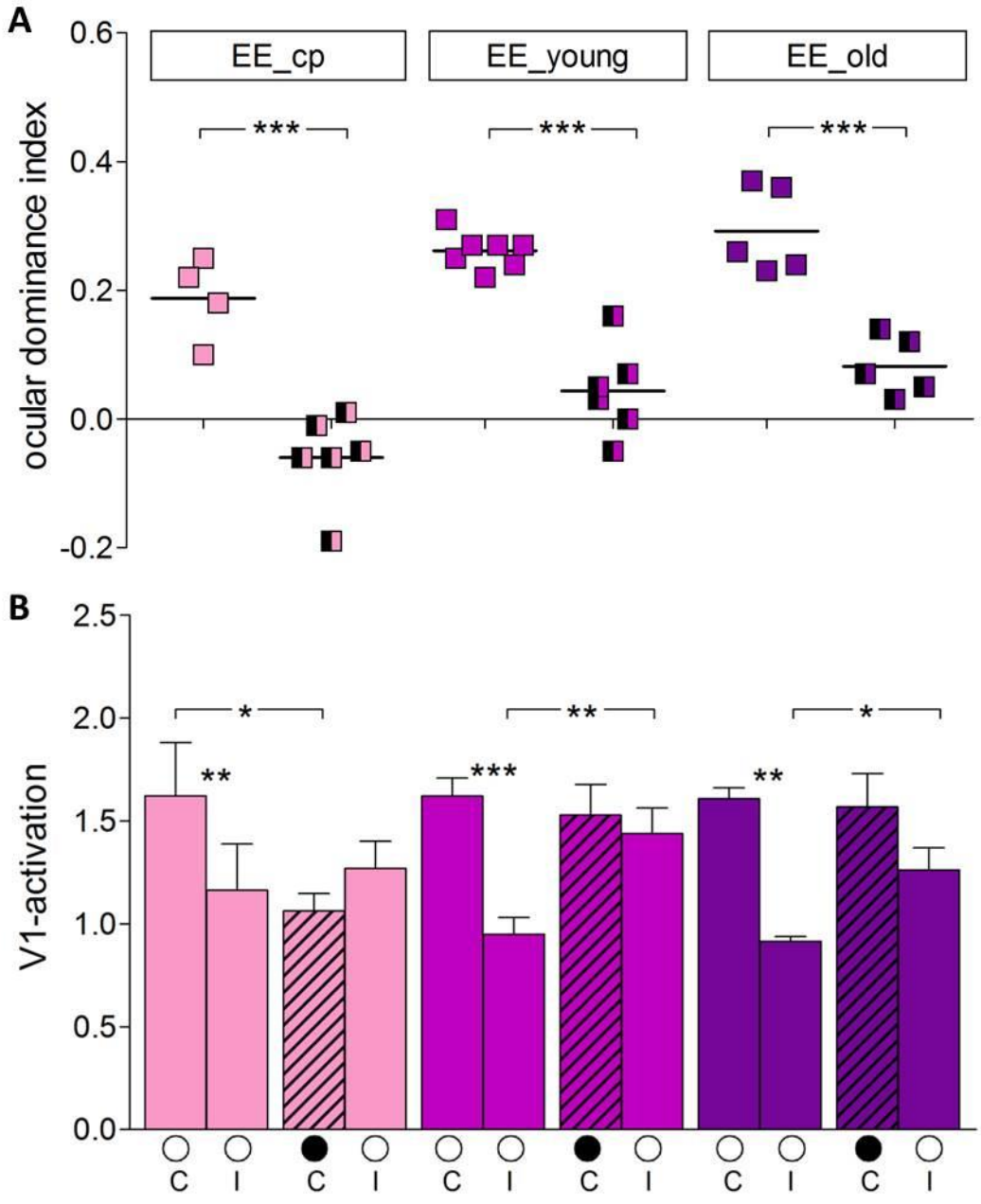


Figure 33: Quantification of V1-activation in different age groups of EE-mice. A. Optically imaged ODIs of mice without and with MD raised in EE during the critical period (EE_cp: PD27-34), young-adults (EE_young: PD80-101) and old-adults (EE_old: PD121-183). Symbols represent ODI-values of individuals, filled boxes refer to mice without MD, half-filled boxes refer to mice with MD and means are marked by horizontal lines. All groups showed an OD-shift after 4 days of MD. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye without and after MD (black filled circles indicate MD eye). The OD-shift was mediated by a reduction of deprived (contralateral: C) eye responses in V1 for the EE_cp group, whereas for the EE_young and EE_old the shift is mediated by an elevation of open eye responses (ipsilateral: I).

3.3.3. Two days of MD also induced an OD-shift in old EE-mice

We observed that 4 days of MD can induce an OD-shift in old EE-mice which was mediated by increased open eye responses in V1. Trying to understand how V1 activity after stimulation of each eye changes immediately after MD, I performed chronic experiments after 2 and 4 days of MD in old EE-mice (>PD110). In detail mice were imaged before MD (first session), then the right eye (contralateral to the imaged hemisphere) was deprived for 2 days, and imaged again (second session). Immediately after the second imaging session the previously deprived eye was again closed for 2 more days and then a third session of imaging took place (4 days after the first deprivation). I managed to get data for all three sessions from 2 animals (PD143 and PD218).

In the first imaging session, V1-activities after visual stimulation of the contra- and ipsilateral eye were determined, and then compared with those after the second and third imaging session. Activity maps recorded before MD (first session) were dominated by input from the contralateral eye, warm colors prevailed in the 2-dimensional OD-map and the average ODI was positive (Figure 34A and B). Already after 2 days of MD (second session), a change in V1-activation was observed: the activities after ipsilateral eye stimulation were slightly elevated which resulted in decreased ODI values, colder colors in the 2-dimensional OD-map and a shifted ODI-histogram to the left (Figure 34C and D). After 4 days of MD (third session), V1-activation via the contralateral eye was equally strong to the one via the ipsilateral eye, colder colors dominated the OD-map, ODI-values were even more reduced and the ODI-histogram was shifted to the left (Figure 34E and F).

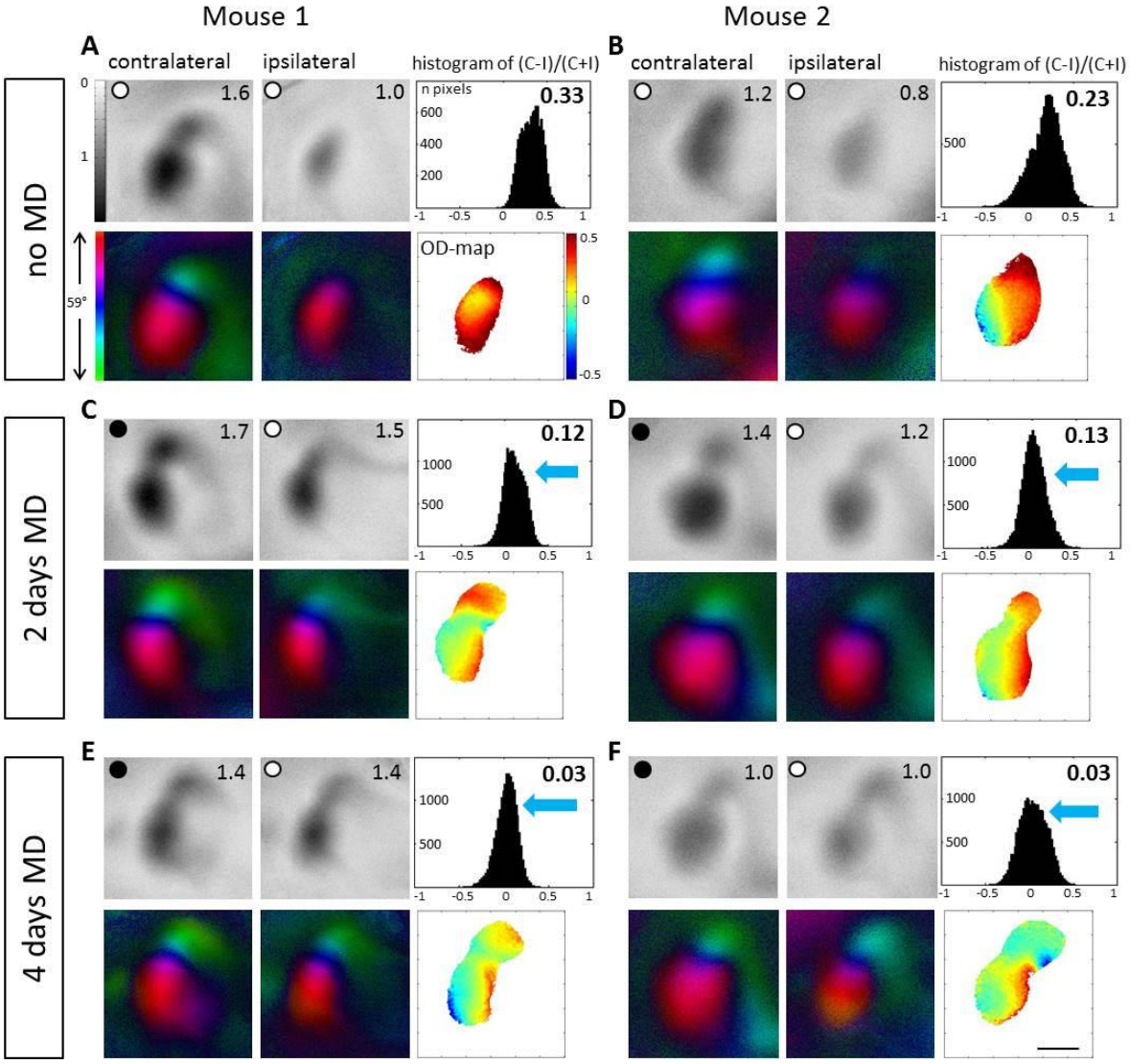


Figure 34: Optical imaging acquired maps in chronically imaged old EE-mice (>PD110). Optically recorded activity maps of the contralateral and ipsilateral eye in the binocular region of mouse V1 are presented for the two mice tested over the three imaging sessions (mouse 1 : **A**, **C** and **E**; mouse 2: **B**, **D** and **F**). Grayscale-coded response magnitude maps with the activity value on the top right corner, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average ODI are illustrated. Before MD (first session), 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 a contralateral dominance (**A** and **B**). After 2 days of MD (second session) an OD-shift was observed mediated by increased open eye responses in V1, colder colors appeared in the 2-dimensional OD-maps and the peak of the OD-histogram was shifted to the left (**B** and **D**). After 4 days of MD (third session) a stronger OD-shift was observed, the contra- and ipsilateral eye activated V1 equally strong, colder colors appeared in the OD-map, and the histogram of OD-scores shifted to the left (**E** and **F**). Blue arrows indicate the shift in the histograms to the left. Scale bar: 1 mm.

Further quantitative analysis showed that the ODI decreased from 0.33 before MD to 0.12 after 2 days of MD and to 0.03 after 4 days MD for the first mouse (mouse 1; PD143 on first imaging session; Figure 35A). Additionally, for the same mouse, the V1-activation after

contralateral eye stimulation was 1.63 before MD and remained unchanged after 2 days of MD to 1.69, whereas a small decreased was observed after 4 days of MD to 1.35 (Figure 35B). Similarly the V1-activation after ipsilateral eye stimulation was 0.99 before MD, 1.45 after 2 days of MD and slightly increased after 4 days of MD to 1.35 (Figure 35B).

For the second mouse tested (mouse 2; PD187 on first imaging session) ODI decreased gradually from 0.23 before MD to 0.13 after 2 days of MD and to 0.03 after 4 days of MD (Figure 35C). Quantification of V1-activation after each eye stimulation for that mouse showed a small increase in V1-activation after contralateral eye stimulation after 2 days of MD but after 4 days of MD the V1-activation was more similar to the one before MD (contralateral V1-activation: before MD/2 days MD/4 days MD: 1.19/1.39/1.03; Figure 35D). V1-activation after ipsilateral eye stimulation was slightly increased after 4 days of MD (ipsilateral V1-activation: before MD/2 days MD/4 days MD: 0.82/1.15/1.00; Figure 35D). Taking together our data showed that ODI is gradually decreasing after MD, V1-activation after contralateral eye stimulation is initially unchanged after MD but then slowly decreasing and V1-activation after ipsilateral eye stimulation is increasing immediately after MD and later showed a small decrease.

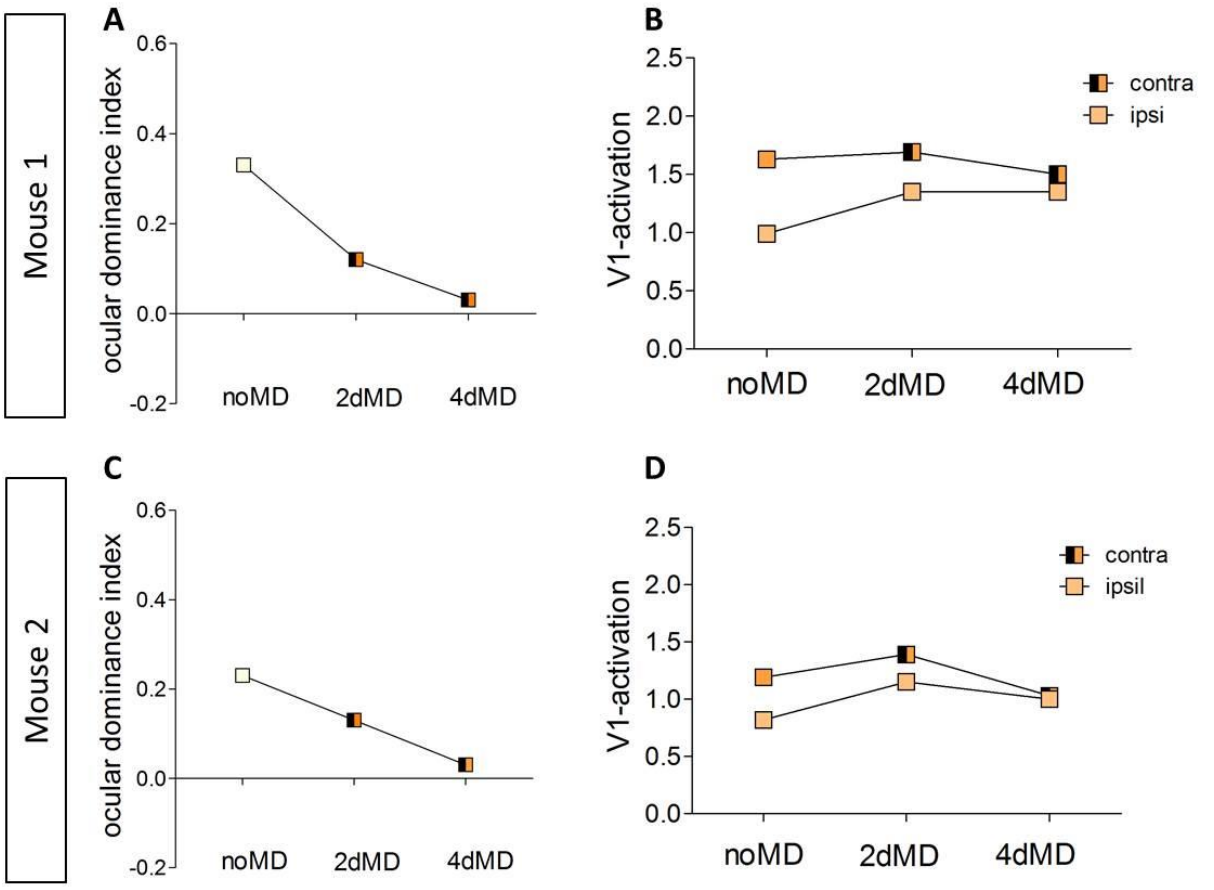


Figure 35: Chronically imaged old EE-mice. A and **C.** ODIs before (empty boxes) and after 2 and 4 days of MD (half-filled boxes) for both mice tested (mouse 1: **A**; mouse 2: **C)**. In both cases the ODI decreased gradually over the days of MD. **B** and **D.** V1-activation after contralateral (contra) and ipsilateral (ipsi) eye stimulation, before and after 2 or 4 days of MD for both mice tested (mouse 1: **B**;

mouse 2:**D**). Half-filled boxes indicate the deprived eye. V1-activation after contralateral eye stimulation was decreased after 4 days of MD, whereas a small increase after ipsilateral eye activation was observed after 4 days of MD.

Interestingly, our data showed that a very short period of MD in old EE-mice resulted in OD-plasticity. Combining these data of the two chronically imaged mice with chronic experiments of two more animals of which I could acquire data only before and after 2 days of MD, we found that 2 days of MD were sufficient to induce an OD-shift in old EE-mice (n=4; PD143-281; Figure 36A). Specifically, the ODI significantly decreased from 0.27±0.02 before MD to 0.14±0.007 after 2 days of MD (p=0.002, t-test). Quantification of the V1-activation did not reveal any significant changes after 2 days of MD (Figure 36B). V1-activation after contralateral eye was 1.46±0.09 before MD and remained unchanged after 2 days of MD (1.47±0.11; p=0.954, t-test). After ipsilateral eye stimulation V1-activation slightly increased after 2 days of MD: from 0.92±0.07 before MD to 1.23±0.13 after MD but the difference was not significant (p=0.072; t-test).

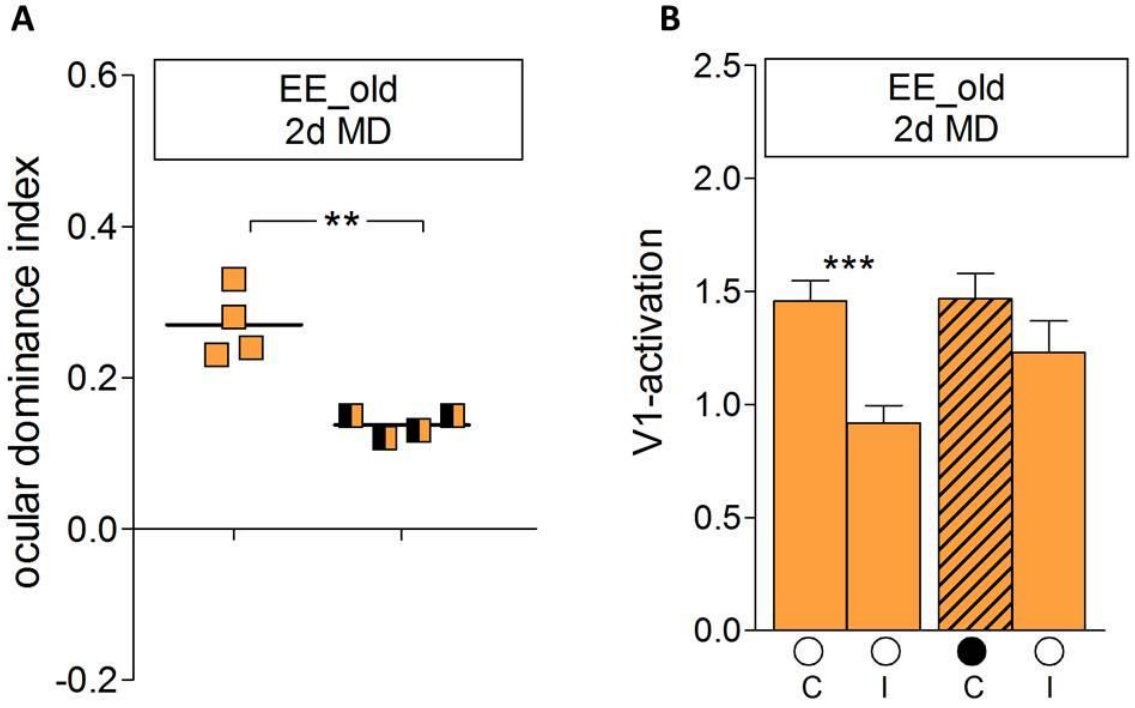


Figure 36: ODIs and V1-activation of old EE-mice after 2 days of MD. A. Optically imaged ODIs of mice before (orange boxes) and after 2 days of MD (half-filled orange boxes). Symbols represent ODI-values of individuals; means are marked by horizontal lines. After 2 days of MD a significant OD-shift was observed. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye. Black circle indicates the deprived eye. Before MD a clear contralateral dominance was observed whereas after MD the V1-activation after ipsilateral eye stimulation was increased but not significantly (p=0.072, t-test).

3.4. Voluntary physical exercise promotes ocular dominance plasticity in adult mouse primary visual cortex (Kalogeraki et al., 2014)

Enriched environment (EE) preserved a juvenile-like OD-plasticity in mice into late adulthood (Greifzu et al., 2014). EE housing provides the mice with more social interactions, voluntary physical exercise and cognitive stimulation compared to SC. The diversity of EE components raised the question whether all are needed or one of them is sufficient to prolong OD-plasticity. To test whether running alone has a positive effect on OD-plasticity of adult mice here I raised mice in SCs with or without a running wheel (RW). When mice were older than PD130 I performed MD to trigger plasticity and visualized V1-activation with optical imaging of intrinsic signals. Mice were additionally tested in the optomotor setup daily during the MD period. Detailed description of the experimental design and the groups of mice used for this study can be found in the materials and method section (part 2.2.2). In mice with a RW but not in mice without, OD-plasticity was preserved into adulthood. Furthermore, I tested whether a short period of physical exercise, during the 7 days MD period resulted in restored OD-plasticity in adult SC mice. Indeed 7 days of running were enough to restore OD-plasticity in mouse V1. Interestingly, all the observed OD-shifts were mediated by reduced deprived eye responses in V1, a signature for juvenile OD-plasticity. Visual abilities and their increase after MD were not different between the different housing conditions. Together our data suggest that voluntary running alone can promote a juvenile-like OD-plasticity in adult mice.

3.4.1. Basic visual abilities and enhanced optomotor reflex after MD were not different between mice with and without a RW

Using the virtual reality optomotor setup (Prusky et al., 2004) the “visual acuity” and contrast sensitivity thresholds of the optomotor reflex were measured before and after MD in mice raised in SCs without (SC-mice) or with a RW (RW-mice). Before MD, the highest spatial frequency that elicited an optomotor reflex in mice with RW was 0.37±0.003 cyc/deg (n=15; PD138-242) and 0.37±0.002 cyc/deg in SC-mice used as controls (n=10; PD137-200). Similarly, for the mice with access to RW only for 7 days after the MD (7dRW group), the highest spatial frequency elicited a reflex of 0.38±0.005 cyc/deg (n=12; PD141-214). No significant differences were observed between the groups (p=0.901, ANOVA; Figure 37). Moreover, the values were similar to those previously published for SC C57BL/6J mice (Prusky et al., 2006; Lehmann and Löwel, 2008).

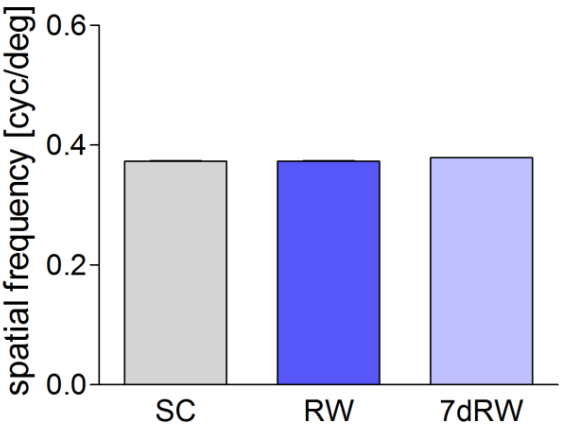


Figure 37: Baseline visual acuity in mice with or without a RW. The highest spatial frequency that elicited a response in optomotor setup was similar between SC (grey), RW (blue) and 7dRW (light blue) mice.

Additionally, using the optomotor setup the contrast sensitivity thresholds for RW- and SC-mice were measured in six different frequencies (0.031, 0.064, 0.092, 0.103, 1.192 and 0.272 cyc/deg) before and after MD. The baseline contrast sensitivity for RW-mice (n=15) was not significantly different from SC-mice (n=10; $p>0.05$ for every spatial frequency, ANOVA; Table 8). Thus, daily voluntary physical exercise did not change basic spatial vision.

Table 8: Baseline contrast sensitivity values of the RW mice and SC mice. For the 6 different spatial frequencies tested the average contrast sensitivity for each group is listed as mean±SEM.

Spatial frequency (cyc/deg)	RW mice (n=10)	SC mice (n=10)
0.031	4±0.1	4±0.01
0.064	14±0.2	14±0.1
0.092	13±0.1	13±0.1
0.103	12±0.1	12±0.1
0.192	7±0.1	7±0.1
0.272	4±0.1	4±0.01

The "visual acuity" and contrast sensitivity thresholds of the optomotor reflex were also tested daily during the MD period. The increase of "visual acuity" and contrast sensitivity thresholds through the open eye was similar in RW, SC and 7dRW mice after MD (Figure 38). For "visual acuity" an increase on average by 29% was observed in RW mice: from 0.37±0.003 cyc/deg before MD to 0.48±0.04cyc/deg after MD (n=8; $p<0.001$, Bonferroni-adjusted t-test). In SC-mice the increase in "visual acuity" was on average 34%: from 0.37±0.002 cyc/deg before MD to 0.50±0.03 cyc/deg after MD (n=5; $p<0.001$, Bonferroni-adjusted t-test). For the 7dRW group "visual acuity" increased on average by 27% from 0.37±0.01 cyc/deg before MD to 0.47±0.02 cyc/deg after MD (n=6; $p<0.001$, Bonferroni-adjusted t-test). The increase in "visual acuity" was not different between all the groups ($p>0.05$, ANOVA). Mice without

MD from all housing conditions did not show any change in "visual acuity" over days (Figure 39B).

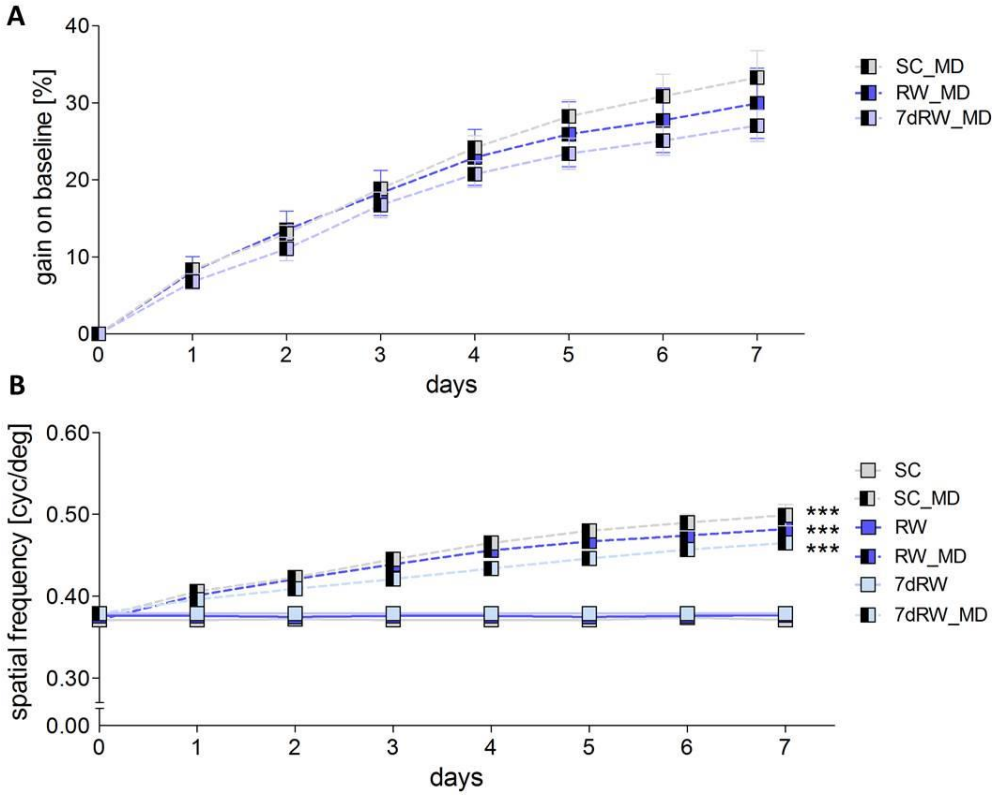


Figure 38: Improvement of "visual acuity" after MD in RW and SC mice over days. A. Gain on baseline is plotted as percent over days of MD for all 3 groups tested (SC: grey, RW: blue and 7dRW: light blue). The gain on baseline was similar for all the groups. **B.** "Visual acuity" values in cyc/deg are plotted against days for all housing conditions. MD mice are illustrated with half-filled boxes whereas mice without MD with filled boxes. All the MD groups improved significantly over days compared to the no MD groups. There were no significant differences between the MD groups.

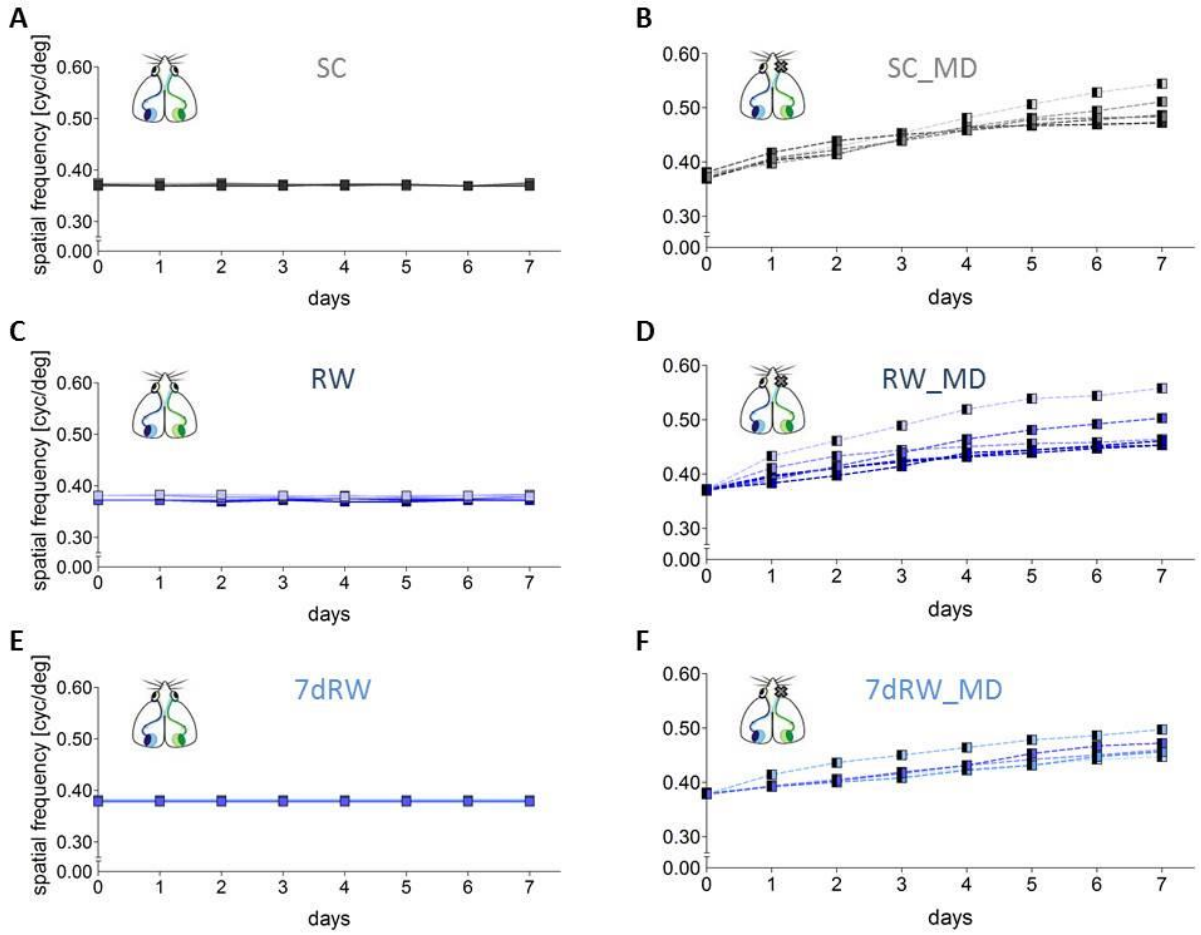


Figure 39: Spatial frequency thresholds measured in mice without or with RW over the 7 days noMD/MD period. Every line represents a single animal. **A.** Mice raised in SC without MD had similar visual acuity thresholds over the days. **B.** MD in SC-mice resulted in an increase of visual acuity thresholds over days. **C.** Mice raised in a cage with a RW without MD did not change over days while **D**. mice of the same group with MD showed a significant improvement over the days. **E**. NoMD mice raised in a SC and transferred to RW-cage for 7 days had same values over day. **F.** In 7dRW mice with MD spatial frequency thresholds of the open eye increased over days.

Additionally, contrast sensitivity thresholds of the optomotor reflex of the open eye were also increased in RW mice with MD (at 0.031, 0.064, 0.092, 0.103, 0.192 and 0.272 cyc/deg: to 6±0.04, 28±0.4, 29±0.5, 32±0.8, 22±0.7 and 9±0.4 on day 7; $p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p>0.05$, compared to values from day 0, ANOVA, Table 9). Similar increase was observed in SC mice with MD (at 0.031, 0.064, 0.092, 0.103, 0.192 and 0.272cyc/deg: to 7±0.04, 30±0.34, 36±0.46, 37±0.52, 24±0.34 and 7±0.08 on day 7; $p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p>0.05$, compare to day 0, ANOVA; Table 9). The values on day 7 were not different between the two groups ($p>0.05$ for every frequency, ANOVA; Figure 40). Contrast sensitivity remained unchanged over the days in mice without MD for both housing conditions (RW or SC).The contrast sensitivity values over the days for mice without and with MD for both housing conditions are presented in figure 41. To summarize, neither basic

spatial vision nor the experience-enabled increase of thresholds after MD were modified by RW-experience during adolescence.

Table 9: Optomotry measured contrast sensitivity improvements of the open eye after MD. Values of contrast sensitivity for every spatial frequency tested on day 0 (before MD) and on day 7 for RW_MD and SC_MD groups.

	Day 0		Day 7	
Spatial frequency (cyc/deg)	**RW_MD (n=8)**	**SC_MD (n=5)**	**RW_MD (n=8)**	**SC_MD (n=5)**
0.031	3.8±0.01	3.7±0.01	5.8±0.04	6.7±0.03
0.064	14.4±0.21	14.2±0.13	28.1±0.36	30.2±0.34
0.092	12.7±0.13	12.6±0.05	29.1±0.47	36.0±0.46
0.103	11.9±0.11	11.8±0.05	32.2±0.76	37.1±0.52
0.192	6.7±0.12	6.6±0.05	21.8±0.66	24.4±0.34
0.272	3.7±0.02	3.7±0.01	9.1±0.36	7.4±0.08

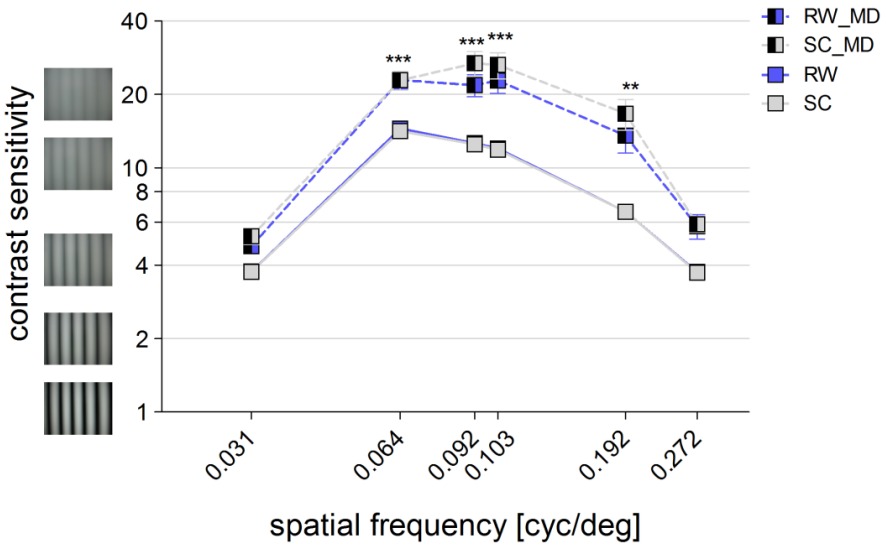


Figure 40: Contrast sensitivity improvements over MD days in RW and SC mice. Mice with a running wheel (blue half-filled boxes) improved significantly in 4 out of 6 frequencies after 7 days of MD. Similar improvement was observed for the SC mice after MD (grey half-filled boxes).

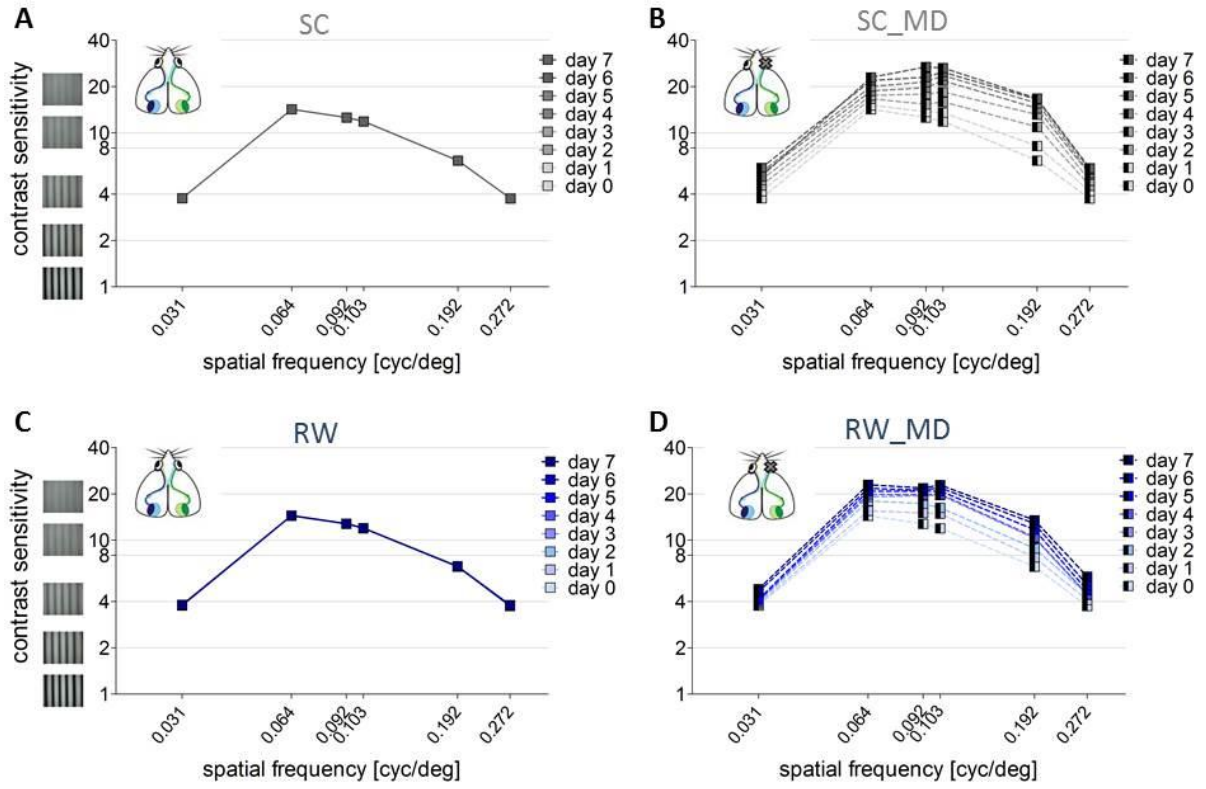


Figure 41: Mean contrast sensitivity thresholds in the 6 spatial frequencies tested for mice raised with or without a RW over days. Day 0 represents the baseline values before MD. **A.** In SC-mice without MD contrast sensitivity remained stable over days. **B.** After 7 days of MD in SC-mice the contrast sensitivity of the open eye increased significantly. **C**. Mice raised in a cage with RW without MD did not improve over days. **D**. RW-mice with MD showed a significant improvement over days in contrast sensitivity thresholds.

3.4.2. Voluntary running prolonged the sensitive phase for OD-plasticity in V1 into adulthood

Using optical imaging of intrinsic signals V1-activation after stimulation of each eye was measured and an ODI was calculated for every mouse. In both RW and SC groups without MD, V1-activation in the binocular zone of V1 was dominated by contralateral eye responses. The activity patches after stimulation of the contralateral eye were always darker than those after ipsilateral eye stimulation, the calculated ODIs were positive, and warm colors prevailed in the 2-dimensional OD-maps (Figure 42A and C). Likewise, after 7 days of MD in SC mice activity patches after contralateral (deprived) eye stimulation in binocular V1 were stronger than those after ipsilateral eye stimulation and the average ODI was positive with warm colors dominating the OD-map (Figure 42B). In contrast, in RW-mice 7 days of MD caused an OD-shift towards the open eye: the V1-activation was equally strong after contralateral (deprived) and ipsilateral (open) eye stimulation, colder colors predominated in the OD-map and the ODI-histogram was shifted to the left (Figure 42D). To test whether long-term running is required for prolonging OD-plasticity or a short period of running is sufficient to promote

plasticity in adult V1, mice raised in SCs until PD134 and then transferred to a cage with a RW only during the 7-day-MD/noMD-period. In this case, mice without MD showed a clear contralateral dominance in the binocular V1 with positive ODIs and warm colors prevailing the 2-dimensional OD-maps whereas in 7dRW mice after MD both eyes stimulation was activating V1 equally strong, the ODI values were closer to zero, the OD-map predominated by colder colors and the OD-histogram shifted to the left (Figure 42E and F).

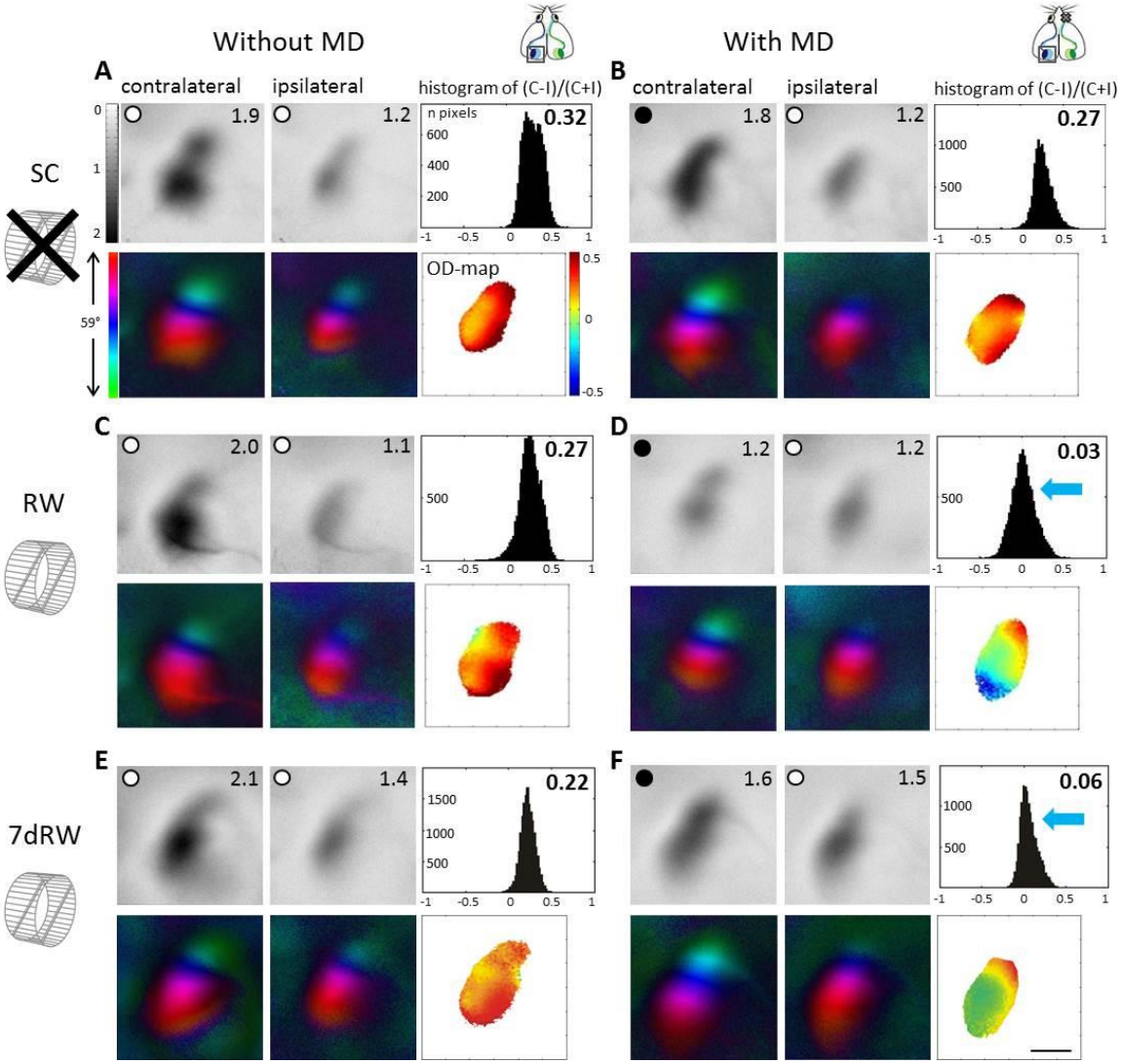


Figure 42: Running prolonged and restored the sensitive phase for OD-plasticity in mice in adulthood (>PD110). Optically recorded activity maps of the contralateral and ipsilateral eye in the binocular region of mouse primary visual cortex (V1) in mice raised in SCs without a running wheel (**A** and **B**), with a running wheel (**C** and **D**) and with 7 days of running wheel (**E** and **F**), before (**A**, **C** and **E**) and after MD (**B**, **D** and **F**). Grayscale-coded response magnitude maps, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. 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 (**A**, **C** and **E**). While 7 days of MD did not induce OD-plasticity in mice without a RW (**B**), it induced a strong OD-shift towards the open eye in age-matched adult RW (**D**) and 7dRW mice (**F**): after MD, the contra- and ipsilateral eye activated V1 about

equally strong, colder colors appeared in the OD-map, and the histogram of OD-scores shifted to the left (blue arrows). Scale bar: 1 mm.

Quantitative analyses of V1-activation for all recorded maps showed that the average ODI of RW mice older than PD130 decreased from 0.26±0.03 without MD (n=7, PD140-240) to 0.06±0.03 (n=8, PD140-240) with MD (n=8, PD138-242; p=0.0003, Bonferroni-adjusted t-test). In contrast, adult SC mice did not show an OD-shift after 7 days of MD: the average ODI was similar in mice without and with MD (noMD/MD: 0.32±0.02/0.25±0.03, n=5/5, PD137-200/145-195; p=0.1126, Bonferroni-adjusted t-test). The ODI values were also significantly different between the RW and SC group with MD (p=0.0009, Bonferroni-adjusted t-test). Additionally, the average ODI for 7dRW mice without MD was 0.21±0.01 (n=6, PD143-217), thus significantly different from the 7dRW mice with MD (0.06±0.01, n=6, PD141-214; p=0.0001, Bonferroni-adjusted t-test). The ODIs of 7dRW mice with MD were also significantly different from the SC mice with MD (p=0.0001, Bonferroni-adjusted t-test) but not from the RW mice with MD (p>0.05, Bonferroni-adjusted t-test; Figure 43A).

Further quantification of V1-responses after each eye stimulation revealed that the observed OD-shifts were mediated by a decrease of deprived eye (contralateral) responses in V1 (Figure 43B). In the RW group V1-activity after stimulating the deprived eye was 1.59±0.10 (n=7) whereas after MD it was 1.18±0.09 (n=8; p=0.008, t-test). In contrast, open (ipsilateral) eye responses remained unchanged between RW mice without and with MD (noMD/MD: 0.95±0.06/1.13±0.09, n=7/8; p=0.143, t-test). V1-activation after stimulating the ipsi- and contralateral eye did not change after MD in SC mice and the binocular part of V1 remained dominated by the contralateral eye. After MD in SC mice the V1-activation after contralateral eye stimulation was 1.67±0.07 and after ipsilateral eye stimulation was 1.11±0.11 (n=5), while SC mice without MD had a V1-activation of 1.60±0.09 after stimulation of the contralateral eye and 0.88±0.09 after ipsilateral eye stimulation (n=5). Neither of these was significant different (contralateral/ipsilateral: p=0.525/0.134, t-test). For the short-term running (7dRW) the observed OD-shift was again mediated by decreased deprived eye responses in V1: V1-activation through the contralateral eye was reduced from 2.00±0.08 to 1.34±0.22 after MD (n=6/6; p=0.037, t-test), whereas ipsilateral eye V1-responses did not change (noMD/MD: 1.40±0.10/1.35±0.13, n=6/6; p=0.77, t-test; Figure 43B).

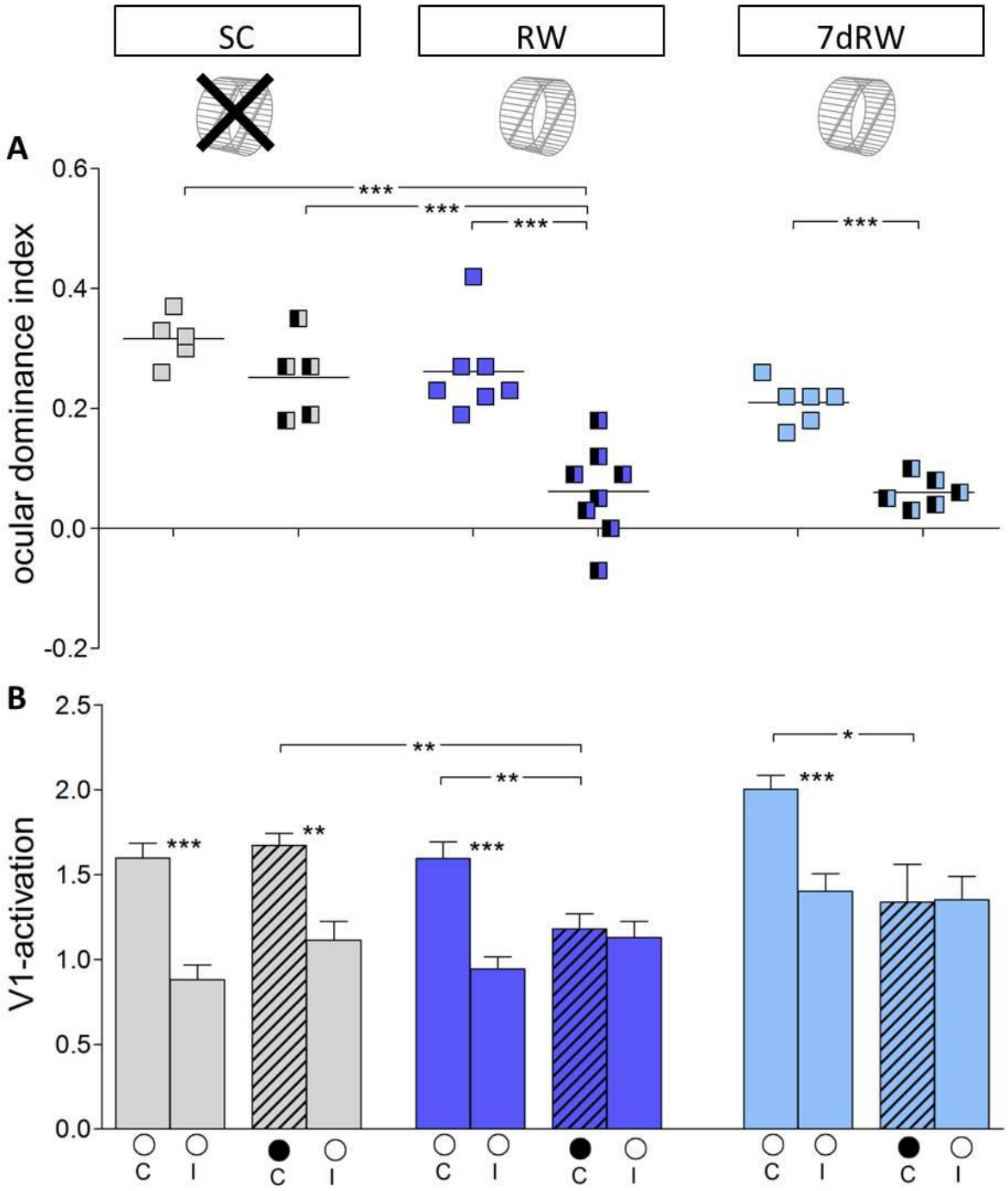


Figure 43: Quantification of V1-activation in adult mice with or without a RW. A. Optically imaged ODIs of mice without and with MD raised in SCs (grey), RW-cages (blue) or with only 7days of RW (7dRW, light blue). Symbols represent ODI-values of individual mice, means are marked by horizontal lines. Only mice that had a RW showed an OD-shift after MD. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye without and after MD (black filled circles indicate MD eye). The OD-shift was mediated by a reduction of deprived (C) eye responses in V1.

Furthermore, to clarify the mechanism underlying the prolonged OD-plasticity in adult RW mice, chronic imaging experiments were performed. For that purpose, 2 mice born and raised in a cage with a RW were imaged before MD (first session), then the right eye (contralateral to the imaged hemisphere) was deprived (MD) for a period of 7 days, and imaged again on the 7^{th} day (second session). In the first imaging session, V1-activities after visual stimulation of the contra- and ipsilateral eye were determined, and then compared with those after the second imaging session. Activity maps recorded before MD (first session) were dominated by input from the contralateral eye, warm colors prevailed in the 2-dimensional OD-map and the average ODI was positive. After 7 days of MD (second session), V1-activation via the contralateral eye was visibly reduced, thus both eyes activated V1 about equally strong, colder colors dominated the OD-map, ODI-values were reduced and the ODI-histogram was shifted to the left (Figure 44A).

Quantitative analysis showed that the ODI decreased from 0.21±0.02 before MD to 0.01±0.08 after 7 days of MD (n=2; Figure 44B). Additionally the OD-shift was mediated by reductions in contralateral (deprived) eye responses in V1: from 1.68±0.16 before MD to 1.06±0.16 after 7 days of MD (Figure 44C), while the V1-responses after ipsilateral (open) eye stimulation remained unchanged (before MD: 1.13±0.05, after MD: 1.10±0.04; Figure 44 D). The preserved OD-shift in the RW-mice was predominantly mediated by a reduction of deprived eye responses in V1, a typical characteristic of juvenile OD-plasticity observed in SC mice. Adult mice in EE showed also juvenile-like plasticity after 7 days of MD suggesting that the mechanism underlying the presence of OD-plasticity into adulthood is the same after enriched housing and housing in a cage with a running wheel.

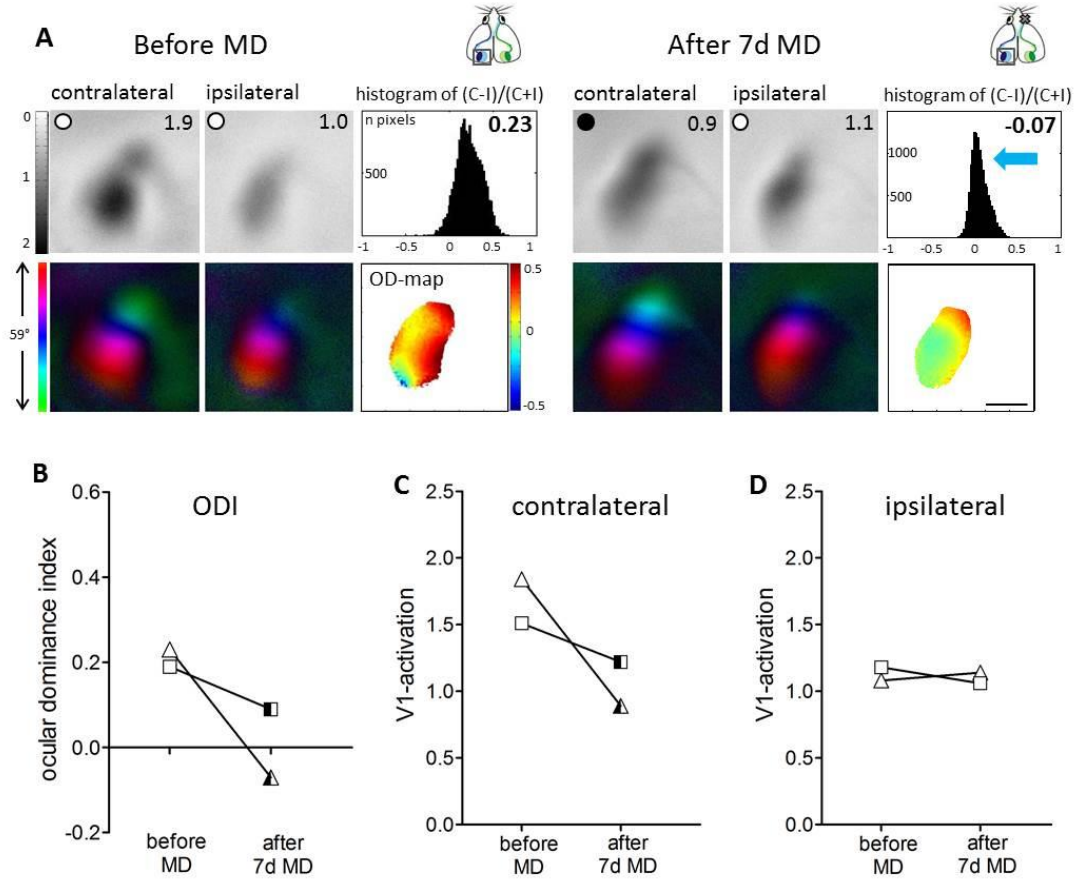


Figure 44: Chronically imaged RW mice. A. Optically recorded activity and polar maps of the contralateral and ipsilateral eye in the binocular region of mouse primary visual cortex (V1) in a RW mouse before and after MD. Grayscale-coded response magnitude maps with the V1-activation value on top right corner, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average ODI are illustrated. Before 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. After 7 days of MD in the same mouse the contra- and ipsilateral eye activated V1 about equally strong, colder colors appeared in the OD-map, and the histogram of OD-scores shifted to the left (blue arrow). Scale bar: 1 mm. **B.** ODIs before (empty boxes) and after MD (half-filled boxes). Every line represents one mouse. In both cases the ODI decreased after MD. **C.** V1-activation after contralateral (deprived) eye stimulation before (empty boxes) and after 7 days of MD (half-filled boxes). V1-activation was reduced after MD in both mice. **D.** V1-activation after ipsilateral (open) eye stimulation before (empty boxes) and after 7 days of MD (half-filled boxes) of the two mice tested. There were no changes observed in V1-activation after ipsilateral eye stimulation before and after MD.

3.4.3. Retinotopic maps and V1-activation were similar in mice with or without RW

Using full screen moving bars in elevation (horizontal) or azimuth (vertical) direction the strength of V1-activation and the quality of the retinotopic maps was calculated by optical

imaging of intrinsic signals for all three housing conditions (RW, SC and 7dRW). There were no detectable differences between the groups after elevation or azimuth stimulation in signal strength and retinotopic map quality (Figure 45). The magnitude of the V1-responses after elevation stimulation for mice in a cage with a RW was 2.78±0.33, for mice in a SC was: 2.46±0.15 and for mice in a cage with RW for only 7 days was: 3.10±0.33 (n=7, 5 and 6 respectively; p=0.17, ANOVA; Figure 46). Azimuth maps were also similar in strength of V1-activation: 2.27±0.22 for RW-mice, 2.18±0.15 for SC-mice and 2.70±0.24 for 7dRW-mice (p=0.26, ANOVA). Additionally, the quality of the retinotopic maps after elevation stimulation was similar for all the groups: 1.5±0.4 for RW-mice, 1.8±0.5 for SC-mice and 1.3±0.5 for 7dRW-mice (p=0.36, ANOVA). The same was true for the azimuth stimulation with: 4.8±2.0 for RW-mice, 2.8±1.0 for SC-mice and 2.6±0.7 for 7dRW-mice (p=0.43, ANOVA; Figure 46)

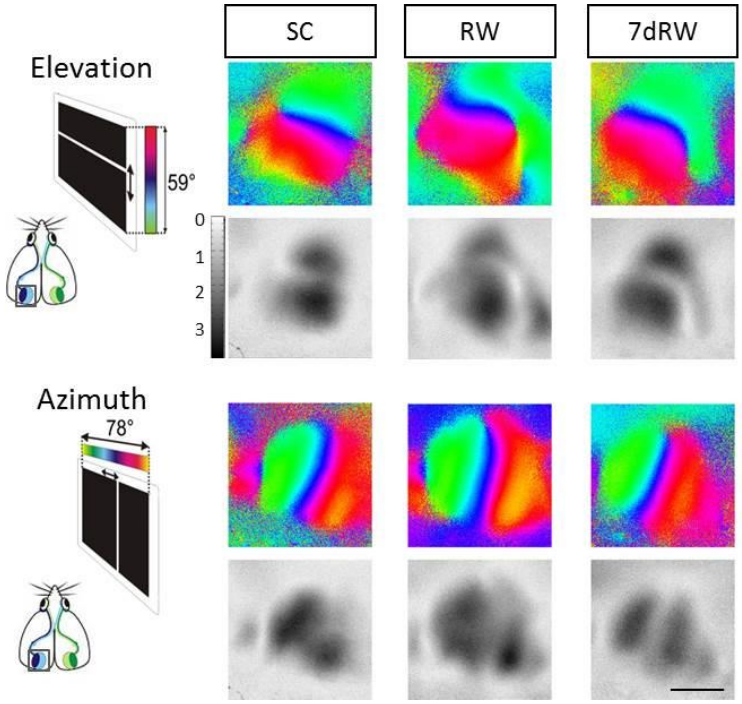


Figure 45: Representative examples of retinotopic and activity maps after elevation and azimuth stimulation recorded from SC, RW and 7dRW mice. There were no differences in the acitivity and quality of the maps between the different housing conditions. Scale bar: 1 mm

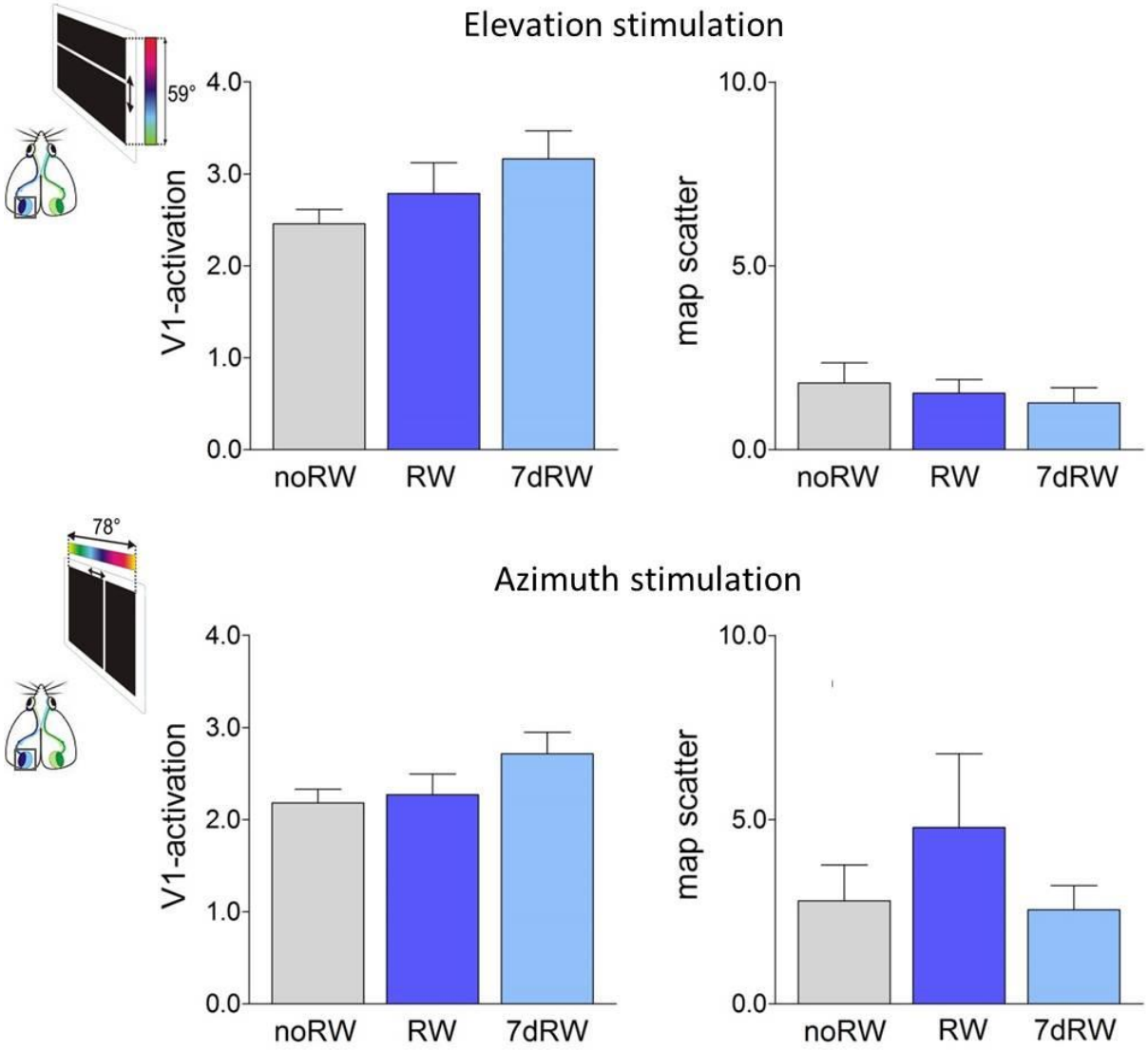


Figure 46: Running had neither an effect on V1-activation nor on the quality of the retinotopic maps. Comparison of V1-activation and map quality after right eye stimulation between SC (grey) and RW (blue) mice. For both groups the acquired maps were identical without significant differences in signal strength or quality of retinotopy for both elevation and azimuth stimulation.

3.4.4. Amount of running

The cages with RW were equipped with a counter which records the turns of the wheel. The counter was checked daily and an average number of turns was calculated for every mouse dividing the counted numbers of turns by the number of mice living in each cage (3-5 mice per cage). The average amount of RW-turns for male RW-mice was 3,229±212 turns/day and 3,991±445 turns/day for female RW-mice. The diameter of the running wheel (12.8 cm) was used to convert the turns into kilometers (km) finding that for male mice was 1.28±0.09 km/day and for female mice was 1.60±0.18 km/day (Table 10). The difference in RW-turns between males and females was not significant ($p>0.05$, t-test). For 7dRW-mice, the average

amount of RW-turns/day was 3,452±626 corresponding to 1.38±0.25 km/day. Comparing with the other groups it was similar (p>0.05, ANOVA).

Table 10: Amount of running. The mice were housed in group of 3-5 animals per cage. The average amount of turns of the wheel for mice that were born and raised in RW-cages is presented as: turns per day, turns per day per animal and corresponding km per animal per day. Additionally, the average amount of running per day per animal is presented for female and male mice.

Number of animals per cage	Average turns/day/ cage	Average turns/day/ animal	Km/day/ animal (R=6.4cm)		Average turns/day / Animal	Km/day/ animal (R=6.4cm)
3 females	11,868	3,956	1.58	**Females**	3,991	1.6
5 females	20,065	4,013	1.61			
3 males	9,180	3,060	1.23	**Males**	3,229	1.28
4 males	13,424	3,356	1.53			

3.4.5. Sex differences had no effect on ODIs, V1-activation and map quality

In this study I used mice of both sexes (males and females). Previously we did not observe any differences between the two sexes in OD-plasticity or any of the parameters we tested. Here, I also checked for sex differences in RW-mice after MD on ODI, V1-activation and quality of the retinotopic maps to exclude this possibility. Average ODI after MD for male mice raised in RW-cage was 0.08±0.02 (n=3) and for females was 0.05±0.04 (n=5), thus not significant different (p=0.57, t-test; Figure 47A). Similarly, V1-activation after contralateral or ipsilateral eye stimulation was similar for both sexes (males/females: contralateral: 1.02±0.11/1.47±0.26, ipsilateral: 0.93±0.13/1.25±0.10; p=0.18/0.13, t-test; Figure 47B). Moreover, retinotopic map quality after elevation or azimuth stimulation was not different between the sexes. After elevation stimulation map quality was 5.07±1.87 for male mice and 5.06±2.83 for females (p=0.99, t-test; Figure 47C). After azimuth stimulation map quality was again similar between males (2.82±0.84) and females (2.61±0.48; p=0.84, t-test; Figure 47D).

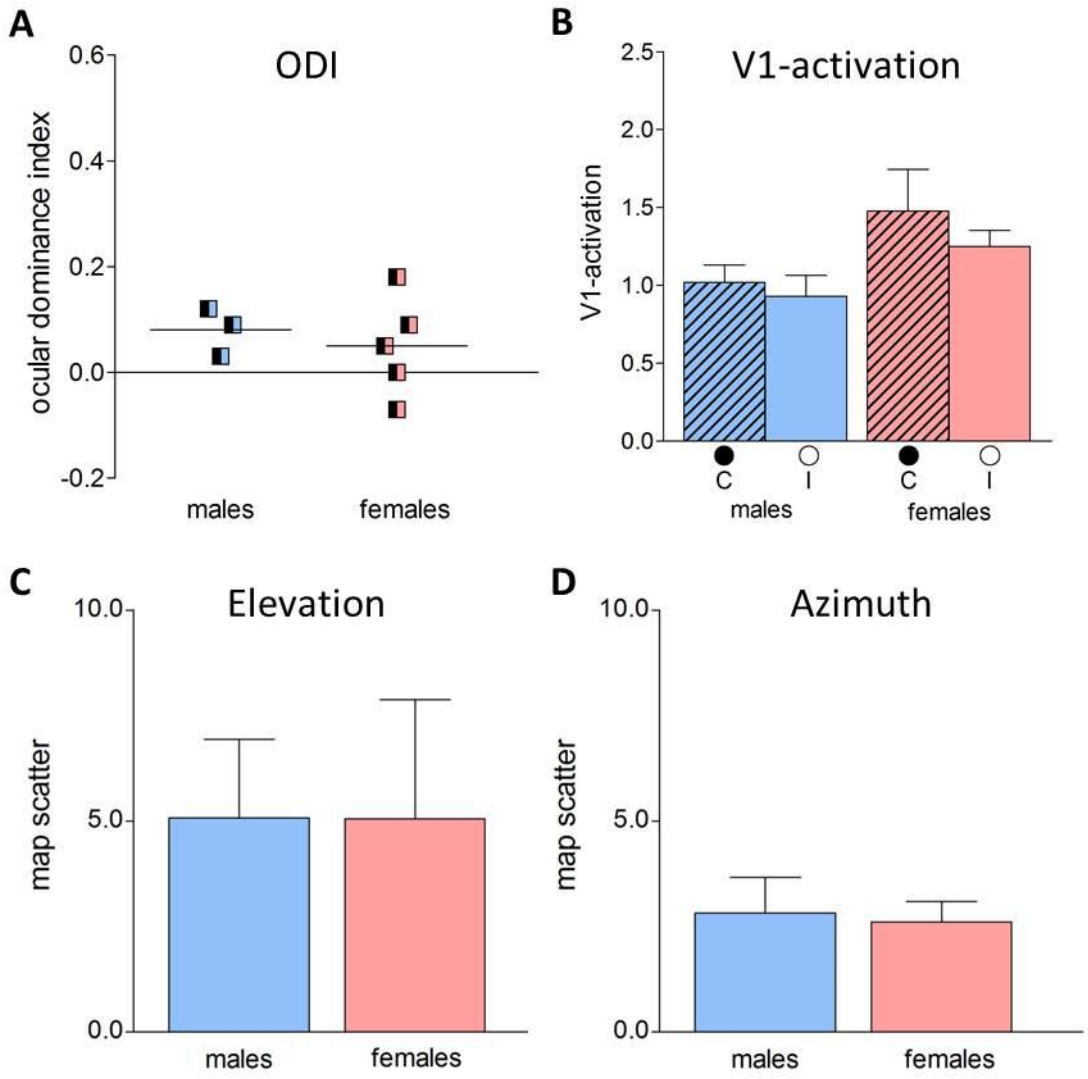


Figure 47: No significant differences between RW males and females mice. Comparison of mice raised in RW-cages after MD. ODI values (**A**), V1-activation after contralateral (C) or ipsilateral (I) eye stimulation (**B**) and retinotopic map quality after either elevation stimulation (**C**) or azimuth stimulation (**D**) were similar for both sexes. Male mice are presented in blue color (n=3) while female mice in pink color (n=5).

3.5. Lifelong running and 14dRW preserved and restored, respectively, OD-plasticity in adult mice after stroke in S1

As it has been previously reported a small cortical lesion in the primary somatosensory cortex (S1) impairs visual plasticity (Greifzu et al., 2011) which can be restored in mice due to a stimulating environment (Greifzu et al., 2014). These findings point out that, active and challenged individuals are more likely to recover faster after brain injuries than non-active individuals. I already described (part 3.4.) that voluntary physical exercise alone has a beneficial effect on OD-plasticity since OD-plasticity was preserved in old animals

(Kalogeraki et al., 2014), yet the impact of physical exercise on mice OD-plasticity after stroke is not well understood. Do adult mice that experience physical exercise show preserved OD-plasticity after stroke injury? Is long-term running required for showing OD-plasticity after stroke or is it still possible to restore OD-plasticity after stroke in old mice by providing them access to RWs? To this end, I investigated whether raising mice in a cage with a RW will preserve OD-plasticity in old animals (>PD110) after a small cortical lesion in S1. Additionally, I tested whether OD-plasticity can be restored in adult mice after a small localized cortical lesion by transferring SC-mice in a cage with a RW directly after induction of a stroke. Detailed description of all the groups and the experimental timeline can be found in the material and methods section (part 2.2.3.). All the PT-injections for induction of the cortical stroke as well as some of the imaging experiments were performed by Dr. Justyna Pielecka-Fortuna. Our data, presented here, suggest that physical exercise not only can preserve but also restore OD-plasticity after a localized cortical stroke. We did not find any differences in visual abilities and their increase after MD measured with the optomotor setup between the different groups.

3.5.1 Improvements in visual abilities after MD were not affected in RW or 14dRW mice after induction of a cortical lesion

Improvements in visual acuity and contrast sensitivity thresholds after MD can be used as an indicator for sensory learning as it was shown previously (Prusky et al., 2006). However, a stroke induction in SC raised mice abolished improvements after MD (Greifzu et al., 2011). After induction of a photothrombotic lesion in mice born and raised in a cage with a RW (RW_PT) or in mice raised in SC but transferred to a RW-cage after stroke for 14 days (14dRW_PT) the spatial frequency and contrast sensitivity thresholds were tested in the virtual reality optomotor setup developed by Prusky et al., (2004). Also, control sham mice were tested for both raising conditions (RW_sham and 14dRW_sham). Initially the baseline values of spatial frequency and contrast sensitivity threshold of the optomotor reflex were determined for all groups pooled. The highest spatial frequency that elicited an optomotor response in mice born and raised in a RW-cage was 0.38±0.001 cyc/deg for sham mice (n=7) and 0.38±0.001 cyc/deg for PT mice (n=10). There were no significant differences between the groups ($p>0.05$, ANOVA). Likewise, in 14dRW mice no differences were detected between sham and PT groups on day 0 (sham/PT: 0.38±0.003/0.39±0.001 cyc/deg, n=6/12; $p>0.05$, ANOVA; Figure 48).

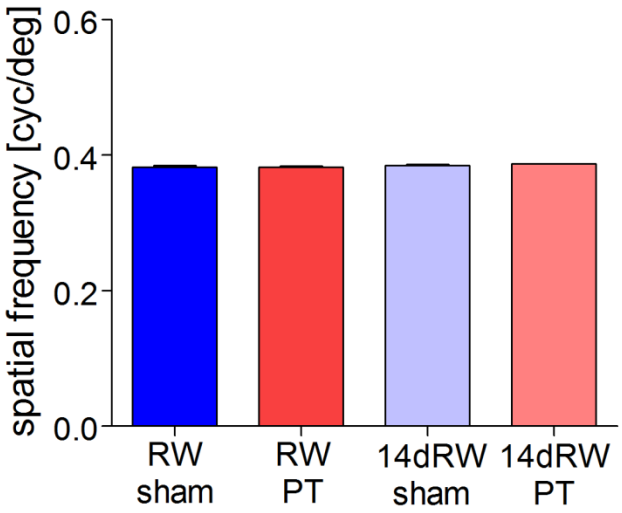


Figure 48: Baseline "visual acuity" in RW and 14dRW mice PT or sham treated. The highest spatial frequency that elicited a response in the optomotor setup was indistinguishable among all mice groups: RW_sham (blue), RW_PT (red), 14dRW_sham (light blue) and 14dRW_PT (light red) mice.

Furthermore, contrast sensitivity thresholds of the optomotor reflex were determined at six different spatial frequencies (0.031, 0.064, 0.092, 0.103, 0.192 and 0.272 cyc/deg) for all the groups. On day 0 (before MD) all mice born and raised in a cage with RW had similar contrast sensitivity values in all frequencies tested (comparison between sham and PT groups: $p>0.05$; for every frequency, ANOVA). Similarly, all 14dRW mice on day 0 had no differences in contrast sensitivity thresholds (comparison between sham and PT groups: $p>0.05$ for every frequency, ANOVA; Table 11). The values did not differ between the different raising conditions (RW and 14RW) and also not from values described before for C57BL/6J mice (Prusky et al., 2006).

Table 11: Baseline contrast sensitivity values of RW and 14dRW mice. The table includes the mean with SEM values of contrast sensitivity for all RW and 14dRW groups for the 6 different spatial frequencies tested (0.031, 0.064, 0.092, 0.103, 0.192, 0.272 cyc/deg).

	Day 0			
Spatial frequency (cyc/deg)	**RW_sham (n=3)**	**RW_sham_MD (n=4)**	**RW_PT (n=5)**	**RW_PT_MD (n=5)**
0.031	3.5±0.01	3.7±0.06	3.6±0.03	3.7±0.03
0.064	13.0±0.89	12.8±0.18	12.3±0.31	12.4±0.28
0.092	11.7±0.55	11.7±0.14	11.5±0.23	11.4±0.25
0.103	11.3±0.29	11.3±0.16	10.9±0.28	10.8±0.23
0.192	7.1±0.17	7.4±0.03	7.3±0.09	7.1±0.07
0.272	3.6±0.05	3.6±0.05	3.6±0.03	3.6±0.03
	Day 0			
Spatial frequency (cyc/deg)	**14dRW_sham (n=3)**	**14dRW_sham_MD (n=3)**	**14dRW_PT (n=5)**	**14dRW_PT_MD (n=7)**
0.031	3.6±0.08	3.6±0.05	3.7±0.02	3.6±0.02

0.064	15.4±0.65	14.5±0.37	13.9±0.18	14.4±0.23
0.092	14.6±0.72	13.3±0.25	13.2±0.17	13.7±0.28
0.103	13.3±0.85	12.8±0.26	12.7±0.14	13.1±0.26
0.192	7.5±0.15	7.6±0.21	7.7±0.11	7.5±0.14
0.272	3.6±0.01	3.7±0.04	3.7±0.04	3.7±0.02

After the induction of MD spatial frequency and contrast sensitivity thresholds of the optomotor reflex of the open eye were measured for all the mice groups for the next 7 days. Mice without MD from every group were also tested daily in the optomotor setup. Starting with the visual acuity thresholds of the optomotor reflex we observed a significant increase in mice with MD. In detail, after 7 days of MD "visual acuity" of the open eye increased in RW_sham mice from 0.38±0.001 cyc/deg to 0.45±0.002 cyc/deg (n=4) and in RW_PT mice from 0.38±0.001 cyc/deg to 0.45±0.002 cyc/deg (n=5; Figure 49; Figure 50). The increase in spatial frequency thresholds was similar for both groups (RW_sham and RW_PT) after MD and equal to 24%. The values on day 7 were significantly different from day 0 for both groups ($p<0.01$, ANOVA; Figure 49). Mice without MD did not show any changes in visual acuity threshold over the days ($p>0.05$, for both groups, ANOVA; Figure 49). Furthermore, neither PT and sham groups without MD nor PT and sham groups with MD showed any significant differences ($p>0.05$ for both comparisons; ANOVA).

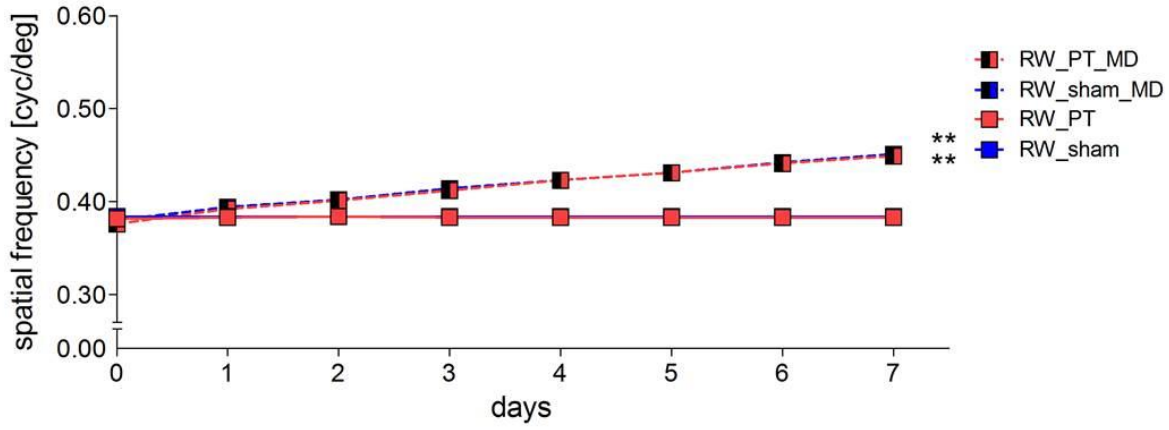


Figure 49: Improvements of visual acuity thresholds over days of MD in mice born in a RW-cage. "Visual acuity" values in cyc/deg are plotted against days for all the groups without MD (boxes) and with MD (half-filled boxes). Mice raised in a cage with a RW showed a significant improvement after 7 days of MD regardless the induction of a lesion. PT groups are presented in red color and sham groups in blue. There was no significant difference between sham and PT mice after MD, or between sham and PT mice without MD.

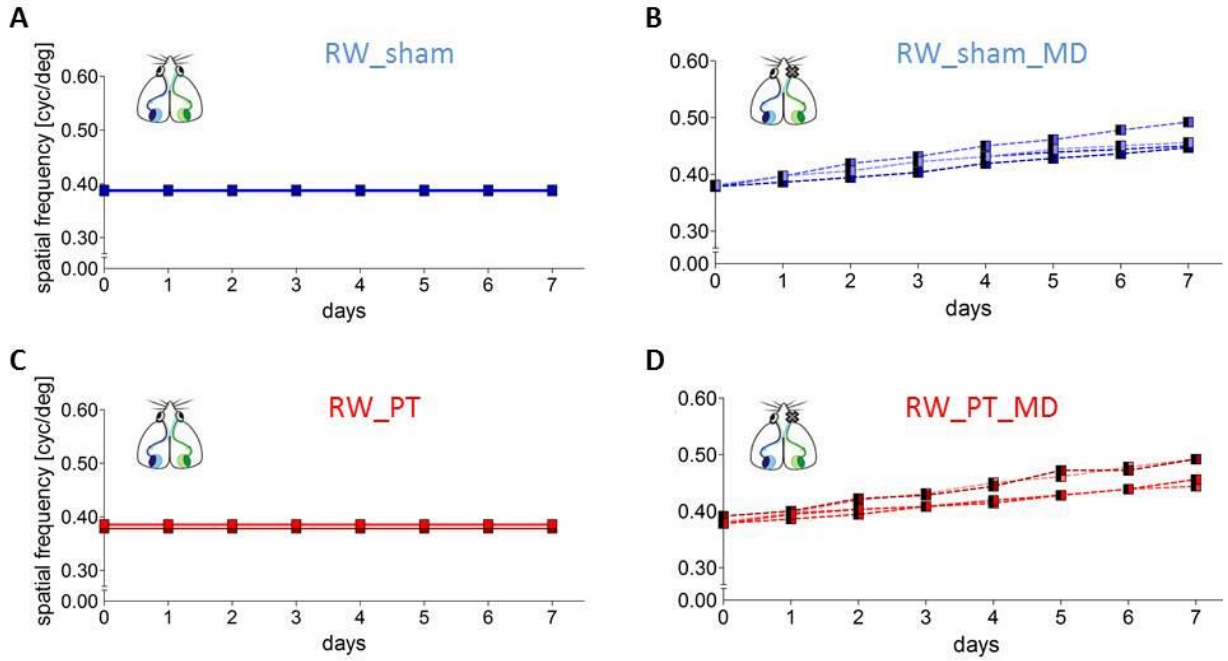


Figure 50: Spatial frequency thresholds measured in RW mice sham or PT treated over the 7 days noMD/MD period. Every line represents a single animal. **A.** RW_sham mice without MD had same visual acuity thresholds over the days. **B.** After 7 days of MD, RW_sham mice showed an increase of visual acuity thresholds. **C.** RW_PT mice without MD did not show any change over days. **D.** RW_PT mice with MD visual acuity thresholds of the open eye increased over days.

Similar to RW mice, mice transferred to a RW-cage directly after the induction of a cortical lesion improved in visual acuity thresholds of the optomotor reflex over the MD period. Visual acuity thresholds of mice from the 14dRW_sham group improved from 0.38±0.003 cyc/deg before MD to 0.48±0.010 cyc/deg after 7 days of MD (n=3; $p<0.001$, t-test; Figure 51, Figure 52). The increase was equal to 27%. Likewise, RW_PT mice after MD increased on average by 28%: from 0.39±0.001 cyc/deg before MD to 0.49±0.005 cyc/deg (n=7; $p<0.001$, t-test; Figure 51, Figure 52). The increase of optomotor threshold of both was similar after the MD period ($p>0.05$, ANOVA; Figure 51). As expected, mice without MD from all groups did not show improvement of "visual acuity" over days (Figure 51).

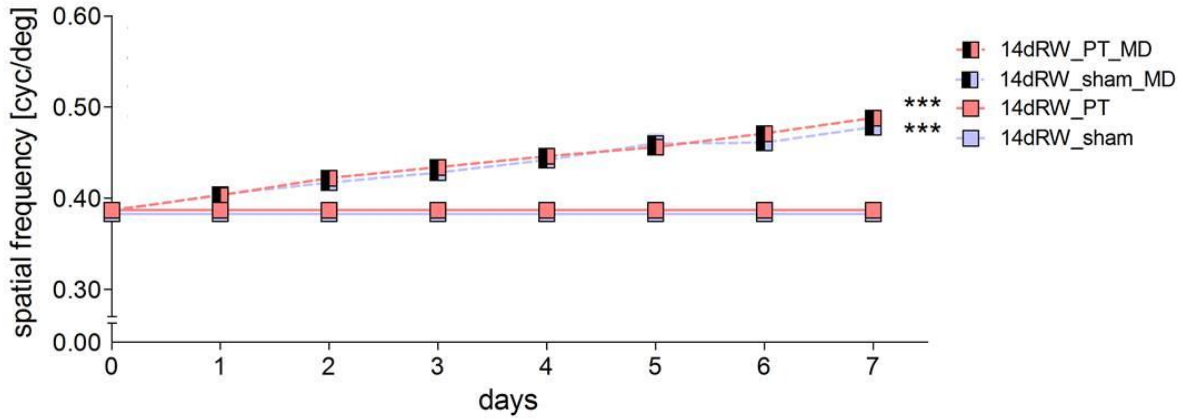


Figure 51: Improvements of visual acuity thresholds over days of MD in 14dRW sham and PT mice. "Visual acuity" values in cyc/deg are plotted against days for all the groups without MD (boxes) and with MD (half-filled boxes). Mice were raised in a SC and then transferred in cage with a RW after the induction of lesion (14dRW_PT: light red) and the control sham (light blue) group improved significantly over days of MD. In mice without MD "visual acuity" did not change over days.

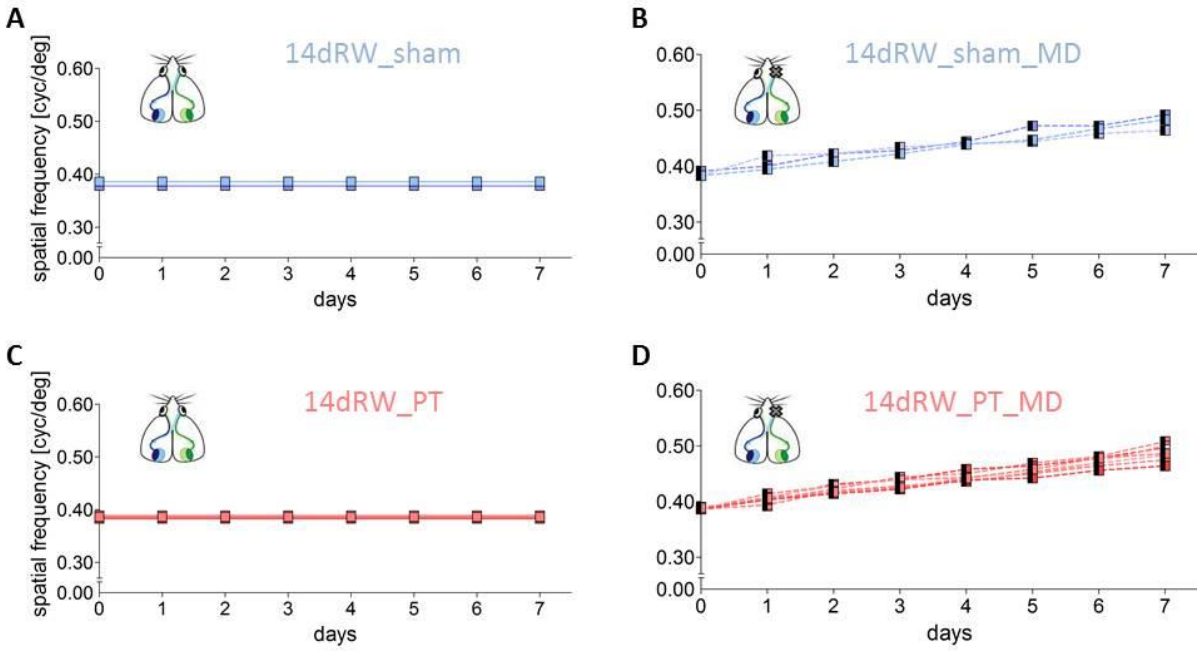


Figure 52: Spatial frequency thresholds measured in 14dRW mice sham or PT treated over the 7 days noMD/MD period. Every line represents a single animal. **A.** 14dRW_sham mice without MD had same visual acuity thresholds over the days. **B.** After 7 days of MD, 14dRW_sham mice showed an increase of visual acuity thresholds. **C.** 14dRW_PT mice without MD did not show any change over days. **D.** 14dRW_PT mice with MD visual acuity thresholds of the open eye increased over days.

Together with spatial frequency thresholds the contrast sensitivity thresholds of the optomotor reflex for all groups were determined during the noMD/MD period. After 7 days of MD both

sham and PT groups of mice raised in RW-cages increased significantly in contrast to mice without MD ($p<0.001$, ANOVA; Figure 53). Specifically in RW_sham mice contrast sensitivity thresholds increased significantly in 3 out of 6 spatial frequencies measured after MD to 4.8±0.05 at 0.031 cycl/deg, 26.2±1.66 at 0.064 cyc/deg, 23.1±1.35 at 0.092 cyc/deg, 21.2±1.19 at 0.103 cyc/deg, 12.6±0.47 at 0.192 cyc/deg and 4.8±0.06 at 0.272 cyc/deg on day 7 ($p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p>0.05$, $p>0.05$, compared to values from day 0, ANOVA; Figure 53; Figure 54B; Table 12). Similar increase was observed in RW_PT mice after MD (at 0.031, 0.064, 0.092, 0.103, 0.192 and 0.272cyc/deg: to 5.0±0.17, 27.0±1.18, 23.4±0.92, 21.3±0.54, 12.9±0.62 and 4.7±0.10 on day 7; $p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p>0.05$, $p>0.05$, compare to day 0, ANOVA; Figure 53; Figure 54D). There were no differences in any spatial frequency between the two groups on day 7 ($p>0.05$, ANONA, Figure 53). As expected, mice without MD from both groups did not show any change over the 7 days tested in optomotor setup ($p>0.05$, for every frequency, ANOVA; Figure 54A and C).

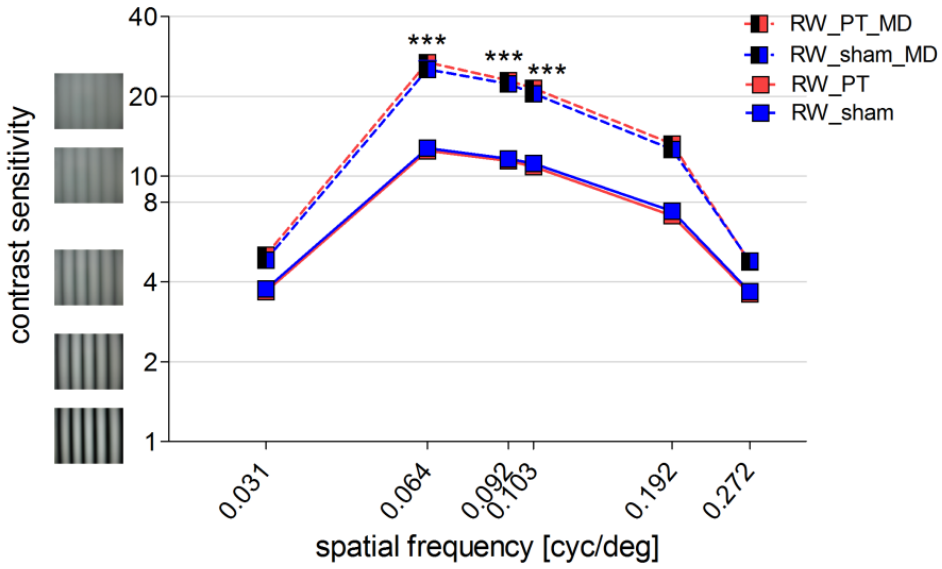


Figure 53: Contrast sensitivity improvements of RW mice after 7 days of MD. Sham (red) and PT (blue) mice raised in a cage with a running wheel improved significantly in 3 out of 6 frequencies after 7 days of MD.

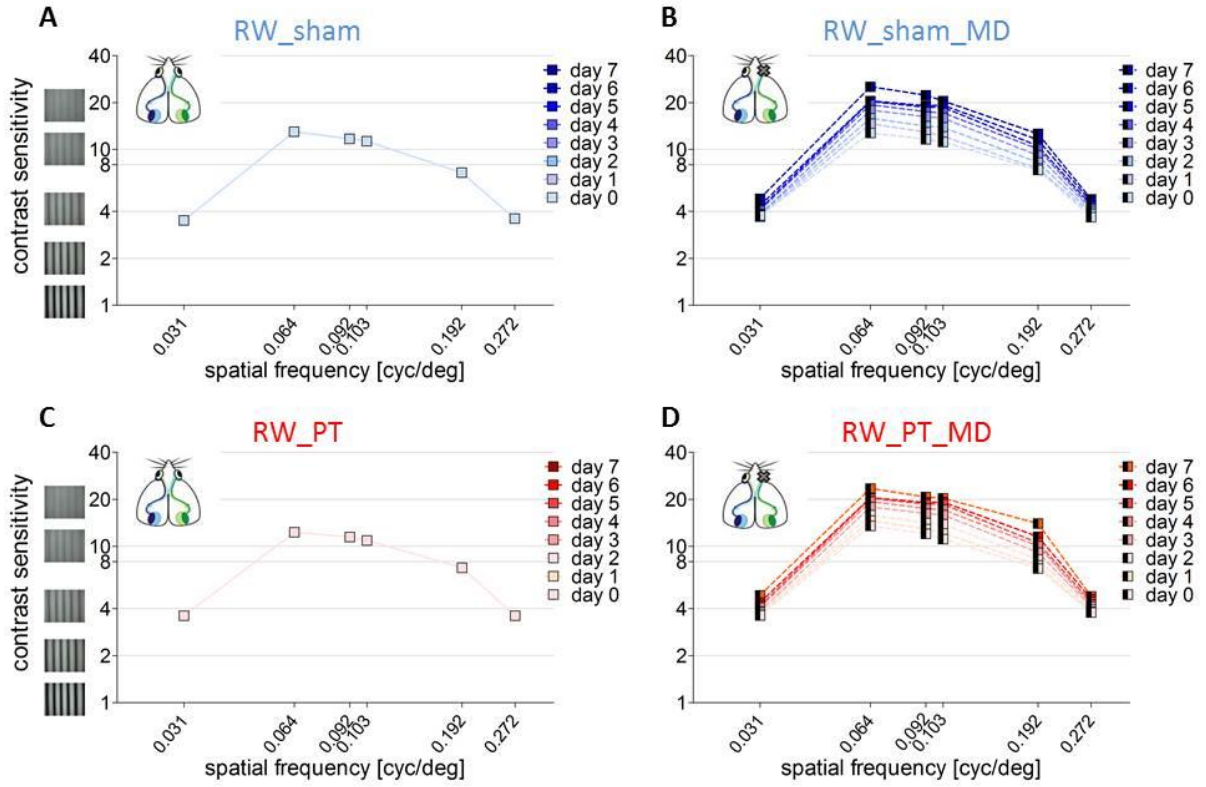


Figure 54: Mean contrast sensitivity thresholds at the 6 spatial frequencies of RW mice sham or PT treated over days. Day 0 represents the baseline values before MD. **A**. In RW_sham mice without MD contrast sensitivity remained stable over days. **B**. After 7 days of MD in RW_sham the contrast sensitivity of the open eye increased significantly. **C**. PT mice raised in a cage with RW without MD did not improve over days. **D**. RW_PT mice with MD showed a significant improvement over days in contrast sensitivity thresholds.

Furthermore, 14dRW_sham or PT with MD showed a significant improvement on the 7th day compare to mice without MD of the same treatment ($p<0.001$ for spatial frequencies 0.064 cyc/deg, 0.092cyc/deg and 0,103 cyc/deg, and $p<0.05$ for 0.192 cyc/deg; ANOVA; Figure 55). After 7 days of MD both sham and PT groups of mice raised in SC and transferred in a cage with a RW after PT for 14 days improved significantly compared to the same groups of mice without MD ($p<0.001$, ANOVA; Figure 55). In detail, 14dRW_sham mice with MD improved significantly in contrast sensitivity thresholds of the open eye at 4 out of 6 spatial frequencies measured (at 0.031, 0.064, 0.092, 0.103, 0.192 and 0.272cyc/deg: to 5.2±0.18, 30.1±1.78, 26.4±1.18, 24.3±1.82, 11.9±0.86 and 5.2±0.16 on day 7; $p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p>0.05$, compared to day 0, ANOVA; Figure 56B). The 14dRW_PT group with MD also showed a significant improvement over the days in contrast sensitivity thresholds to 4.7±0.12 at 0.031 cyc/deg, 36.3±2.17 at 0.064 cyc/deg, 31.8±1.48 at 0.092 cyc/deg, 27.8±1.43 at 0.103 cyc/deg, 15.6±0.72 at 0.192 cyc/deg and 5.3±0.20 at 0.272 cyc/deg on day 7 ($p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p>0.05$, compared to values from day 0, ANOVA; Figure 56D). The increase after MD was not significantly different between the sham and PT treated mice at any spatial frequency ($p>0.05$, ANONA, Figure 55).

Again sham or PT mice without MD had no improvement over the 7 days testing period in the optomotor setup (p>0.05, for every frequency, ANOVA; Figure 56A and C).

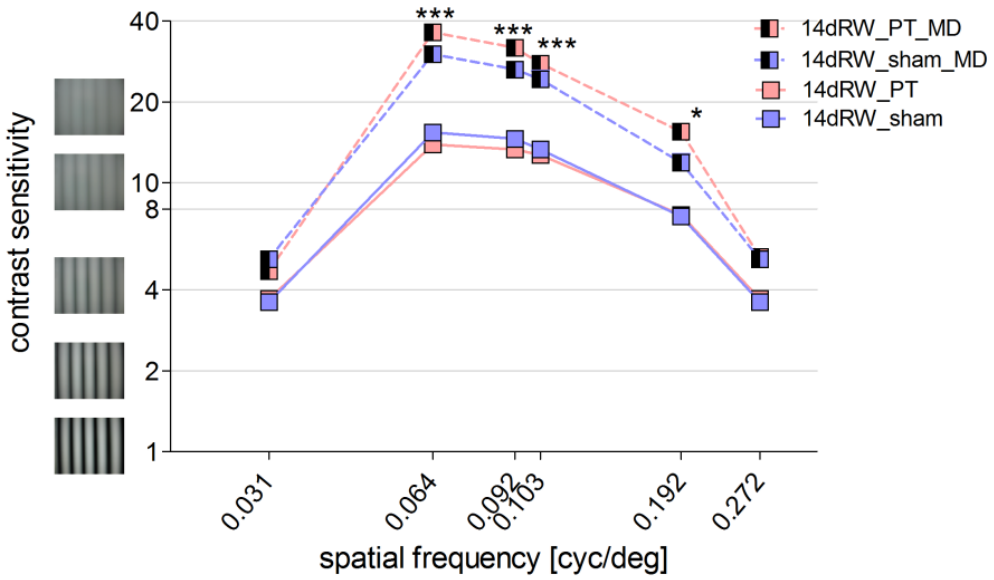


Figure 55: Contrast sensitivity improvements of 14RW mice after 7 days of MD. Sham (light red) and PT (light blue) mice transferred in a cage with RW for 14 days improved significantly in 4 out of 6 frequencies comparing the values on day 0 and on day 7 after MD.

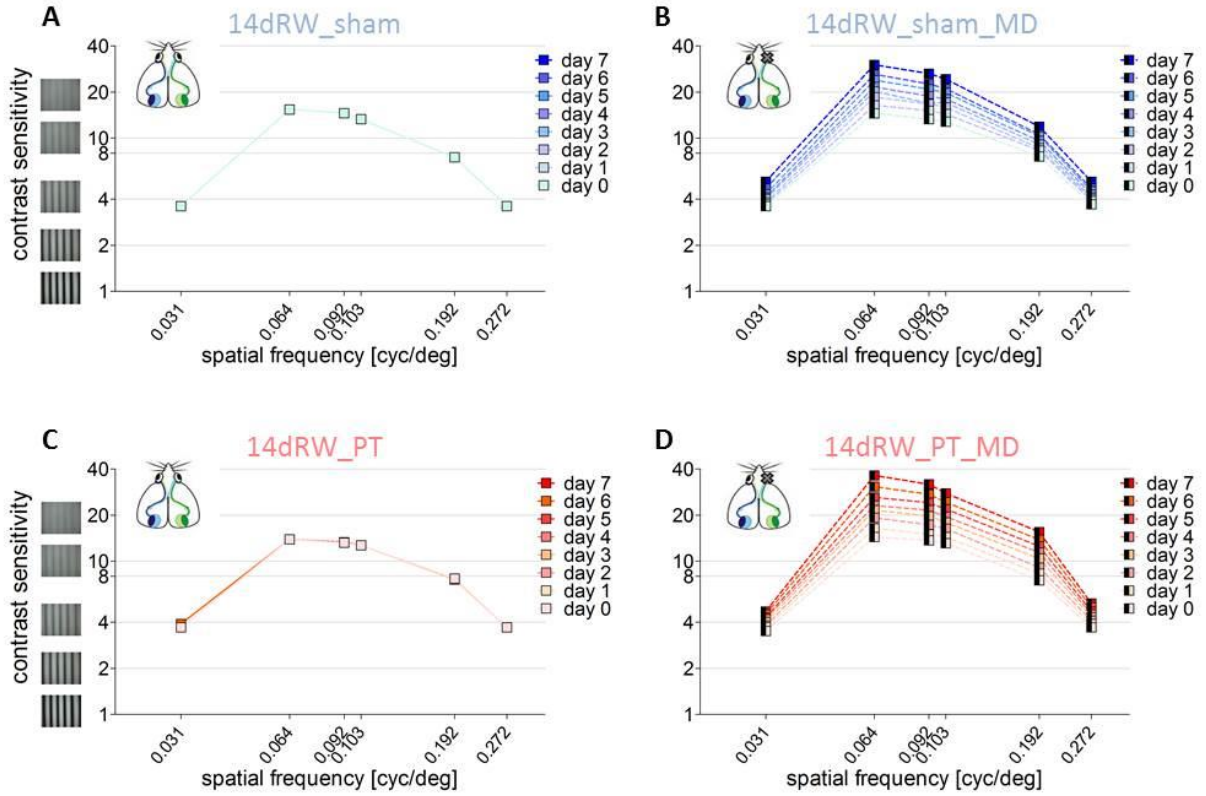


Figure 56: Mean contrast sensitivity thresholds in the 6 spatial frequencies tested, of the 14dRW sham or PT treated mice over days. Day 0 represents the baseline values before MD. **A.** In

14dRW_sham mice without MD contrast sensitivity remained stable over days. **B.** After 7 days of MD in 14RW_sham mice the contrast sensitivity of the open eye increased significantly. **C**. 14dRW mice with PT without MD did not improve over days. **D**. 14dRW_PT mice with MD showed a significant improvement over days in contrast sensitivity thresholds.

Comparing contrast sensitivity thresholds after 7 days of MD of RW with 14dRW mice, resulted in a significant difference in improvement of 3 of the spatial frequencies measured. The contrast sensitivity thresholds of 14dRW_PT_MD mice increased to 36.3±2.17 at 0.064 cycl/deg, to 31.8±1.48 at 0.092 cycl/deg and to 27.8±1.43 at 0.103 cyc/deg whereas in RW_PT mice with MD the contrast sensitivity thresholds at the same frequencies were 27.0±1.18, 23.4±0.92 and 21.3±0.54 ($p<0.05$, for every frequency, ANOVA; Table 12). Altogether life-long running (RW) or 14dRW groups showed improvements in visual capabilities over 7 days of MD in the optomotor system after the induction of a photothrombotic stroke in S1.

Table 12: Contrast sensitivity values of all RW and 14dRW groups at day 7. The table includes the mean with SEM values of contrast sensitivity for all RW and 14dRW mice for the 6 different spatial frequencies tested (0.031, 0.064, 0.092, 0.103, 0.192, 0.272 cyc/deg).

	Day 7			
Spatial frequency (cyc/deg)	**RW_sham (n=3)**	**RW_sham_MD (n=4)**	**RW_PT (n=5)**	**RW_PT_MD (n=5)**
0.031	3.5±0.01	4.8±0.05	3.6±0.03	5.0±0.17
0.064	13.0±0.89	26.2±1.66	12.3±0.30	27.0±1.18
0.092	11.7±0.55	23.1±1.35	11.5±0.23	23.4±0.92
0.103	11.3±0.29	21.2±1.19	10.9±0.28	21.3±0.54
0.192	7.1±0.17	12.6±0.47	7.3±0.09	12.9±0.62
0.272	3.6±0.05	4.8±0.06	3.6±0.03	4.7±0.10
	Day 7			
Spatial frequency (cyc/deg)	**14dRW_sham (n=3)**	**14dRW_sham_MD (n=3)**	**14dRW_PT (n=5)**	**14dRW_PT_MD (n=7)**
0.031	3.6±0.08	5.2±0.18	3.9±0.01	4.7±0.12
0.064	15.4±0.63	30.1±1.78	13.9±0.18	36.3±2.17
0.092	14.6±0.72	26.4±1.18	13.3±0.15	31.8±1.48
0.103	13.3±0.75	24.3±1.82	12.7±0.14	27.8±1.43
0.192	7.5±0.15	11.9±0.86	7.6±0.10	15.6±0.72
0.272	3.6±0.02	5.2±0.16	3.7±0.04	5.3±0.20

3.5.2. Voluntary physical exercise preserved OD-plasticity in adult mice after stroke

To visualize and calculate V1-activation and determine the ODI for every mouse the optical imaging of intrinsic signals was used. Using the 150x150 camera objective we were able to capture V1-activation from both hemispheres. Initially, to test whether life-long running or short-term running has an effect on mice OD-plasticity after a cortical lesion in the left S1 ODIs of the left hemisphere of all groups were measured (all mice tested were >PD110). To stimulate the left binocular part of V1 20 degrees moving bar restricted on the -5 to +15 degrees of visual field was projected in a monitor in front of the mouse.

As expected, the sham mice raised in RW-cages without MD showed a contralateral dominance in the binocular part of V1. The activity patches induced by the stimulation of the contralateral eye were darker than those after stimulation of the ipsilateral eye, the ODI values were positive and warm color dominated the 2-dimensioned OD-map (Figure 57A). Induction of MD in RW_sham mice changed the contralateral dominance resulting in an OD-shift. In this group the binocular part of V1 was activated almost equally strong after stimulation of contra- or ipsilateral eye, ODI values were closer to zero, colder colors appeared in the 2-dimensional OD-map and the histogram was shifted to the left (Figure 57B). After a cortical lesion RW mice without MD (RW_PT group) were also contralaterally dominated: the V1-activation patch was darker after contralateral eye stimulation compared to the one after ipsilateral eye stimulation, the ODI was positive and warm colors prevailed the 2-dimensional OD-map, suggesting that PT alone does not affect the contralateral dominance and the activation of V1 (Figure 57C). Like in the sham treated mice, MD in RW mice that received PT resulted in an OD-shift: V1 was activated approximately with the same strength via stimulation of both eyes, resulting in lower ODI values, colder colors in the 2-dimensional OD-map and shift of the OD-histogram towards the left (Figure 57D).

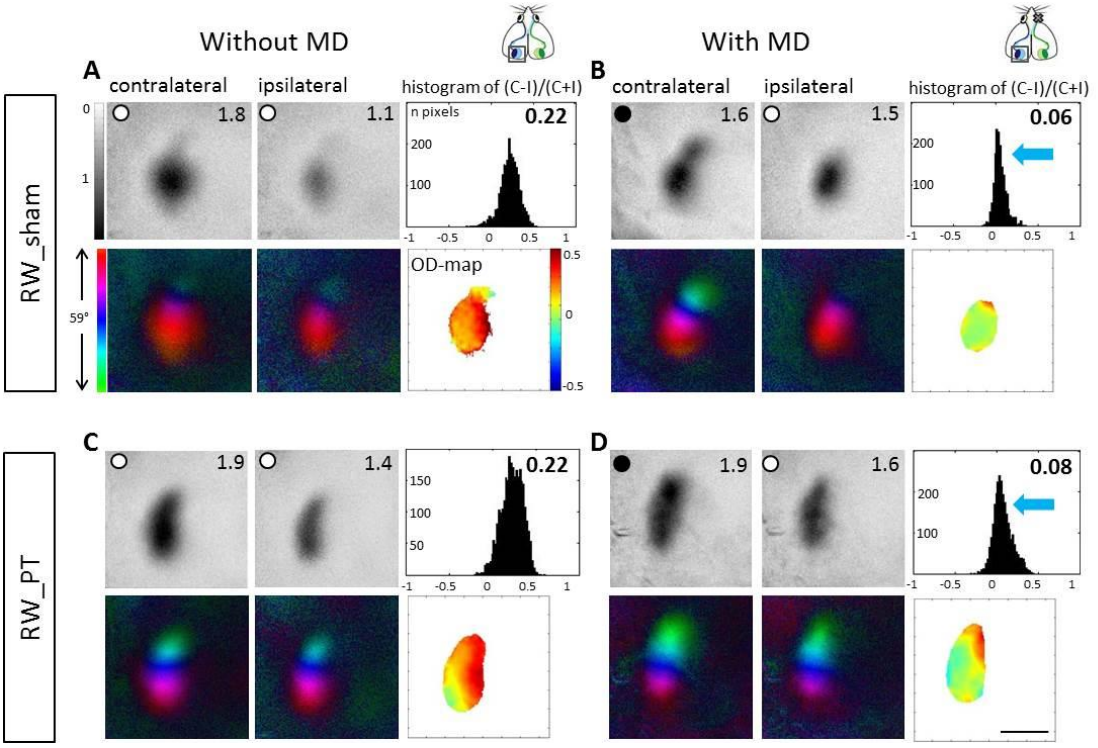


Figure 57: Examples of optical imaging recorded maps from lifelong running groups of the left hemisphere. Activity and polar maps of the binocular part of V1 and ODI values of the left hemisphere measured with optical imaging of intrinsic signals in RW sham (**A, B**) or PT (**C, D**) treated animals. Mice without MD (**A, C**) showed a contralateral dominance with stronger activities after stimulation of the contralateral eye, positive ODI values and warm colors in the 2-dimensional OD-map. Seven days of MD in both groups (sham: **B** and PT: **D**) resulted in a strong OD-shift, both eyes activated V1 equally strong, the ODI values were lower, colder colors prevailed in the OD-maps and the histograms shifted to the left (blue arrows). Scale bar: 1 mm.

Like the lifelong RW mice, 14dRW_sham mice without MD showed a contralateral dominance: activity patches induced after stimulation of the contralateral eye were darker than those after ipsilateral eye stimulation, the calculated ODI had positive values and the 2-dimensional OD-map was dominated by warm colors (Figure 58A). Whereas in the 14dRW_sham group after MD V1-activation was equally strong after both eyes (contra-, or ipsilateral) stimulation, ODI values were reduced, the 2-dimensional OD-map was colored with colder colors and the histogram shifted to the left (Figure 58B). Furthermore, 14dRW_PT mice without MD showed a contralateral dominance with stronger V1-activation after contralateral eye compare to the one after ipsilateral eye stimulation, positive ODI values and warm colors in the 2-dimensional OD-map (Figure 58C). However, after MD in the 14dRW_PT mice the strength of V1-activation was approximately the same after stimulation of both eyes, ODI values were closer to zero, the OD-map was dominated by colder colors and the histogram shifted to the left (Figure 58D).

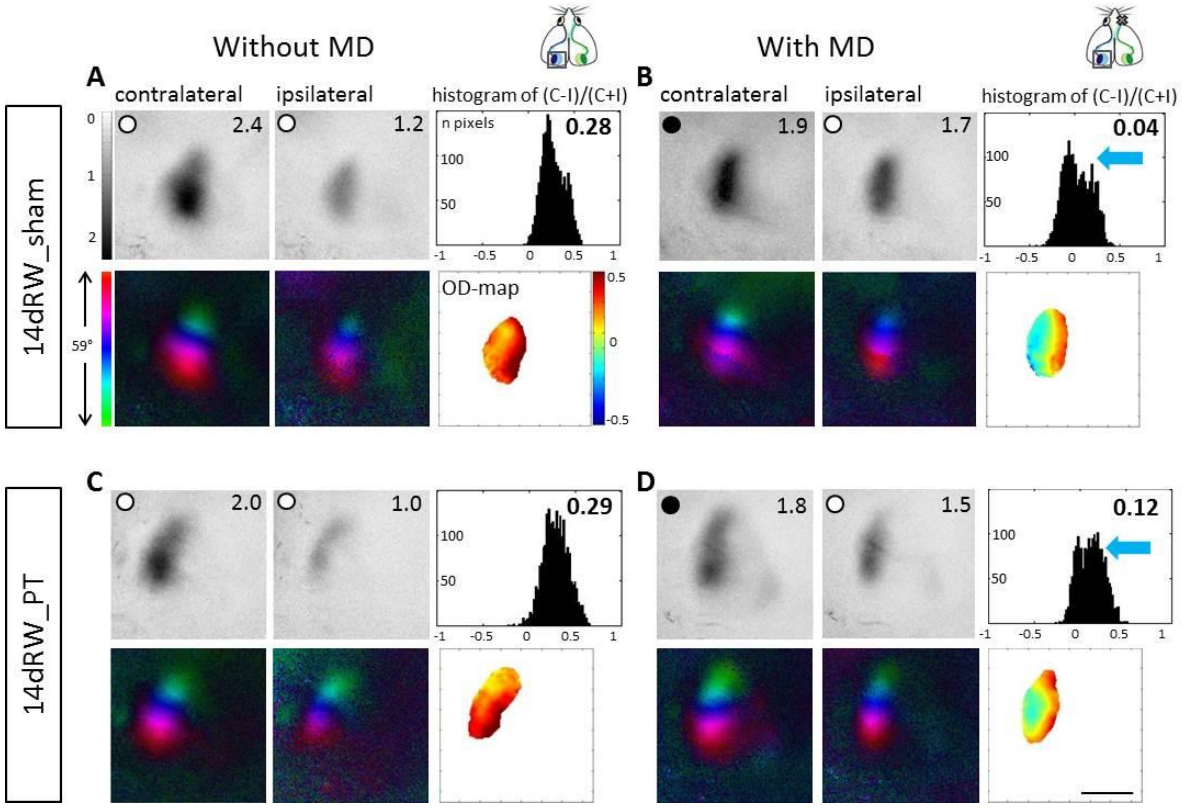


Figure 58: Optical imaged maps recorded from 14dRW groups of the left hemisphere. Data presented as in figure 57**.** Activity and polar maps of the binocular part of V1 and ODI values of the left hemisphere 2-dimensional OD-maps and ODI histograms for 14dRW sham (**A, B**) and PT (**C, D**), without (**A, C**) or with MD (**B, D**). Animals with MD showed a significant OD-shift in both groups: both eyes activated V1 about equally strong, the ODI values were lower, colder colors prevailed in the OD-maps and the histogram shifted to the left (blue arrows), regardless of the treatment (sham or PT). Scale bar: 1 mm.

Quantitative analysis of the ODIs for every group showed that all MD mice displayed an OD-shift independent of the presence of a cortical lesion. In RW_sham mice the ODI decreased significantly from 0.25±0.05 without MD (n=3, PD180-215) to 0.05±0.05 after 7 days of MD (n=4, PD156-218; p=0.04, t-test). Similarly, the RW_PT group showed an OD-shift: from 0.21±0.01 without MD (n=5; PD174-222) decreased to 0.08±0.01 with MD (n=5, PD149-204; p<0.0001, t-test). The 14dRW_sham group without MD had an ODI of 0.28±0.09 (n=3, PD119-162) which was significantly reduced in 14RW_sham mice after MD (0.04±0.06; n=3, PD120-197; p=0.012, t-test). Likewise, in the 14dRW_PT group the ODI in mice without MD was 0.25±0.04 (n=5, PD124-258), while in MD mice was significantly lower (0.05±0.04; n=7, PD=119-213; p=0.006, t-test; Figure 59A). Differences between mice with MD in all conditions were not significant (p>0.05, ANOVA). Also, mice without MD from all groups had similar mean ODI values (p>0.05, ANOVA).

Further, quantitative analyses of V1-activation showed neither significant reduction in the activation after contralateral eye stimulation or significant increase in the activation after

ipsilateral eye activation between mice without and with MD, in any of the tested groups. In detail for the life-long running groups the V1-activation after contralateral eye stimulation was 1.79±0.20 in sham mice without MD and 1.40±0.10 in sham mice with MD (p=0.116, t-test), and for PT mice was 1.76±0.17 without MD and 1.3±0.16 with MD (p=0.076, t-test). After simulation of the ipsilateral eye the activation of V1 was 1.04±0.09 in sham mice without MD and 1.35±0.14 in sham mice with MD (p=0.139, t-test). Additionally for PT mice without MD the V1-activation after ipsilateral eye stimulation was 1.14±0.14 and not significant different from PT mice with MD (1.12±0.15; p=0.931, t-test). Similarly for the 14 days running groups the V1-activation after contralateral eye stimulation was in sham groups without or with MD was not significant different (14dRW_sham/14dRW_sham_MD: 2.14±0.15/ 1.81±0.23; p=0.305, t-test) and in the PT groups without or with MD also not significant different (14dRW_PT/14dRW_PT_MD: 2.27±0.21/ 1.85±0.14; p=0.119, t-test). Additionally, V1-activation after ipsilateral eye stimulation was 1.30±0.17 in sham mice without MD and 1.59±0.13 in and for PT mice (p=0.249, t-test). V1-activation remained also unchanged in 14dRW_PT mice without or with MD (without/with MD: 1.42±0.25/1.66±0.11; p=0.344, t-test).

Although we did not find any significant differences comparing the V1-activation in mice without and with MD, the p-value after comparing the RW_PT mice without MD with RW_PT after MD was 0.076, indicating a trend towards a reduction of the contralateral eye activation (Figure 59B).

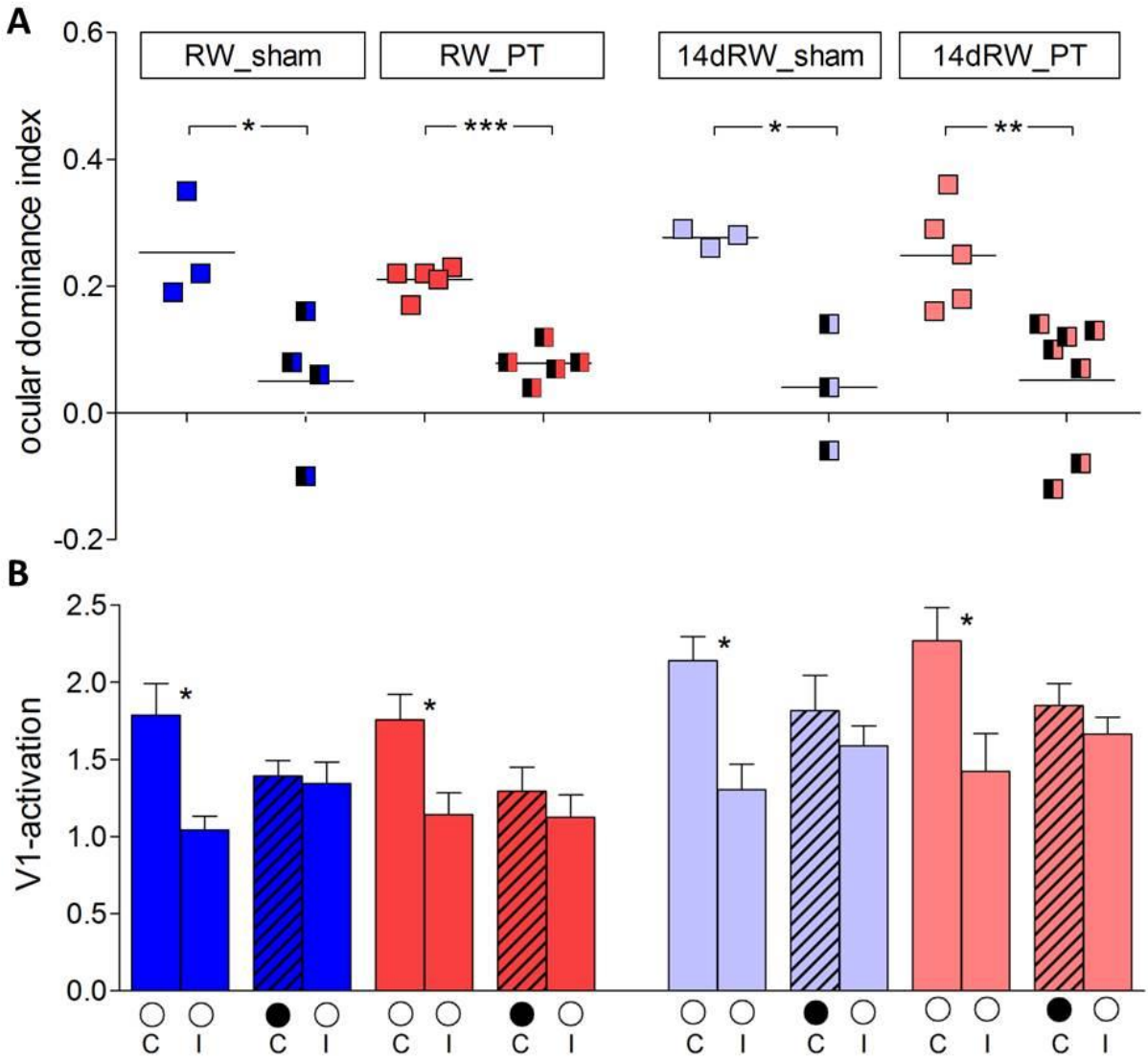


Figure 59: Quantification of ODIs and V1-activation RW and 14dRW groups after PT or sham treatment. A. Optically imaged ODIs without and with MD of sham treated (blue) and PT (red) mice raised in RW-cages, as well as sham (light blue) and PT (light red) treated mice with only 14 days in RW-cage. Symbols represent ODI-values of individuals; means are marked by horizontal lines. All MD mice showed an OD-shift regardless the treatment. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye without and with MD (black filled circle indicates MD eye). No statistical significant changes in V1-activation were detected.

3.5.3. Optical imaging data of the right hemisphere in the RW and 14dRW mice

Plasticity changes were also investigated in the non-lesioned (right) hemisphere. To stimulate the binocular part of V1 of right hemisphere a 20° moving bar restricted on the -15 to +5 degrees of the left visual field was used. In this case the deprived eye is ipsilateral to the imaged V1, whereas the open eye is contralateral to the imaged hemisphere. All tested groups showed a clear contralateral dominance in the binocular part of V1 in the right hemisphere.

V1-activation after contralateral eye (open) stimulation was always higher that those after ipsilateral eye (deprived eye) stimulation. The ODI values of all groups were positive and warm color dominated the 2-dimensinal OD-map. After 7 days of MD, the activities of V1 after ipsilateral (deprived) eye stimulation were reduced resulted in even stronger contralateral dominance, indicator of plasticity changes in the right hemisphere (Figure 60).

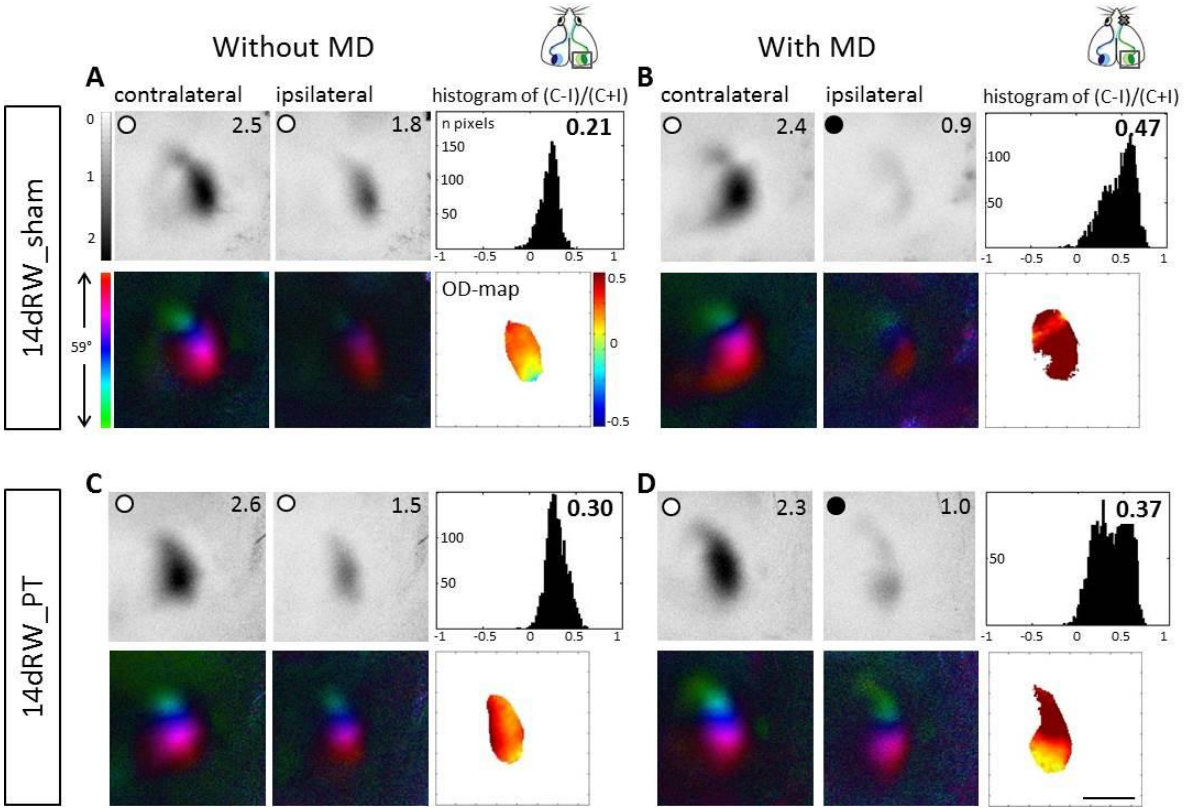


Figure 60: Examples of optical imaged maps of 14dRW groups of the right hemisphere. Data presented as in figure 57**.** Activity and polar maps of the binocular part of V1 and ODI values of the right hemisphere 2-dimensional OD-maps and ODI histograms for 14dRW sham (**A, B**) and PT (**C, D**), without (**A, C**) or with MD (**B, D**). In all groups contralateral eye activities in V1 were stronger compared to the ipsilateral, the ODI values were positive and the 2D OD-maps were dominated by warm colors. Scale bar: 1 mm.

The average ODI value of RW_sham mice without MD was 0.21±0.02 (n=3), whereas in the RW_sham mice with MD was 0.29±0.02 (n=3). Although the ODI is higher in mice after 7 days of MD the difference was not significant (p=0.064, t-test). In the RW_PT group without MD the ODI value was 0.23±0.04 (n=5) and not significant different from the RW_PT group after MD (0.29±0.03, n=4; p=0.357, t-test). Additional to the lifelong running groups the 14dRW_sham mice had an average ODI of 0.24±0.01 (n=3) which was significantly lower than the 14dRW_sham after MD (0.44±0.03, n=3; p=0.003, t-test). Similarly, the ODI of the 14dRW_PT mice increased significantly from 0.25±0.03 (n=4) without MD to 0.4±0.03 after MD (n=7; p=0.031, t-test; Figure 61A).

Quantitative analysis of the V1-activation revealed that in most of the cases the deprived eye responses (ipsilateral) in binocular part of V1 were significantly reduced. In detail, for the RW_sham mice with MD V1-activation after ipsilateral eye stimulation was 1.06±0.08 and significantly lower than in mice without MD (1.75±0.01; p=0.009, t-test), whereas the V1-activation after contralateral stimulation was unchanged (MD/noMD: 2.22±0.12/2.58±0.15, p=0.14, t-test). In RW_PT group the V1-activation after ipsilateral eye was lower after 7 days of MD than the one without MD but not significant (MD/noMD: 0.86±0.13/1.17±0.09, p=0.07, t-test). After contralateral eye stimulation in RW_PT group no significant difference was observed in V1-activation (MD/noMD: 1.58±0.19/1.83±0.02, p=0.18, t-test). V1-activation after ipsilateral eye stimulation was significantly reduced in 14dRW_sham mice after MD (0.89±0.05) compared to 14dRW_sham mice without MD (1.67±0.08, p=0.001, t-test), while no difference was detected after contralateral eye stimulation in V1 activation (MD/noMD: 2.41±0.37/2.37±0.10, p=0.92, t-test). After MD the 14dRW_PT group showed a V1-activation of 1.08±0.16 after ipsilateral eye stimulation and 2.65±0.30 after contralateral eye stimulation. There was no significant difference from the 14dRW_PT group without MD (ipsilateral: 1.43±0.37, contralateral: 2.54±0.63; p=0.33/p=0.85, compared to MD group, t-test; Figure 62B). Together these data propose that OD-plasticity is not affected on the non-lesioned hemisphere and these observations are comparable to previously published results (Greifzu et al., 2011).

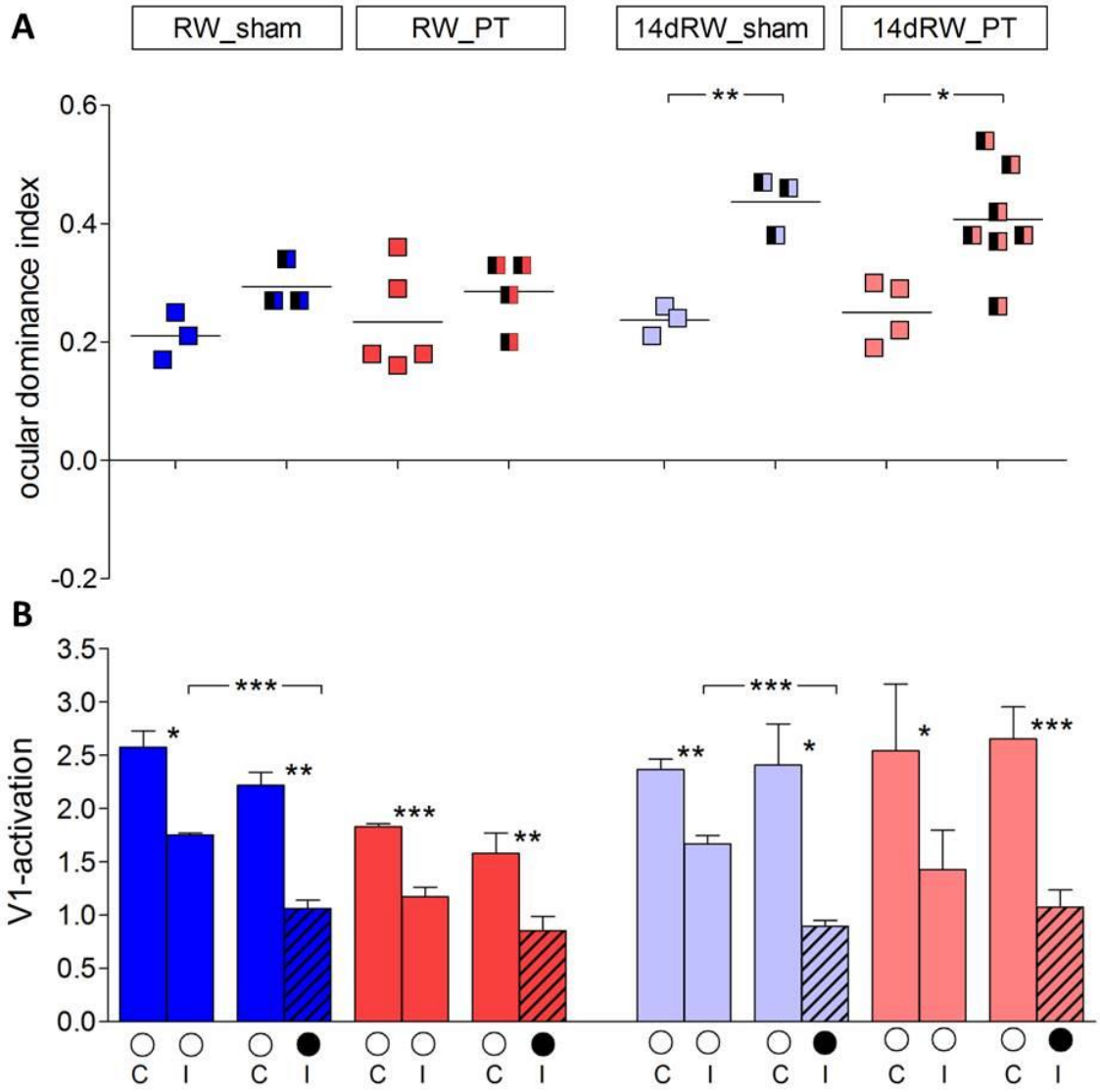


Figure 62: ODIs and V1-activation of the right hemisphere in lifelong running (RW) and 14dRW groups after PT or sham treatment. A. Optically imaged ODIs without and with MD of sham treated (blue) and PT (red) mice raised in RW-cages, as well as sham (light blue) and PT (light red) treated mice with only 14 days in RW-cage. Symbols represent ODI-values of individuals; means are marked by horizontal lines. All mice showed a contralateral dominance which in case of 14dRW mice after 7 days of MD was even stronger causing a significant increase in ODI values. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye without and after MD (black filled circles indicate MD eye). The differences in ODIs in mice without or with MD were mediated by decreases in V1-responses after ipsilateral (deprived) eye stimulation in RW_sham and 14dRW_sham mice. In RW_PT and 14dRW_PT mice V1-activities after contralateral eye stimulation were not changed while we observed a decrease in ipsilateral eye responses after MD, although not significant.

3.5.4. Analysis of cortical lesions

In order to determine the exact position as well as the size of the lesions, after the experiments mice were perfused and brains were removed, then sectioned and stained for GFAP (Glia Fibrillary Acidic Protein). GFAP is a common marker used to reveal brain lesions (Lai, 2014), by staining the astrocytes that are accumulating on the lesion site (Figure 63). Lesions were visible in all mice that received a PT-stroke. Unfortunately, the quality of sections in 3 out of 10 brains of RW_PT mice was poor due to brakes in the tissue during slicing, making calculations of exact size impossible. Therefore they were excluded from the calculations.

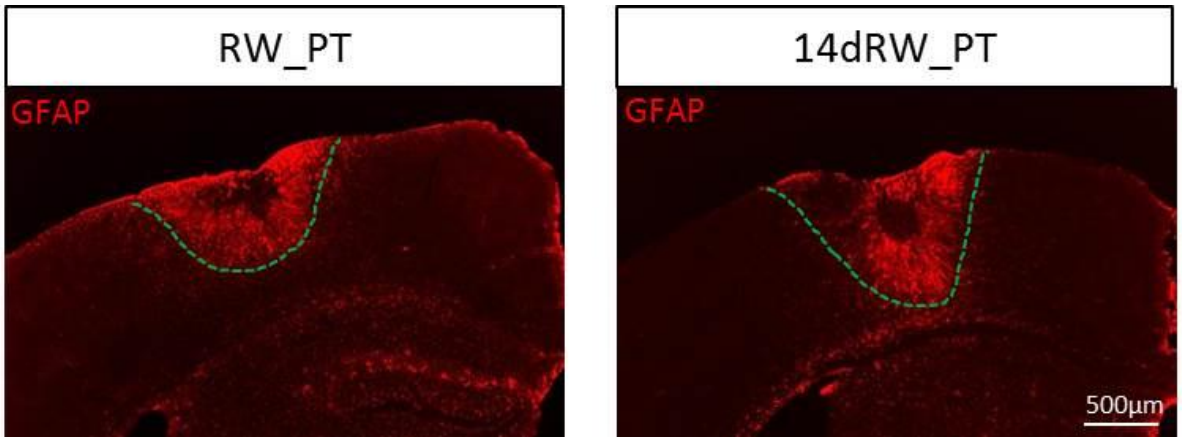


Figure 63: Examples of representative PT-lesion sites after GFAP labelling in RW and 14dRW mice. The boarders of lesions are marked with a green dashed line. On the left is a lesion site section of a RW_PT mouse and on the right a section of a 14dRW_PT mouse.

To characterize the position of the lesions, the distance from the center of each lesion to the midline and also to the anterior border of V1 was calculated. Then, an average position was calculated for each group. In RW_PT mice the lesion center was positioned 1.63±0.06 mm lateral to the midline and 0.98±0.19 mm anterior to the anterior border of V1 (Figure 64A). The location of the lesion was not different in 14dRW_PT mice: 1.82±0.07 mm lateral of the midline and 0.95±0.16 mm anterior of the anterior border of V1 ($p>0.05$, for each distance, t-test; Figure 64B).

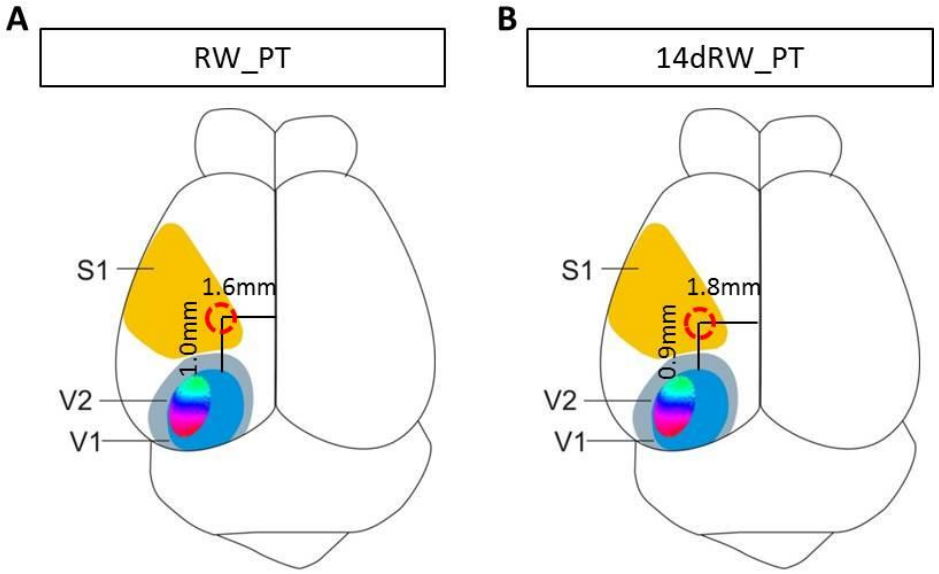


Figure 64: Schematic representation of the average position of the lesion center in lifelong running (RW) and 14dRW mice. Schemes show a top view of a mouse brain. The somatosensory cortex (S1) is colored in yellow, the secondary visual cortex (V2) is colored in grey, and the primary visual cortex (V1) in blue. A retinotopic map of the binocular zone in V1 is also displayed in the binocular part of V1. The positon of the lesion is marked with a red circle and the distance from midline and anterior border of V1 are displayed. In RW_PT mice (**A**) the center of the lesion was 1.63 mm lateral from the midline and 0.98 mm anterior to the beginning of V1, while in 14dRW_PT mice (**B**) the lesion was located 1.82 mm lateral from the midline and 0.95 mm anterior to the beginning of V1.

Furthermore the size of the lesions was determined by measuring the length (anterior-posterior), the diameter (medial-lateral), the depth and the total volume for every lesioned brain. The average lesion length in RW_PT mice was 1.38±0.24 mm (n=7 and in 14dRW mice was 1.24±0.14 mm (n=12). There was no significant difference in length between the two groups (p=0.59, t-test; Figure 65A). The average lesion diameter (Figure 65B) was 1.18±0.16 mm for the RW_PT mice and 0.79±0.07 mm for the 14dRW_PT mice. The diameter of the lesion in the 14dRW_PT mice was significantly smaller for the RW_PT mice (p=0.017, t-test). The average lesion depth (Figure 65C) of the RW_PT group was 0.47±0.11 mm and did not differ from the 14dRW_PT group (0.57±0.06 mm; p=0.478, t-test). Although the average lesion diameter was smaller for the RW_PT mice the total volume of the lesion was not significantly different between the groups (RW_PT/14dRW_PT: 2.75±0.93/2.04±0.41 mm^3; p=0.401, t=test; Figure 65D). The observation that there is no difference in the lesion size between the two groups, suggests that the time does not have an influence on the size lesion: the lesion is not getting smaller with the time.

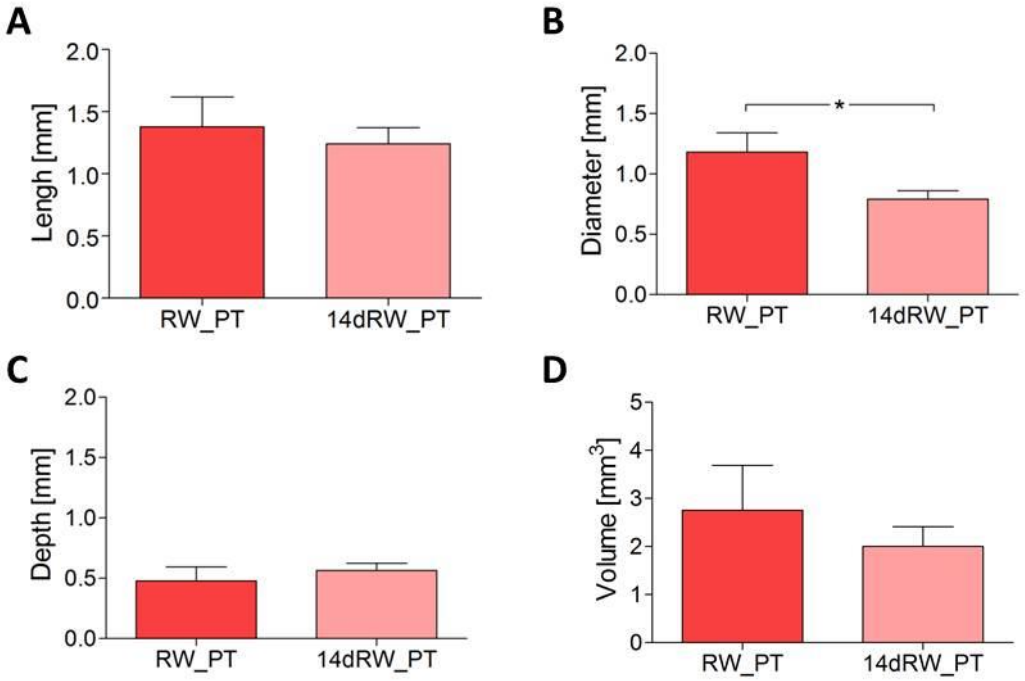


Figure 65: Measurements of lesions in RW_PT and 14dRW_PT mice. The graphs present on the y-axis the length (anterior-posterior) of the lesion in mm (**A**), the diameter (medial-lateral) of the lesion in mm (**B**), the depth of the lesion in mm (**C**) and the total volume of the lesion in mm^3 (**D**) for RW_PT (red) and 14dRW_PT (light red) groups. Only the lesion diameter was significantly different between the two groups but the total volume of the lesion was not significantly different between the groups.

3.5.5. Amount of running in 14dRW mice during the MD/noMD period

To test whether a cortical lesion in S1 will affect the ability of the mice to run in a RW but also if the amount of running has an effect on OD-plasticity after PT, the turns of the RW were measured daily in 14dRW after PT or sham treatment. For this study, mice were housed alone (1 mouse per cage) after PT/sham treatment in a RW-cage for 14 days. The amount of turns of the RW was measured daily and an average amount of turns was calculated after the experiment. The sham groups (noMD and MD; n=6) ran on average 6.69±1.22 km per day which corresponds to 16665±3047 turns of the wheel per day, while the PT groups (noMD and MD; n=12) ran on average 4.92±0.99 km per day which is equal to 12235±2470 turns of the wheel per day (Figure 66A). The running amount did not differ significantly between the sham and PT groups (p=0.29, t-test), suggesting that a cortical lesion in S1 is not interfering with the ability of mice to run on a RW. Furthermore, there were not significant differences on the running amount between the sham and PT mice that received an MD (sham/PT: 4.85±1.66/6.55±1.21 km, n=3/7; p=0.427, t-test; Figure 66B) suggesting that the running is not influencing OD-plasticity.

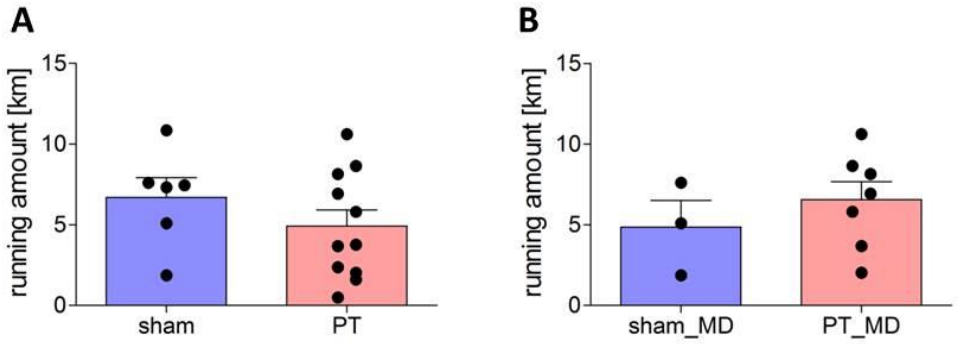


Figure 66: Amount of running in km per day for 14dRW mice. Every black circle represents a single mouse **A.** The running amount in km per day for every mouse was not different between sham (light blue) and PT mice (light red). **B**. No significant difference was detected comparing only the MD mice for sham (light blue) and PT (light red) treatment.

Moreover, in order to reveal possible correlation between the amount of running and the ODI value, the ODIs for every mouse were plotted against the kilometers per day that the mouse run for all the mice that received a PT and MD (Figure 67). Analysis of the data showed that there is no correlation between the running amount and the ODI values ($p=0.96$, $R^2=0.00051$, correlation test).

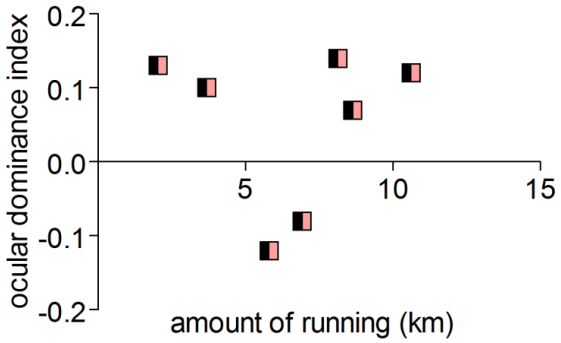


Figure 67: Correlation of amount of running and ODI values for 14dRW_PT group after MD. Every box represents a single mouse. No correlation of the amount of running and the ODI was detected.

3.6. Running but not fluoxetine treatment restored OD-plasticity in EE-mice transferred in SC

As we showed previously EE housing not only can extent the sensitive phase of OD-plasticity in mice V1 into late adulthood but also can restore OD-plasticity in adult mice transferred from SC to EE (Greifzu et al., 2014). The question that rises is how long OD-plasticity is lasting when mice are transferred to a less stimulating environment like a SC. To address this question, mice raised in EE from birth until PD130 which were then transferred to normal SCs. We observed that already after 1 week in SC mice did not show OD-plasticity any more, suggesting that plasticity is rapidly lost. There is a variety of molecules and mechanisms that

are known to be affected by EE-housing and involved in regulating OD-plasticity, as the brain-derived neurotrophic factor, perineuronal nets, insulin-like growth factor I, serotonin and histone acetylation (for review see Sale et al. (2014)). It has been shown that the selective serotonin reuptake inhibitor fluoxetine can restore OD-plasticity in adult rat's visual system as EE (Maya-Vetencourt et al., 2008). We hypothetized that treatment with fluoxetine after tranferring the mice to SCs could preserve OD-plasticity. I could not find any changes in ODI of mice treated with fluoxetine compared to non treated animals after MD suggesting that fluoxetine treatment alone could not preserve OD-pasticity. On the other hand, when mice were tranferred from EE to a SC which was equiped with a RW OD-plasticity was preserved. Together these observations suggest that running but not fluoxetine treatment can preserve OD-plasticity in mouse V1 after transferring them from EE to a SC.

3.6.1. OD-plasticity is lost in EE mice transferred to SCs already after 1 week

Here I address the question whether transferring mice that were born and raised in EE to SCs resulted in an OD-plasticity loss and if yes after how many days in SC OD-plasticity is lost. The mice were housed alone in SCs for either 1 day or 1 week before MD was performed for 7 days followed by optical imaging of intrinsic signals. During the MD period daily optometry was performed. The experimental design and details for the mice used for this study are described in the material and methods part (2.2.4).

3.6.1.1. Basic visual abilities and improvements of the optomotor reflex in EEtoSC mice after MD were comparable to EE or SC mice

All mice transferred from an EE to SC were measured in the optomotor setup to determine the visual acuity and contrast sensitivity threshold before and during the MD period. The baseline visual acuity threshold was 0.38±0.004 cyc/deg while the contrast sensitivity thresholds for the 6 spatial frequencies tested were: for 0.031 cyc/deg: 3.9±0.04, for 0.064 cyc/deg: 13.7±0.26, for 0.092 cyc/deg: 12.9±0.24, for 0.103 cyc/deg: 11.8±0.19, for 0.192cyc/deg: 8.4±0.34 and for 0.272 cyc/deg: 3.8±0.04 for all mice pooled. These values were similar with what was described before for SC and EE mice (Prusky et al., 2006; Greifzu et al., 2014).

After MD the visual acuity was increased with the days with the lowest spatial frequency elicited a response on day 7 at 0.44±0.002 cyc/deg ($p<0.001$ compared to day 0, Bonferroni-adjusted t-test). The increase was not significantly different from values of either SC or EE mice described before (Lehman and Löwel 2008, Greifzu et al., 2014; Figure 68).

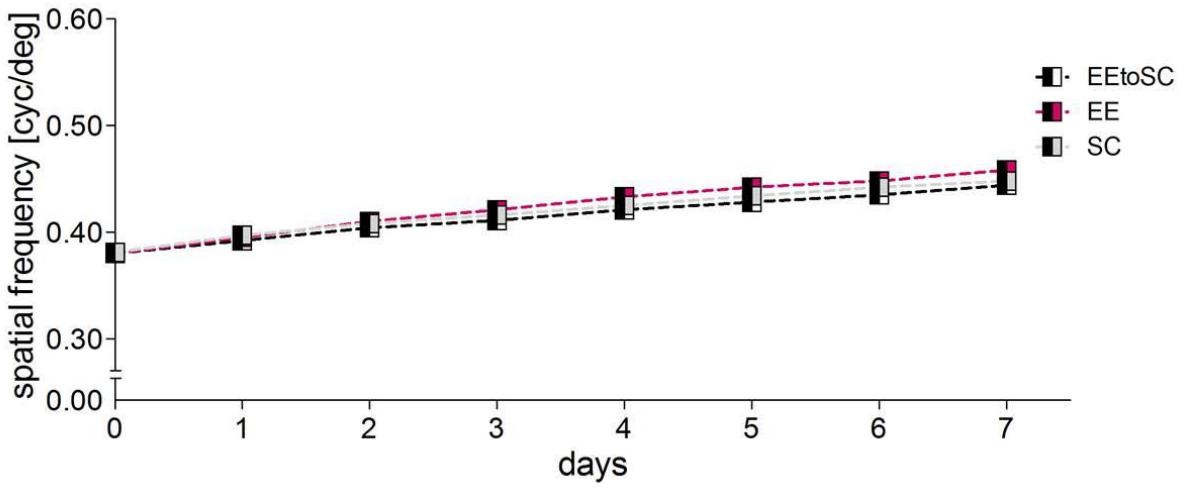


Figure 68: Comparison of improvements of visual acuity during the MD period between EE and SC mice. Visual acuity values in cyc/deg are plotted against days. Mice transferred from EE to SC (EEtoSC) illustrated with half-filled white boxes, EE-mice with half-filled purple boxes and SC mice with half-filled grey boxes. All groups showed similar improvement in visual acuity over MD days.

Likewise, contrast sensitivity thresholds were also increased after 7 days of MD. In detail on day 7 the values were: for 0.031 cyc/deg: 4.8±0.07, for 0.064 cyc/deg: 25.1±1.38, for 0.092 cyc/deg: 21.4±0.67, for 0.103 cyc/deg: 19.5±1.42, for 0.192 cyc/deg: 11.9±0.75 and for 0.272 cyc/deg: 4.5±0.11 ($p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p>0.05$, compared to baseline values on day 0, ANOVA). Again the increase was not significantly different from the previously observed increase in SC and EE mice after MD (Lehman and Löwel, 2008; Greifzu et al., 2014; Figure 69).

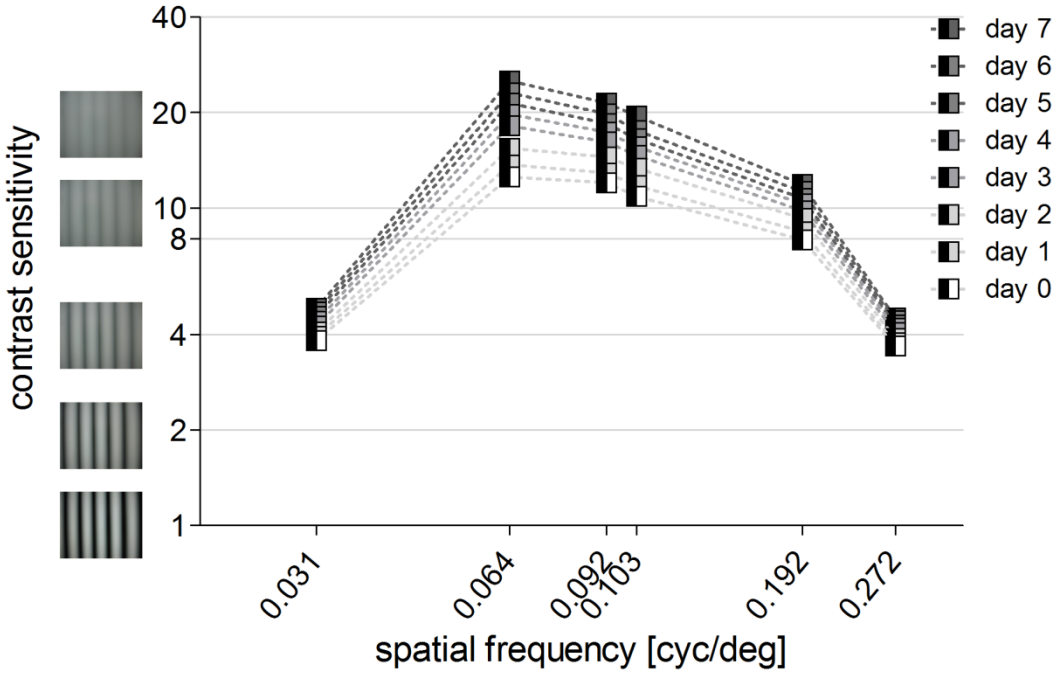


Figure 69: Contrast sensitivity improvements over 7 days of MD. Mice transferred from EE to SCs showed a significant improvement of contrast sensitivity over the MD days in all the tested spatial frequencies.

3.6.1.2. OD-plasticity was abolished in mice transferred to SC from an EE

After the mice have been transferred to SCs mice received an MD for 7 days either after 1 week in SC or after 1 day in SC to induce OD-plasticity. After the end of the MD period the mice were optically imaged to determine V1-activation and calculate ODIs using the optical imaging of intrinsic signals. All mice tested here showed a contralateral dominance in the binocular part of V1. The activity patches induced by the stimulation of the contralateral eye were darker than those after stimulation of the ipsilateral eye, the ODI values were positive and warm color dominated the 2-dimensioned OD-map (Figure 70).

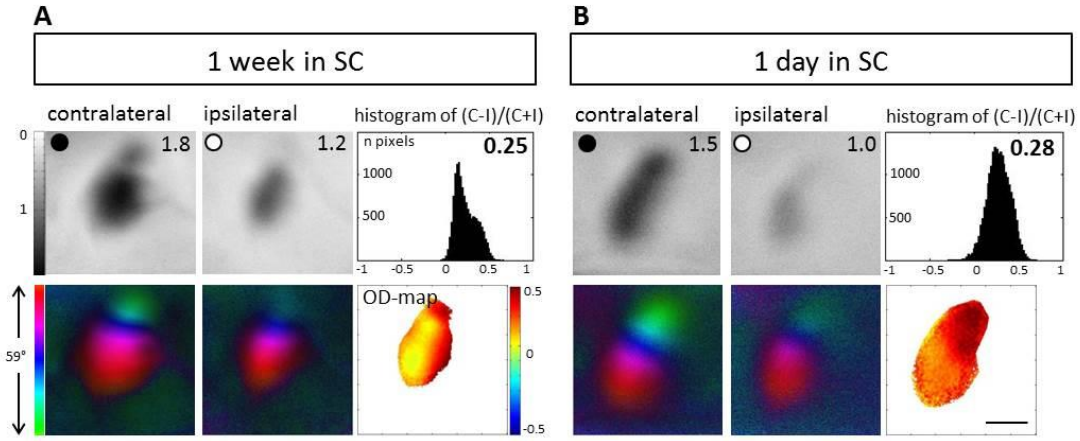


Figure 70: Examples of optically imaged maps from EEtoSC mice. Activity and polar maps of the binocular part of the left V1,ODI values, 2-dimensional OD-maps and ODI histograms for EEtoSC mice that received an MD 1 week (**A**) or 1 day (**B**) after the transfer from EE to SC. All mice showed a contralateral dominance: activity patches from the contralateral eye were darker than those from the ipsilateral eye, with positive ODI values and warm colors in the OD-map. Scale bar: 1 mm.

After quantitative analysis of the ODIs for both groups of EE-mice transferred to SC either 1 week before MD (EEtoSC_1week) or 1 day before MD (EEtoSC_1 day) we found that all mice did not display an OD-plasticity. Specifically, EEtoSC_1week mice had an average ODI of 0.23±0.01 (n=4, PD162-281), as well as EEtoSC_1day mice had an average ODI of 0.23±0.03 (n=4, PD164-278). The difference between the two groups was not significant ($p=0.759$, t-test). Comparing the ODIs of EEtoSC mice with age matched EE-mice with MD (Greifzu et al., 2014) there was a significant difference ($p<0.0001$, for both groups, t-test). In contrast no significant difference was found between EEtoSC mice and SC-mice with MD (Lehmann and Löwel, 2008) of the same age ($p=0.525$ and $p=0.426$ for 1 week and 1 day EEtoSC mice respectively, t-test; Figure 71A).

Further quantitative analyses of V1-activation showed a clear contralateral dominance in both EEtoSC groups. In EEtoSC_1week mice V1-activation after contralateral eye stimulation was 1.54±0.24 while after ipsilateral eye stimulation was 1.08±1.18. Similarly in EEtoSC_1day mice V1-activation after contralateral eye stimulation was 1.92±0.07 and after ipsilateral eye stimulation was 1.28±0.08 (Figure 71B). Summarizing our data suggest that already after one week in SC OD-plasticity which was preserved in EE-mice is lost.

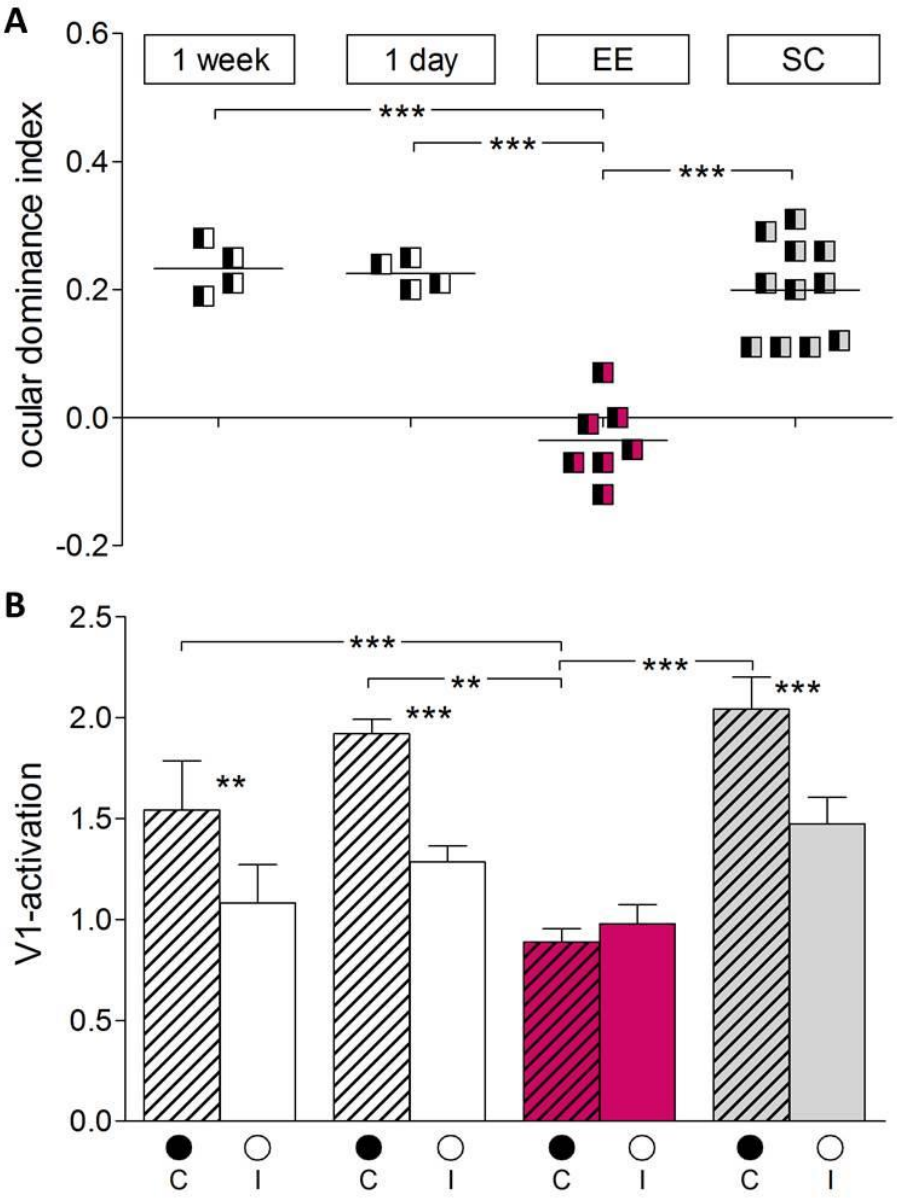


Figure 71: Quantification of ODIs and V1-activation of EEtoSC mice and comparison with EE and SC mice of the same age.in different age groups of EE-mice after MD. A. Optically imaged ODIs of EEtoSC mice (white), EE-mice (magenta; Greifzu et al., 2014) and SC-mice (grey; Lehman and Löwel, 2008). Symbols represent ODI-values of individuals; means are marked by horizontal lines. EEtoSC mice like SC-mice did not show an OD-shift and were significantly different from EE-mice of the same age. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye after MD (black filled circle indicates MD eye). SCtoEE mice showed a clear contralateral dominance.

3.6.2. Running but not fluoxetine treatment preserved OD-plasticity in mice transferred from EE to SCs

Transferring mice from a stimulating EE to more deprived environment (SC) resulted in loss of OD-plasticity already after 1 day of SC housing before MD. Among the factors that are descpribed to be changed in EE compared to SC is serotonin (for review see Sale et al. (2014)). Levels of serotonin were found elevated in EE-mice and administration of fluoxetine could mimic the effects of EE (Maya-Vetencourt et al., 2008), thus I attempted to restore OD-plasticity in EE-mice older than PD130 transferred to SCs by keeping the serotonin levels elevated. To this end, the antidepressant drug fluoxetine was used as a selective serotonin reuptake inhibitor. Administration of fluoxetine was done via drinking water for 3 weeks period and aftwerwards optical imaging was performed. Additionally, mice were transferred from EE to SCs that were equipped with a running wheel to test wheather running is sufficient to restore OD-plasticity in those mice. Detailed description of the treatment, the experimental design and the mice groups used for this study can be found in the material and method part 2.2.4.. In the following paragraph I will describe my findings that running but not fluoxetine treatment restored OD-plasticity in old EE-mice tranferred to SCs.

3.6.2.1. Basic visual abilities and improvements of the optomotor reflex after MD were not affected by fluoxetine treatment or running

Before and during the MD period all mice were tested in the optomotor setup (Prusky et al., 2004) daily to access visual acuity and contrast sensitivity thresholds of the optomotor reflex. Baseline visual acuity values measured on day 0 were not different between treated and non-treated animals. Specifically baseline visual acuity threshold for EEtoSC mice without fluoxetine treatment was 0.373±0.001 cyc/deg (n=7) and for EEtoSC mice that received fluoxetine treatment was 0.373±0.0002 cyc/deg, (n=9). Moreover the mice transferred from EE to SC with RW had a baseline visual acuity of 0.374±0.0005 cyc/deg (n=3). The differences among all the groups were not significant (p=0.884, ANOVA; Figure 72).

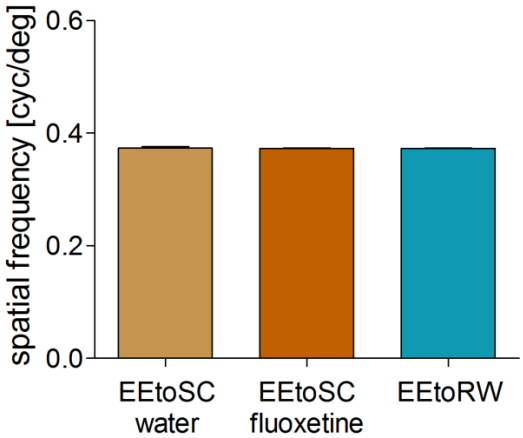


Figure 72: Baseline visual acuity for mice transferred from EE to SCs with water, fluoxetine or RW. The highest spatial frequency elicited a response in optomotor setup on day 0 was similar between EEtoSC_water (light brown), EEtoSC_fluoxetine (dark brown) and EEtoRW (blue) mice.

Together with visual acuity, contrast sensitivity thresholds of the optomotor reflex were determined for all groups on day 0. All mice had similar baseline contrast sensitivity values in every one of the 6 spatial frequencies tested irrespective of the fluoxetine treatment or RW housing ($p>0.05$ for every frequency; ANOVA; Table 13).

Table 13: Baseline contrast sensitivity values of the EEtoSC_water, EEtoSC_fluoxetine and EEtoRW mice. For the 6 different spatial frequencies the average contrast sensitivity for each group is listed as mean±SEM.

Spatial frequency (cyc/deg)	EEtoSC water (n=7)	EEtoSC fluoxetine (n=9)	EEtoRW (n=3)
0.031	3.7±0.07	3.7±0.002	3.7±0.002
0.064	13.2±0.14	13.3±0.07	13.3±0.19
0.092	12.6±0.23	12.8±0.06	12.5±0.20
0.103	12.1±0.09	12.4±0.02	12.0±0.11
0.192	7.5±0.01	7.4±0.02	7.4±0.04
0.272	3.7±0.005	3.7±0.01	3.7±0.005

Visual acuity thresholds were also measured during the noMD/MD period for treated and non-treated animals transferred from EE to SC. As expected all mice without MD did not improve in visual acuity thresholds over days (EEtoSC_water/EEtoSC_fluoxetine on day 7: 0.374±0.001/0.373±0.001 cyc/deg, n=4/4; $p>0.05$ for both groups compared to day 0, ANOVA; Figure 73B). Additionally, the visual acuity values on day 7 of treated and non-treated with fluoxetine mice without MD was not significant ($p>0.05$, Bonferroni adjusted t-test). In contrast there was a significant increase in visual acuity thresholds on day 7 for all the MD groups. Mice transferred from EE to SC with just water treatment and MD showed an increase of 21.8% which correspond to 0.455±0.002 cyc/deg on day 7 (n=3, $p=0.0012$, compared to day 0, ANOVA). Similarly, mice transferred from EE to SC with fluoxetine treatment and MD showed an increase of 21.5%, which is equal to 0.453±0.003 cyc/deg (n=3, $p=0.0016$, compared to day 0, ANOVA). The difference of visual acuity on day 7 between the MD groups (treated and non-treated was not significant ($p>0.05$, ANOVA; Figure 73). Moreover, EE-mice transferred to SC with RW increased significantly in visual acuity from 0.373±0.005 on day 0 to 0.452±0.001 on day 7 after MD (n=3, $p=0.0019$, ANOVA). The increase was equal to 21.2%, thus not different from the other two groups (EEtoSC_water/EEtoSC_fluoxetine: $p=0.973/0.856$, ANOVA). Spatial frequency thresholds of the optomotor reflex over days for each mouse are presented in figure 74.

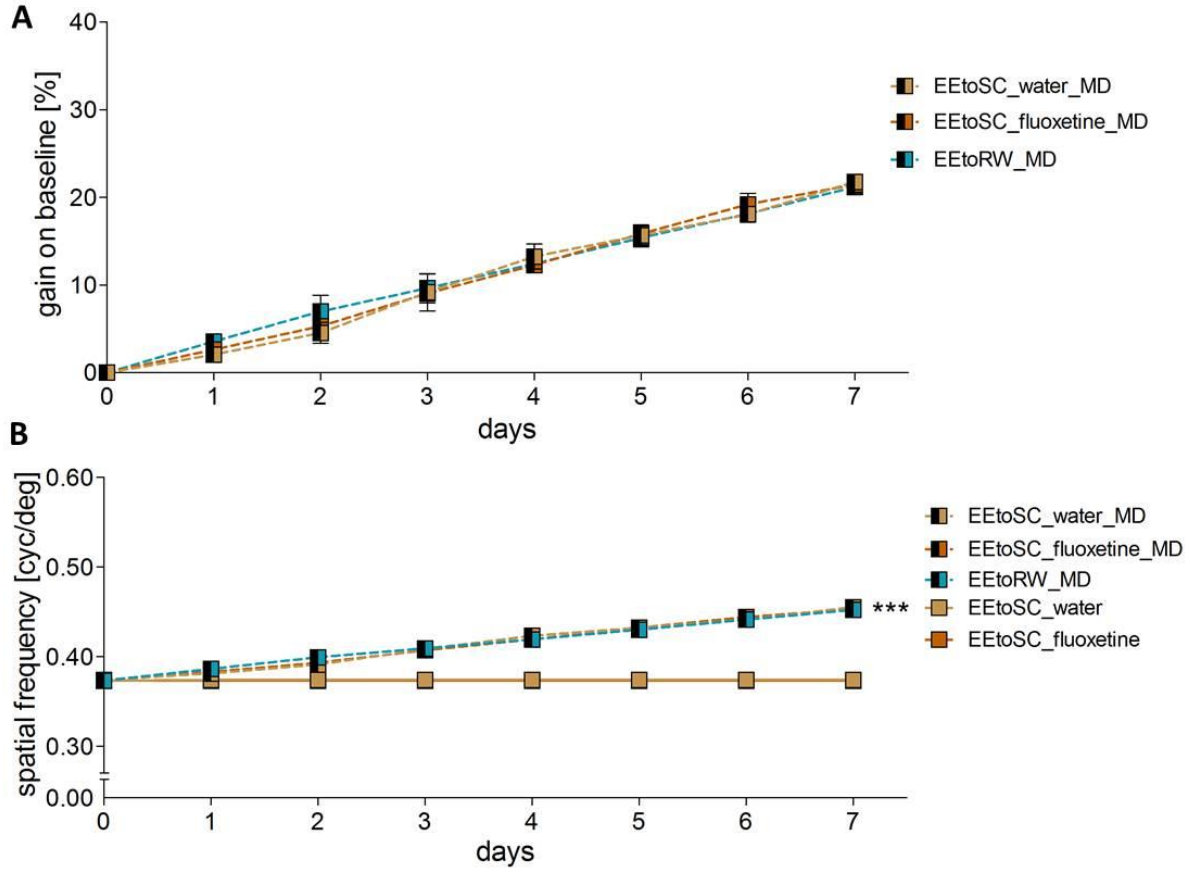


Figure 73: Improvement of visual acuity thresholds over days of MD in non-treated and treated with fluoxetine and EEtoRW mice. A. Gain on baseline is plotted as percent over days of MD for groups of EE-mice transferred to SC after MD. No significant difference observed between the groups over MD days. **B.** Visual acuity values in cyc/deg are plotted against days, for mice without (boxes) and with MD (half-filled boxes) for groups. After 7 days of MD, visual acuity improved significantly compared to mice without MD for the groups. MD mice did not show any differences over days.

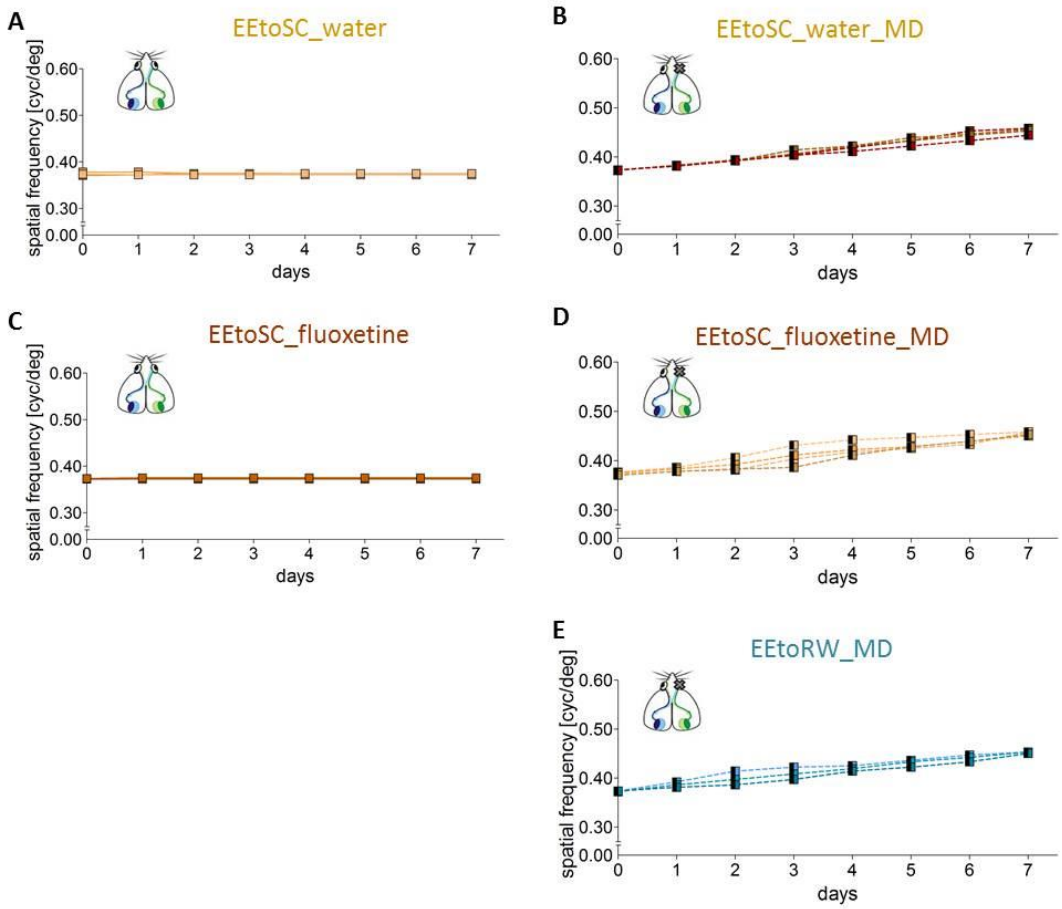


Figure 74: Spatial frequency thresholds measured in EE-mice transferred to SC after treatment or not with fluoxetine mice as well as mice transferred to RW cage over the 7 days of noMD/MD period. Every line represents a single animal. **A.** EEtoSC mice with water without MD had the same visual acuity thresholds over the days. **B.** After 7 days of MD, EEtoSC_water mice showed an increase of visual acuity thresholds. **C.** Fluoxetine treated mice transferred from EE to SC mice without MD did not show any change over days. **D.** In EEtoSC_fluoxetine mice with MD visual acuity thresholds of the open eye increased over days. **E.** Mice transferred from EE to SC cage with a RW after MD showed a significant improvement in visual acuity thresholds over the MD period.

Additionally, to the visual acuity thresholds, contrast sensitivity thresholds were determined over the 7 days of MD/noMD period at six different spatial frequencies for all groups. After 7 days of MD both EEtoSC_water and EEtoSC_fluoxetine mice increased significantly in contrast to mice without MD ($p<0.001$ for both groups, ANOVA; Table 14, Figure 75). Also EEtoRW mice showed a significant improvement on the 7th day compare to day 0 ($p<0.001$, ANOVA; Table 14; Figure 75). The differences among the MD groups were not significant ($p>0.05$, ANOVA; Figure 75). The contrast sensitivity values over the days for each condition are presented in figure 76. All MD mice showed similar improvements with previously described data from C57BL/6 mice (Prusky et al., 2006). All together visual capabilities of all mice were not affected by the treatment or the housing conditions.

Table 14: Contrast sensitivity values of the EEtoSC_water, EEtoSC_fluoxetine and EEtoRW mice on the 7th day after the MD/noMD period. For the 6 different spatial frequencies tested the average contrast sensitivity for each group is listed as mean±SEM.

Spatial frequency (cyc/deg)	EEtoSC water (n=4)	EEtoSC water_MD (n=3)	EEtoSC fluoxetine (n=4)	EEtoSC fluoxetine_MD (n=5)	EEtoRW MD (n=3)
0.031	3.7±0.008	5.2±0.13	3.7±0.004	5.0±0.06	35.0±0.13
0.064	13.2±0.13	34.8±0.88	13.2±0.07	48.4±4.12	52.6±1.88
0.092	12.6±0.19	32.2±0.66	12.8±0.04	42.8±3.47	45.6±1.77
0.103	12.0±0.04	26.9±0.60	12.2±0.11	38.2±2.56	41.8±1.18
0.192	7.4±0.03	14.0±0.52	7.5±0.03	14.6±0.77	14.5±0.35
0.272	3.7±0.01	5.0±0.13	3.7±0.003	4.9±0.11	4.9±0.05

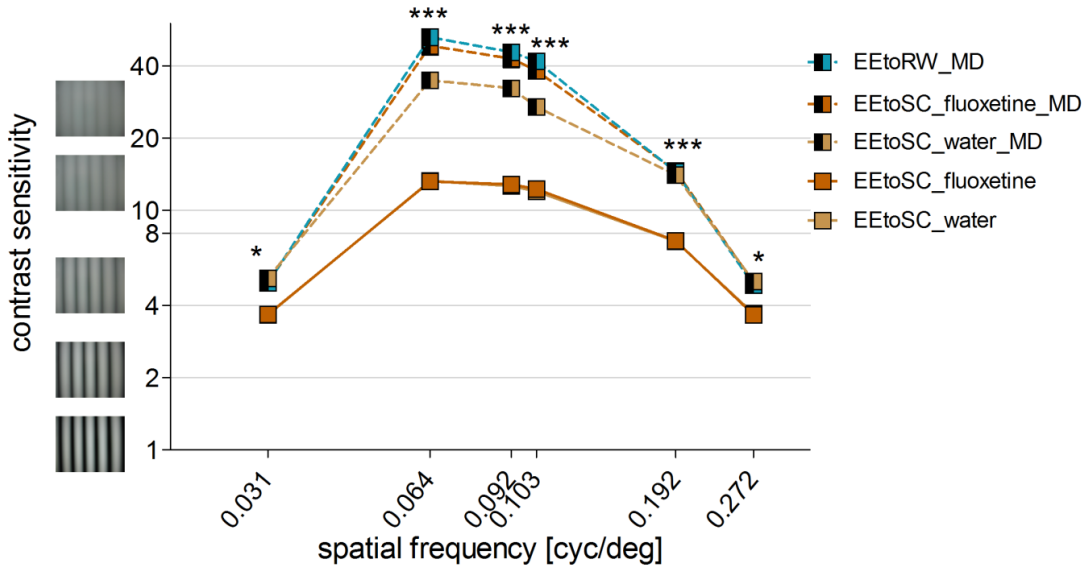


Figure 75: Contrast sensitivity improvements on the 7th day of EEtoSC_water, EEtoSC_fluoxetine and EEtoRW mice with or without MD. Mice with MD (half-filled boxes) of all groups improved significantly in all the tested frequencies compared to the noMD groups (filled boxes) on the same day. The improvement was similar in all of the MD groups.

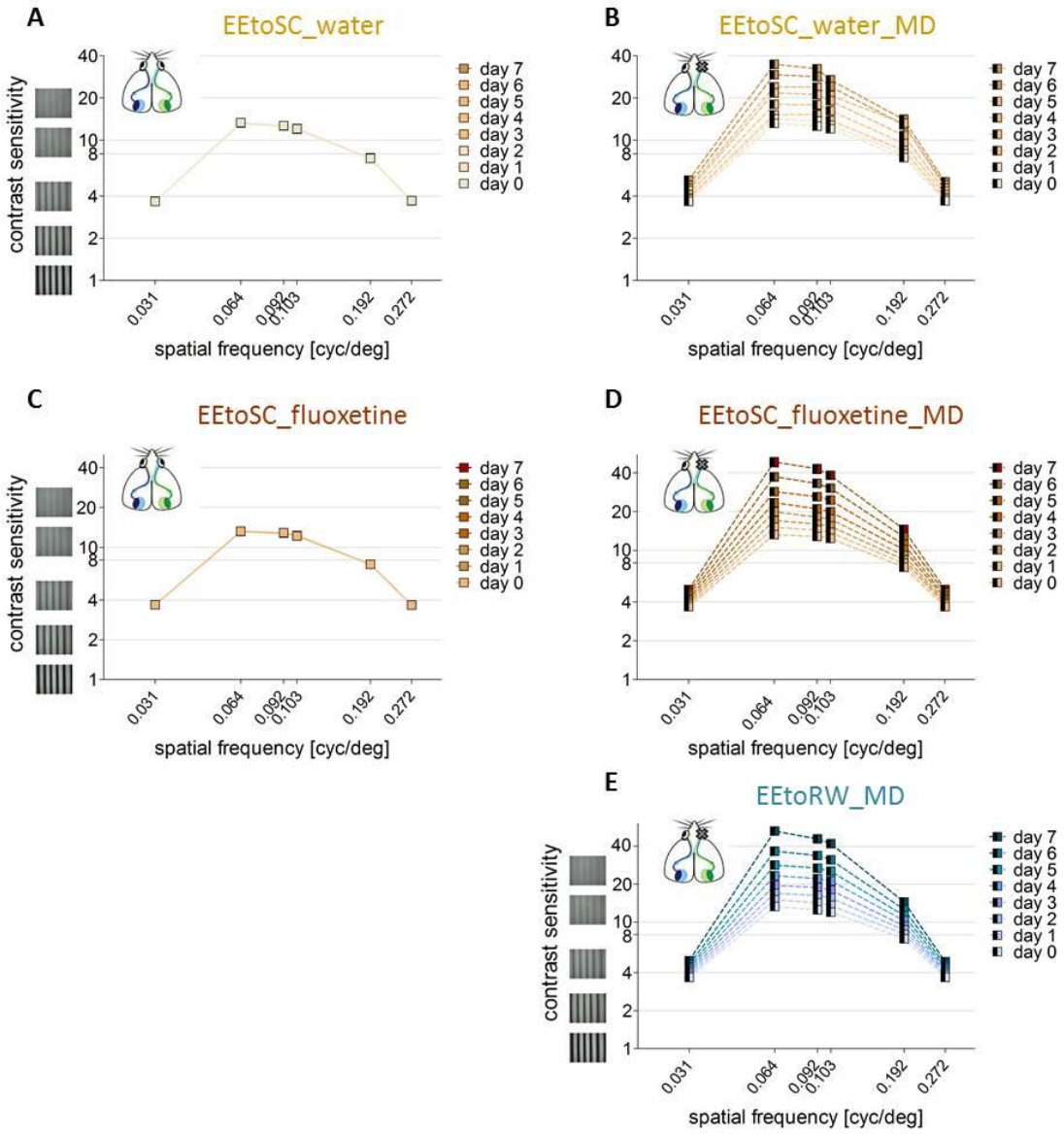


Figure 76: Mean contrast sensitivity thresholds over days of mice transferred from EE to SC with or without fluoxetine treatment and mice transferred to RW cage. Day 0 represents the baseline values before MD. **A.** In EEtoSC_water mice without MD contrast sensitivity remained stable over days. **B.** After 7 days of MD in the same group of mice the contrast sensitivity of the open eye increased significantly. **C**. EEtoSC mice with fluoxetine treatment without MD did not improve over days. **D**. EEtoSC_fluoxetine mice with MD showed a significant improvement over days in contrast sensitivity thresholds. **E.** EE-mice transferred to RW cage with MD showed a significant improvement over the MD period.

3.6.2.2. An OD-shift was observed in mice transferred to RW cage but not in fluoxetine-treated animals

To investigate whether fluoxetine treatment or running restored OD-plasticity in mice transferred from EE to SC, optical imaging was performed after three weeks of treatment in

mice moved to SC. V1-activation after stimulation of each eye and ODIs were calculated for every mouse. Treated and non-treated with fluoxetine mice without MD showed a contralateral dominance in the binocular part of V1. V1-activity patches after stimulation of the contralateral eye were always darker than those after ipsilateral eye stimulation, the calculated average ODIs were positive, and warm colors prevailed in the 2-dimensional OD-maps (Figure 77A and C). Likewise, MD in EE-mice transferred to SC without fluoxetine treatment did not change the ocular dominance: activity patches after contralateral (deprived) eye stimulation in binocular V1 were stronger than those after ipsilateral eye stimulation and the average ODI was positive with warm colors dominating the 2-dimensional OD-map (Figure 77B). Fluoxetine treatment also did not affect the OD in EEtoSC mice with MD. Again here, V1-activation after contralateral eye stimulation was stronger than after ipsilateral eye stimulation, ODI values were positive and the 2-dimensional OD-map was colored mainly with warm colors (Figure 77D). In contrast, MD in mice transferred from EE to a cage with a RW caused an OD-shift towards the open eye: V1-activation was equally strong after contralateral (deprived) and ipsilateral (open) eye stimulation, colder colors predominated in the OD-map and the ODI-histogram was shifted to the left (Figure 7 E).

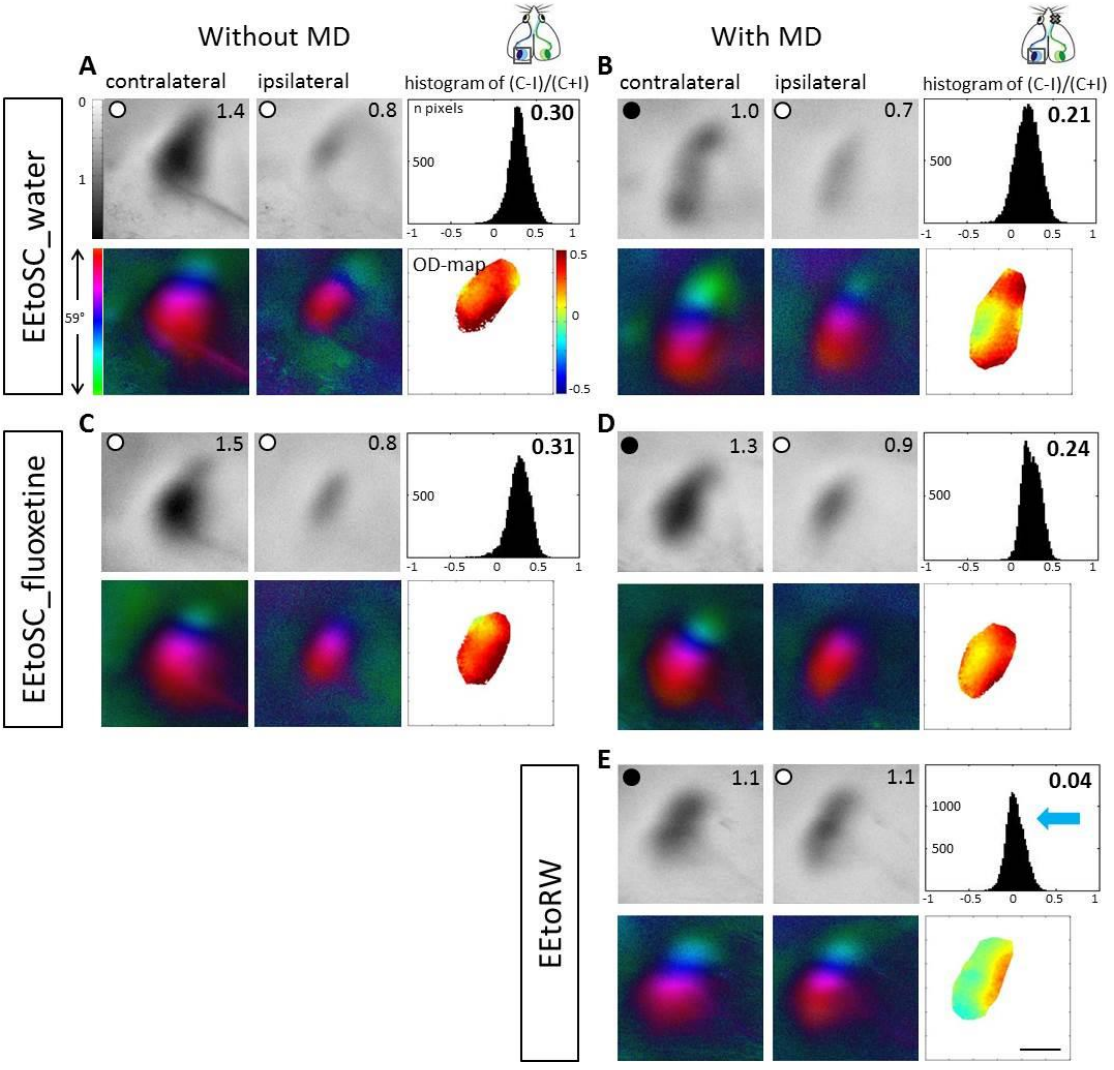


Figure 77: Examples of maps acquired from EE-mice transferred to SCs without or with MD after treatment with water, fluoxetine or RW. Optically recorded activity maps after stimulation of the contralateral and ipsilateral eye in the binocular region of mouse primary visual cortex (V1), of EEtoSC mice with water (**A** and **B**), with fluoxetine (**C** and **D**) or with RW (**E**), before (**A** and **C**) and after MD (**B**, **D** and **E**) are illustrated. For every example grayscale-coded response magnitude maps, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. In all groups transferred to SC with fluoxetine treatment or water only, MD did not change the contralateral dominance: 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 (**A**, **B**, **C** and **D**). In mice transferred from an EE to a SC with a RW, MD induced a strong OD-shift towards the open eye (**E**): the contra- and ipsilateral eye activated V1 about equally strong, colder colors appeared in the OD-map, and the histogram of OD-scores shifted to the left (blue arrow). Scale bar: 1 mm.

The calculated ODIs after analyses of V1-activation for all recorded maps did not reveal any OD-shift in treated and non-treated with fluoxetine EE-mice transferred to SC after MD. The average ODI of EEtoSC_water mice without MD was 0.22±0.05 (n=4, PD266-274) and remained unchanged after MD: 0.22±0.01 (n=3, PD266-269; p=0.956, Bonferroni-adjusted t-test). Additionally, EEtoSC mice treated with fluoxetine for 3 week displayed an average ODI of 0.24±0.04 without MD (n=4, PD260-284) and 0.26±0.01 after MD (n=3, PD278-283). There was not a significant difference in mice with and without MD (p=0.732, Bonferroni-adjusted t-test). The ODI values were also not significantly different between treated and non-treated with fluoxetine mice after MD (p=0.327, Bonferroni-adjusted t-test). However, the average ODI for EEtoRW mice with MD was closer to zero, thus significantly different to the other MD groups (0.21±0.01, n=3, PD211-298; $p<0.01$, for every group comparison, Bonferroni-adjusted t-test; Figure 78A).

V1-responses after each eye stimulation were further analyzed for all the mice transferred from EE to SC or RW cage. As MD did not change the OD in fluoxetine treated or non-treated mice transferred to SC the V1-activities were stronger after contralateral stimulation than after ipsilateral and remained unchanged after MD. In more detail, in EEtoSC_water group V1-activity after stimulating the contralateral eye was 1.57±0.08 whereas after MD was 0.95±0.08. Similarly, ipsilateral eye responses remained mainly unchanged without and with MD (noMD/MD: 1.03±0.12/0.72±0.04). Although the V1-activation in EEtoSC_water group with MD where reduced for both eyes compare to noMD group, the difference was not significant and V1 was still dominated by contralateral responses (MD/noMD comparison: contra-/ipsilateral: p=0.173/0.443, t-test; contra-/ipsilateral comparison for the MD group: p=0.026, t-test). After MD in EEtoSC_fluoxetine mice the V1-activation after contralateral eye stimulation was 1.48±0.18 and after ipsilateral eye stimulation was 0.84±0.14, while without MD had a V1-activation of 1.40±0.09 after stimulation of the contralateral eye and 0.95±0.14 after ipsilateral eye stimulation. Neither of these were significantly different (contralateral: p=0.707, t-test; ipsilateral: p=0.609, t-test). At last, mice transferred from EE to RW-cage after MD showed equally strong V1-activation after each eye stimulation: contralateral: 1.08±0.07, ispilateral: 1.17±0.66 (p=0.323, t-test; Figure 78B). Comparing the

V1-activation after contralateral eye stimulation of the EEtoRW mice with the contralateral activation of all the other groups, it was significantly lower ($p<0.05$, for all comparisons, t-test) suggesting that the OD-shift in those mice is mediated by reduction in deprive eye responses in V1. In conclusion, 3 weeks of fluoxetine treatment did not restore OD-plasticity in EE-mice transferred to SCs, while running could restore OD-plasticity in those mice.

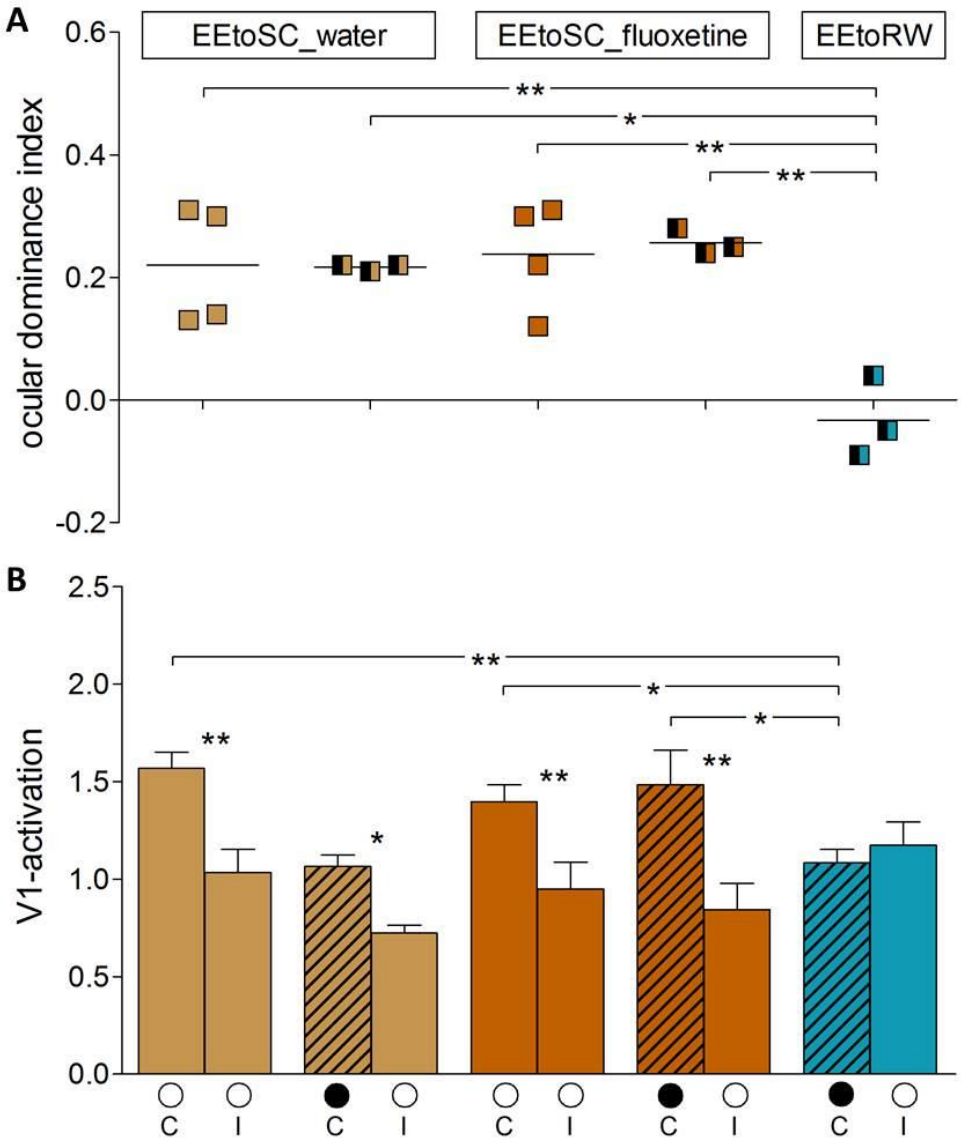


Figure 78: ODIs and V1-activation of EE-mice transferred to SCs. A. Optically imaged ODIs without and with MD of non-treated (light brown), treated with fluoxetine (brown) and mice transferred to a cage with a RW (blue). Symbols represent ODI-values of individuals; means are marked by horizontal lines. Only EEtoRW mice showed an OD-shift after MD. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye without and after MD (black filled circle indicates MD eye). In EEtoSC treated and non-treated mice there is a clear contralateral dominance, whereas in EEtoRW mice V1-activation of both eye is equally strong. The OD-shift in EEtoRW mice is mediated by reduced contralateral eye responses in V1.

3.6.2.3. Fluoxetine treatment did not affect the formation and the strength of V1 maps

In order to study whether fluoxetine has an impact on the signaling circuits of V1, V1-activation and map quality measurements were performed in EE-mice transferred to SC after water or fluoxetine treatment with optical imaging. Data were acquired, while stimulating the right eye with a full length moving bar in elevation (horizontal) or azimuth (vertical) direction. No differences were found concerning the signal strength or the quality of the retinotopic maps between non-treated or treated mice with fluoxetine (Figure 79). After elevation stimulation V1-responses of EEtoSC_water mice were on average 2.45±0.44 and not significantly different from V1-responses of EEtoSC_fluoxetine mice which was 2.55±0.25 (p=0.833, t-test). Likewise after azimuth stimulation the V1-activities evoked by right eye stimulation were similar between the non-treated and fluoxetine-treated mice (water/fluoxetine: 2.13±0.22/2.12±0.24; p=0.97, t-test). Additionally, map quality measurements were performed, as an indicator for the quality of the retinotopic maps. After elevation stimulation the map quality for the EEtoSC_water group was 1.88±0.70, and for the EEtoSC_fluoxetine group was 3.09±1.15. The differences on map quality among the two groups were not significant (p=0.403, t-test). Also no differences were detected after azimuth stimulation between the two tested groups with map quality of 2.85±0.91 for EEtoSC_water mice and 4.71±1.09 for EEtoSC_fluoxetine mice (p=0.232, t-test; Figure 80). Altogether these data suggest that fluoxetine treatment did not affect V1-activation or quality of the retinotopic maps.

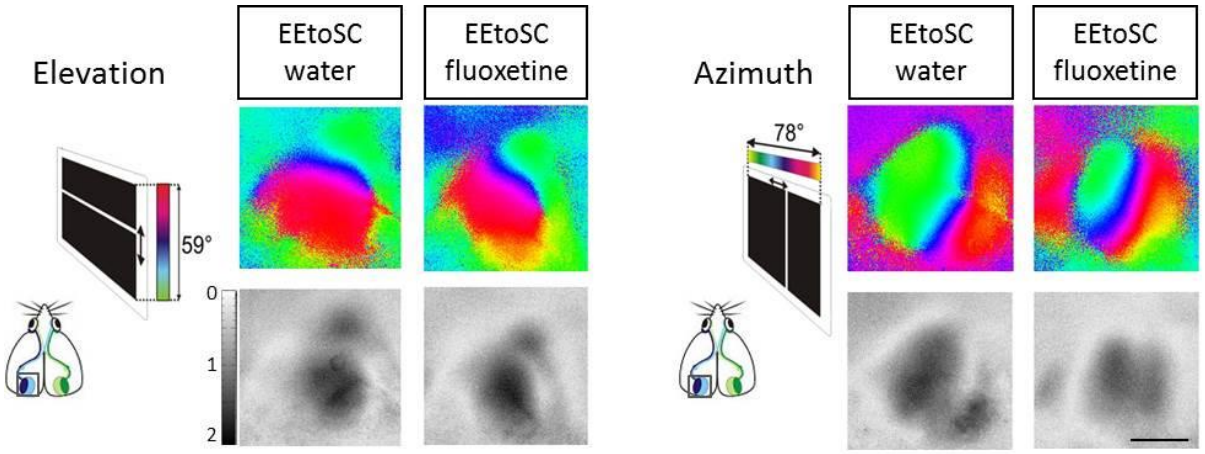


Figure 79: Examples of retinotopic and activity maps after elevation and azimuth stimulation recorded from EEtoSC mice without or with fluoxetine treatment. There were no differences in the quality or activation strength of the maps between the treated and non-treated mice neither for elevation nor for azimuth stimulation. Scale bar: 1 mm.

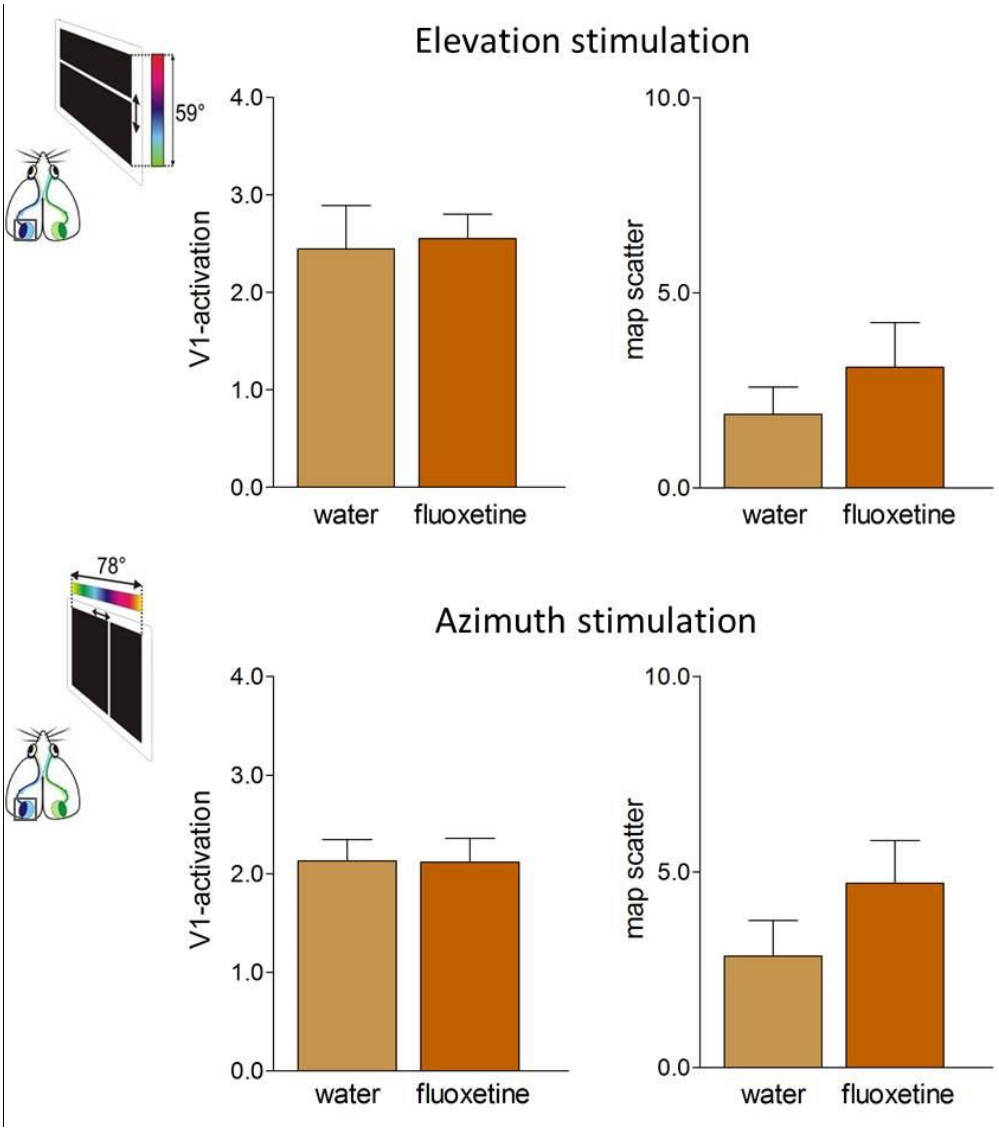


Figure 80: Fluoxetine treatment had no effect either on V1-activation or on the quality of the retinotopic maps. Comparison of V1-activation (left side) and map quality (right side) after right eye stimulation non-treated (light brown) and fluoxetine-treated (brown) mice after transfer from EE to SC, for both elevation (top) and azimuth (bottom) stimulation. For both groups the acquired maps were identical without significant differences in signal strength or quality of retinotopy for both stimuli.

3.6.2.4. Fluoxetine treatment did neither change the average water consumption nor the body weight of the mice

The administration of fluoxetine to the mice was done through drinking water. For this study dripping free bottles were used, the amount of the water consumption (with or without fluoxetine) was measured daily during the 3 weeks of treatment and an average daily consumption was calculated. As mice were housed in groups of 3 to 5 animals per cage the amount of water consumed per cage was divided by the number of the mice housed in every cage to calculate an average consumption per mouse per day. Mice drinking just water

consumed on average 4.01±0.18 ml per day and mice drinking water with fluoxetine drunk on average 3.64±0.25 ml per day. Although fluoxetine-treated mice drunk less, the difference was not significant from the water group (p=0.237, t-test; Figure 81A). For this study the desired concertation of fluoxetine per mouse (10 mg/kg) was calculated based on the average mouse weight and assuming that the mice drink on average 5 ml of water per day. We observed that our mice drunk less than 5 ml per day (3.64±0.25 ml in case of fluoxetine) which is translated to 7.28 mg/kg fluoxetine per day.

Together with the amount of drinking water the weight of each mouse was measured daily during the 3 weeks treatment. Mice that drunk only water, had an average weight of 19.81±0.50 g when transferred to SCs which increased to 23.7±0.21 g on the last day of treatment. Similarly, fluoxetine-treated mice had an average weight of 23.10±0.30 g when transferred to SC and reached 24.3±0.38 g on the last day of treatment. There was no significant change of weight observed in animals during the SC housing period (p=0.078, ANOVA) and both groups had comparable weight on the last day (p=0.146, t-test; Figure 81B). Furthermore, the weight of the mice was similar to the weight of comparable age mice described in the literature (23.6±2.3 g, JAX® Mice, Clinical & Research Services).

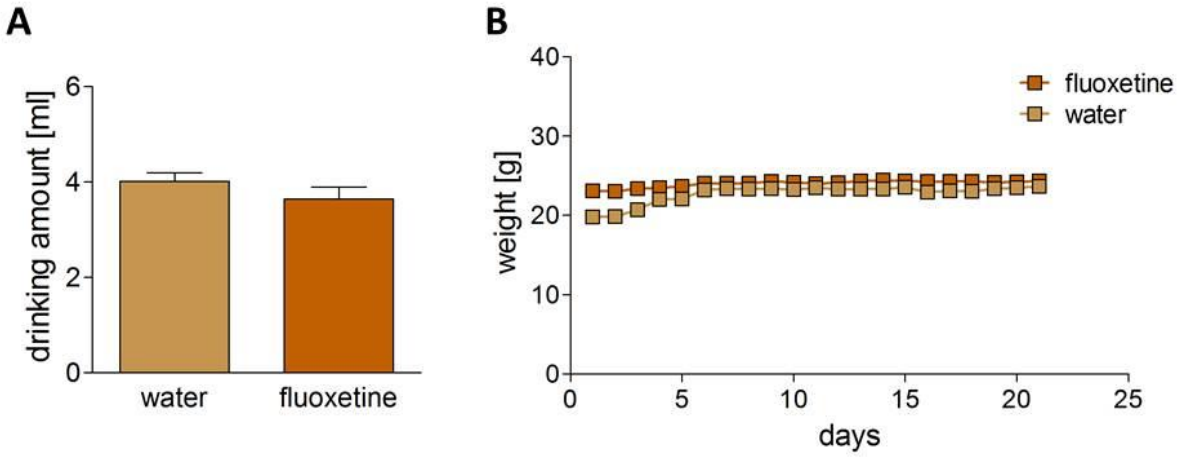


Figure 81: No differences in drinking amount and body weight between water and fluoxetine treated mice. A. Average drinking amount per day per mouse in ml for water (light brown) and fluoxetine (brown) treated animals. No significant differences in drinking amount observed. **B.** Average weight of water (light brown) and fluoxetine (brown) treated mice over the 21 days of treatment. The weight of the mice was comparable and no weight gain or loss was observed.

3.3.2.5. Social grouping did not affect OD-plasticity

Initially when mice transferred from EE to SC to investigate for how long OD-plasticity persist (part 3.6.1.) mice were housed alone in a SC. Afterwards, non-treated mice transferred from EE to SC were housed in group of 3 to 5 animals per cage (part 3.6.2.) By comparing these two groups we can reveal any possible effect of social grouping in OD-plasticity. As there were no significant differences between the EEtoSC_1week and EEtoSC_1day groups, I

pooled the data for this comparison. Mice that were transferred from EE to SC alone had an average ODI of 0.23±0.01 and were not significantly different from the mean ODI of EE-mice transferred in SCs in groups without treatment (EEtoSC_water group) which was 0.22±0.05 after MD (p=0.564, t-test; Figure 82A). Analysis of the V1-activation after contra or ipsilateral eye stimulation for both groups also did not show any difference. V1-activation after contralateral eye stimulation was 1.73±0.13 for the EEtoSC group and similar to the EEtoSC_water group: 1.56±0.08 (p=0.447, t-test). Likewise V1-activation after ipsilateral eye stimulation was not different between the groups (EEtoSC/EEtoSC_water: 1.18±1.10/1.03±0.12, p=0.393, t-test; Figure 82B). These data suggest that social grouping is not one of the major players regarding the presence of OD-plasticity.

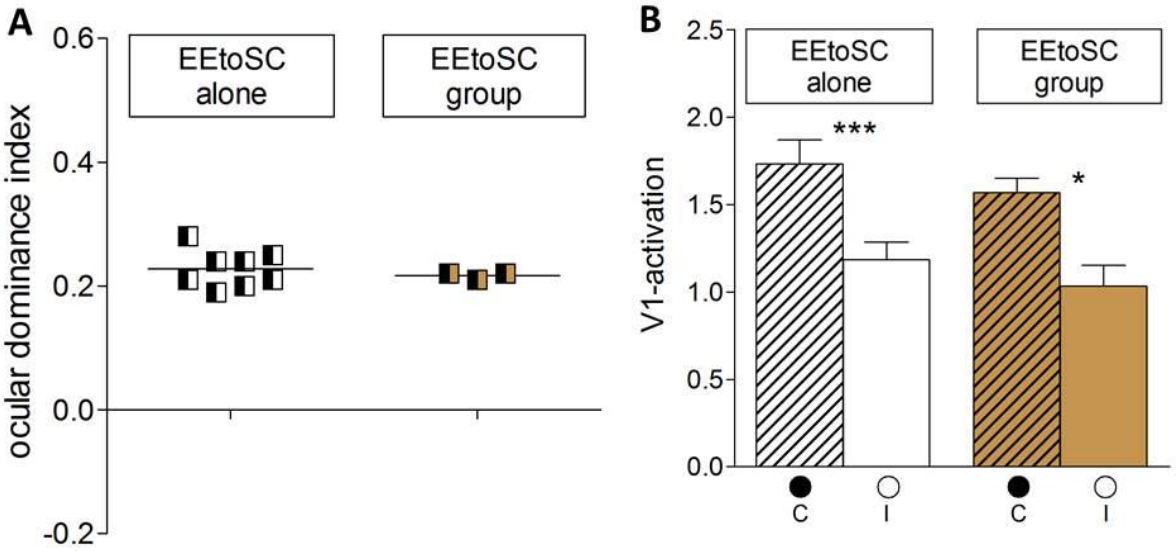


Figure 82: ODIs and V1-activation of EE-mice housed alone or in groups after being transferred to SCs A. Optically imaged ODIs of mice house alone (white) and mice house in groups (light brown). Symbols represent ODI-values of individuals; means are marked by horizontal lines. Social grouping had no effect on ODIs. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye. In both cases mice displayed a clear contralateral dominance.

3.7. Adult mice born in SC from EE parents still showed OD-plasticity

OD-plasticity in the mouse V1 declines with age in SC raised animals but can be preserved in mice raised in an EE (Greifzu et al., 2014). Motivated by a recent study showing that juvenile enrichment rescued a genetic defect in long-term potentiation also in the non-enriched offspring of the enriched mice (Arai et al., 2009), I was interested to investigate if the plasticity-promoting effect of EE could also be transferred from EE-parents to pups born and raised exclusively in SCs. To this point, pregnant EE-mothers were transferred to SCs a few days before delivery (6-10 days). Offspring were raised in SCs into adulthood (>PD120) and then OD-plasticity after MD was analyzed using intrinsic signal optical imaging. Additionally, I tested which parent is responsible for transmitting the positive effect of EE on

OD-plasticity to the next generation of non-enriched parents. For this purpose EE-females were mated with SC-males, or EE-males with SC-females. All the pups were born and raised in SCs at least until they were at least 120 days old before induction of MD and then imaging was performed. Details of the mice groups used for this study as well as the experimental design are described in Material and Method section (part 2.2.5.). Results from this investigation, which revealed that the positive effect of EE can be transferred to the next generation and most likely by the mothers are described below.

3.7.1. Basis visual abilities and improvements of the optomotor reflex are not changed in mice born in SCs from EE-parents, EE-fathers or EE-mothers

Initially using the virtual reality optomotor setup the baseline visual acuity and contrast sensitivity thresholds of the optomotor reflex were determined in adult mice (>PD120) born and raised in SCs from EE-parents, only EE-father and only EE-mother. The baseline visual acuity of EE-parents mice was 0.38±0.001 cyc/deg (n=11), of EE_father mice was 0.38±0.001 cyc/deg (n=11) and for EE-mother mice was 0.39±0.001 cyc/deg (n=9). There were no significant differences observed between the groups (p=0.392, ANOVA; Figure 83). Moreover, the values were similar to those previously published for EE (Greifzu et al., 2014) or SC C57BL/6J mice (Prusky et al., 2006).

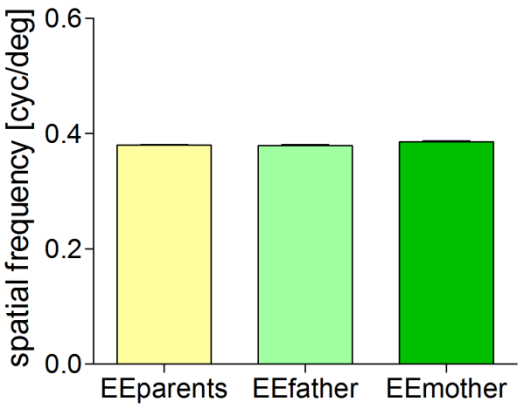


Figure 83: Baseline visual acuity of mice born in SC from EE-parents, EE-father or EE-mother. The highest spatial frequency that elicited a response in the optomotor setup on day 0 was similar between EE-parents (yellow), EE-father (light green) and EE-mother (green) mice.

The baseline contrast sensitivity thresholds of the optomotor reflex were also determined for the three mice groups of non-enriched offspring at six different frequencies (0.031, 0.064, 0.092, 0.103, 1.192 and 0.272 cyc/deg; Table 15). The baseline contrast sensitivity values measured were similar for all the groups (p>0.05 for every spatial frequency and comparison, ANOVA). Our data were also comparable to those described before for EE (Greifzu et al.,

2014). Taking together our observations so far there was no effect on baseline visual abilities of mice born in SCs from EE-parents, EE-father or EE-mother.

Table 15: Baseline contrast sensitivity values of mice born in SC from EE-parents, EE-father or EE-mother. For the 6 different spatial frequencies tested the average contrast sensitivity for each group is listed as mean±SEM.

Spatial frequency (cyc/deg)	EE-parents (n=11)	EE-father (n=11)	EE-mother (n=9)
0.031	3.7±0.06	3.7±0.04	3.6±0.03
0.064	12.3±0.31	13.0±0.43	14.3±0.29
0.092	11.6±0.28	12.1±0.30	13.7±0.27
0.103	11.0±0.20	11.4±0.26	13.1±0.22
0.192	7.7±0.14	7.5±0.10	7.7±0.07
0.272	3.7±0.05	3.6±0.04	3.6±0.03

Afterwards, MD was performed is some of the animals and the visual acuity and contrast sensitivity thresholds of the optomotor reflex were tested in the virtual reality optomotor setup daily for all mice groups (without and with MD). The highest spatial frequency elicited a response on the 7th day was 0.45±0.003 cyc/deg for the EE-parents mice after MD (n=7; Figure 85B), while for the EE-parents mice without MD was 0.39±0.001 cyc/deg (n=5; Figure 85A). The difference on day 7 between mice without and with MD was significant (p=0.0008, Bonferroni adjusted t-test; Figure 84). EE-father mice had a “visual acuity” of 0.46±0.002 cyc/deg on day 7 after MD (n=5; Figure 85D), which was significantly higher of the EE-father mice without MD (0.38±0.001 cyc/deg, n=6; Figure 85C; p=0.0007, Bonferroni adjusted t-test). Likewise, EE-mother mice with MD had a “visual acuity” of 0.44±0.003 cyc/deg on day 7 (n=5; Figure 85E), while EE-mother mice without MD had a value of 0.39±0.002 cyc/deg on the 7th day (n=5; Figure 85E). The “visual acuity” values between EE-mother without and with MD on day 7 were significant different (p=0.0084, Bonferroni adjusted t-test). All the MD groups increased significantly in visual acuity thresholds from day 0 to day 7 ($p<0.001$ for EE-parents and EE-father, $p<0.01$ for EE-mothers, ANOVA), but no significant difference was observed among the MD groups on day 7 ($p>0.05$, ANOVA). On the other hand all mice without MD did not show any improvement in visual acuity thresholds over the days ($p>0.05$, ANOVA; Figure 84; Figure 85).

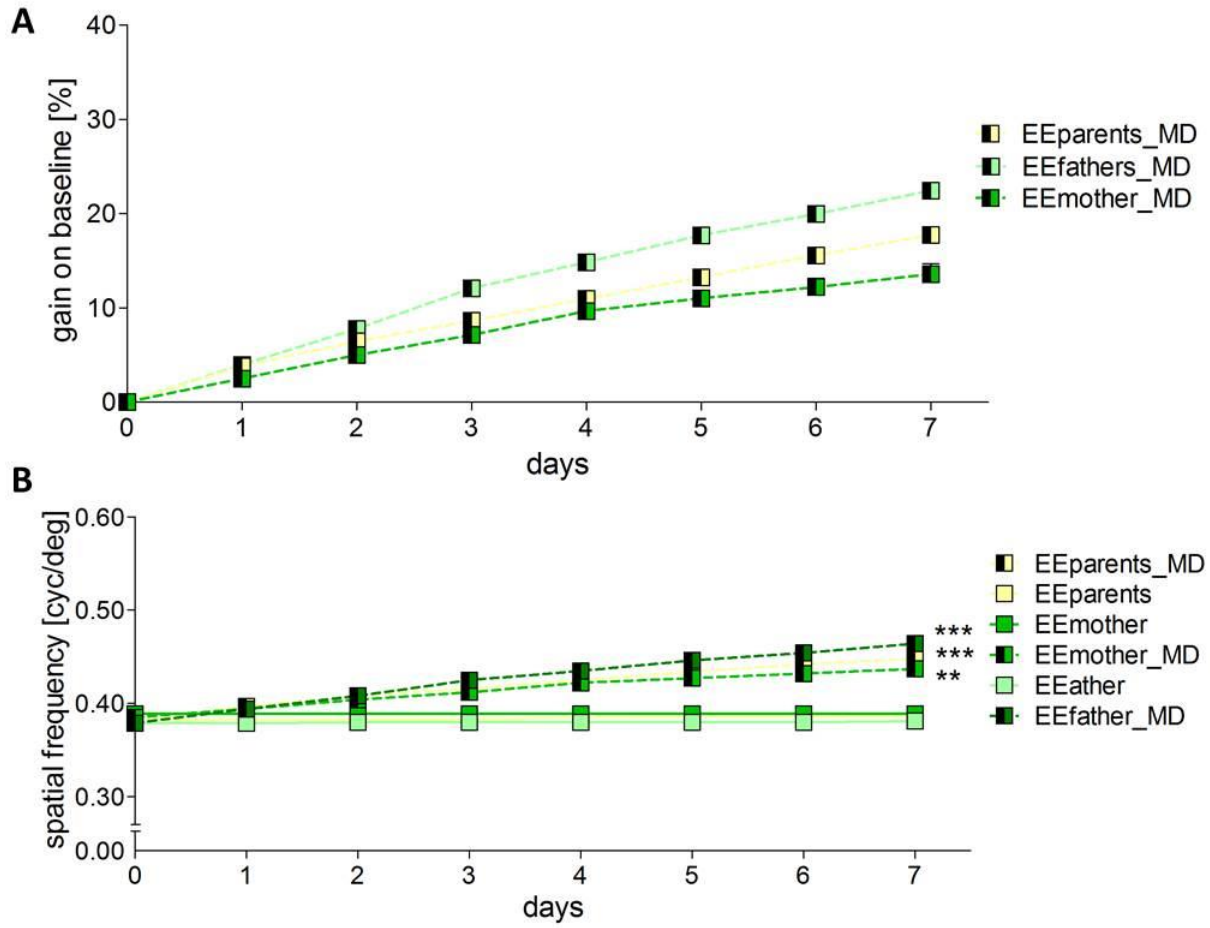


Figure 84: Improvement of visual acuity thresholds over days of MD in mice born in SCs from EE-parents, EE-father and EE-mothers. A. Gain on baseline is plotted as percent over days of MD for groups of EE-parents, EE-fathers and EE-mothers mice. No significant difference observed between the groups over MD days. **B.** Visual acuity values in cyc/deg are plotted against days, for mice without (boxes) and with MD (half-filled boxes) for all groups. After 7 days of MD, visual acuity improved significantly compared to mice without MD for all the groups. Mice without MD did not show any differences over days.

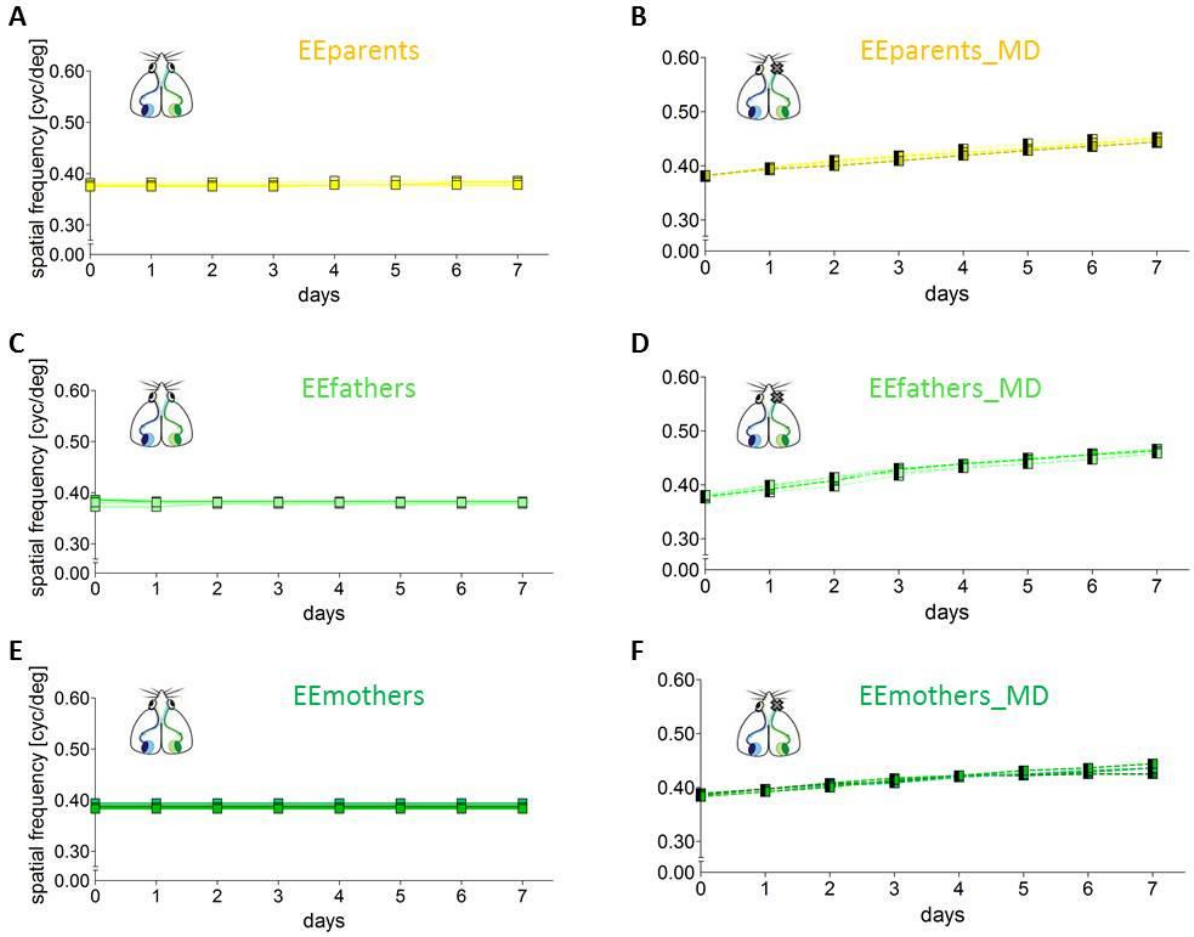


Figure 85: Spatial frequency thresholds over days measured in mice born in SCs from EE-parents, EE-fathers or EE-mothers. Every line represents a single animal. **A.** Offspring of EE-parents born in SCs without MD had same visual acuity thresholds over the days. **B.** After 7 days of MD, non-EE mice of EE parents showed an increase of visual acuity thresholds. **C.** Mice born in SCs from EE fathers without MD did not show any change over days. **D.** In EE-fathers group after MD visual acuity thresholds of the open eye increased over days. **E.** Non-enriched offspring of EE-mothers without MD did not show any change over the days. **F.** Mice born in SCs from EE-mothers showed a significant improvement in visual acuity thresholds over the MD period.

The contrast sensitivity thresholds of the optomotor reflex of the open eye were also increased significantly in offspring of EE-parents after MD compared to day 0 ($p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p>0.01$ for the six spatial frequencies tested, compared to values from day 0, ANOVA). Similar increase was observed in mice from EE-father after MD ($p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p>0.05$, compared to day 0, ANOVA; Table 16) and in mice from EE-mother ($p>0.05$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$, $p>0.05$, compared to day 0, ANOVA; Table 16). The values on day 7 were not different between the three groups ($p>0.05$ for every frequency and group comparison, ANOVA; Figure 86; Figure 87). In mice without MD from all groups contrast sensitivity thresholds remained unchanged over the days ($p>0.05$ for every frequency in each group, ANOVA).

Table 16: Contrast sensitivity values of mice born in SC from EE-parents, EE-father or EE-mother on the 7th day of MD/noMD period. For the 6 different spatial frequencies tested the average contrast sensitivity for each group is listed as mean±SEM.

Spatial frequency (cyc/deg)	EEparents (n=4)	EEparents MD (n=7)	EEfathers (n=6)	EEfathers MD (n=5)	EEmothers (n=5)	EEmothers MD (n=4)
0.031	3.8±0.35	4.8±0.18	3.7±0.08	4.9±0.12	3.6±0.04	4.3±0.06
0.064	12.9±3.26	24.9±1.12	13.3±0.21	25.9±0.51	14.1±0.37	22.6±0.27
0.092	11.9±2.64	22.5±0.89	12.3±0.20	22.0±0.44	13.7±0.38	21.5±0.34
0.103	11.4±2.44	20.8±0.69	11.4±0.09	20.2±0.31	13.0±0.31	20.5±0.38
0.192	8.2±1.31	12.4±0.26	7.7±0.12	11.7±0.28	7.7±0.15	11.4±0.25
0.272	3.8±0.27	4.7±0.11	3.7±0.11	4.7±0.09	3.6±0.03	4.3±0.03

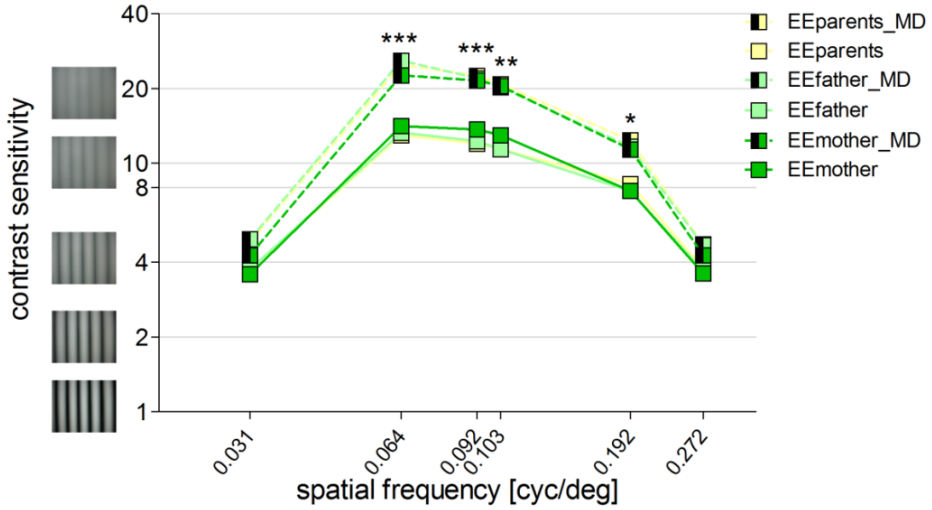


Figure 86: Contrast sensitivity improvements after 7 days of MD in mice born in SCs from EE-parents, EE-fathers and EE-mothers. Mice with MD (half-filled boxes) of all groups improved significantly in four out of six tested frequencies compared to the noMD groups (filled boxes) on the same day. The improvement was similar in all of the MD groups.

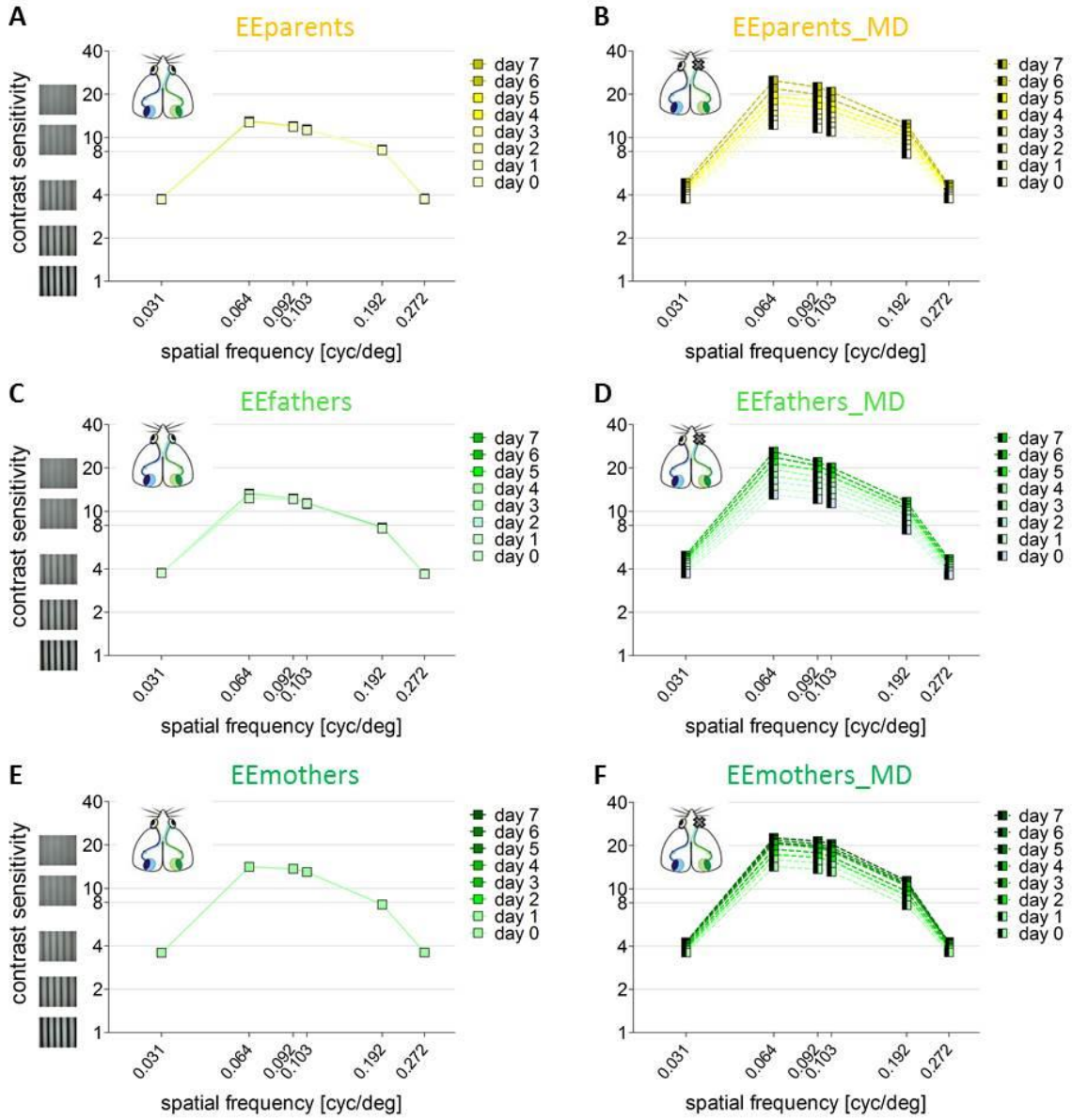


Figure 87: Mean contrast sensitivity thresholds over days in mice born in SCs from EE-parents, EE-fathers or EE-mothers. Day 0 represents the baseline values before MD. **A.** In mice born in SCs from EE-parents without MD contrast sensitivity remained stable over days. **B.** After 7 days of MD in the same group of mice (EEparents) the contrast sensitivity of the open eye increased significantly. **C**. Mice born in SCs from EE-fathers without MD did not improve over days. **D**. The EEfathers group after MD showed a significant improvement over days in contrast sensitivity thresholds. **E.** Offspring of EE-mothers born in SCs without MD did not show any change over the days. **F.** Same group of mice (EEmothers) after MD showed a significant improvement over the MD period.

3.7.2. OD-shift observed in mice born in SC from EE-parents and EE-mothers but not in mice from EE-fathers.

Plasticity changes were investigated in mice born in SCs from EE-parents, EE-father, or EE-mother using the method of optical imaging of intrinsic signal. Mice without MD for all groups showed a contralateral dominance: V1-activation in the binocular zone of V1 were always stronger than those after ipsilateral eye stimulation, the calculated average ODIs were positive, and warm colors prevailed in the 2-dimensional OD-maps (Figure 88A, C and E). Mice from EE-parents with MD showed an OD-shift: activity patches after contralateral (deprived) eye stimulation in binocular V1 were equally strong to the activity patches after ipsilateral eye stimulation in V1, the average ODI had values closer to zero, colder colors dominated the 2-dimensional OD-map and the histogram was shifted to the left (Figure 88B). In contrast, non-EE mice from EE-father and SC-mother after MD did not show any change after MD. Here, V1 was still dominated by contralateral eye activation whereas ipsilateral eye activation in V1 was significantly lower, the calculated ODIs were positive and warm colors dominated the 2-dimensional OD-map (Figure 88D). In case of non-EE mice born from EE-mother and SC-fathers after MD each eye stimulation was activating V1 equally strong, the ODI values were closer to zero, the OD-map predominated by colder colors and the OD-histogram shifted to the left, suggesting the presence of OD-plasticity (Figure 88F).

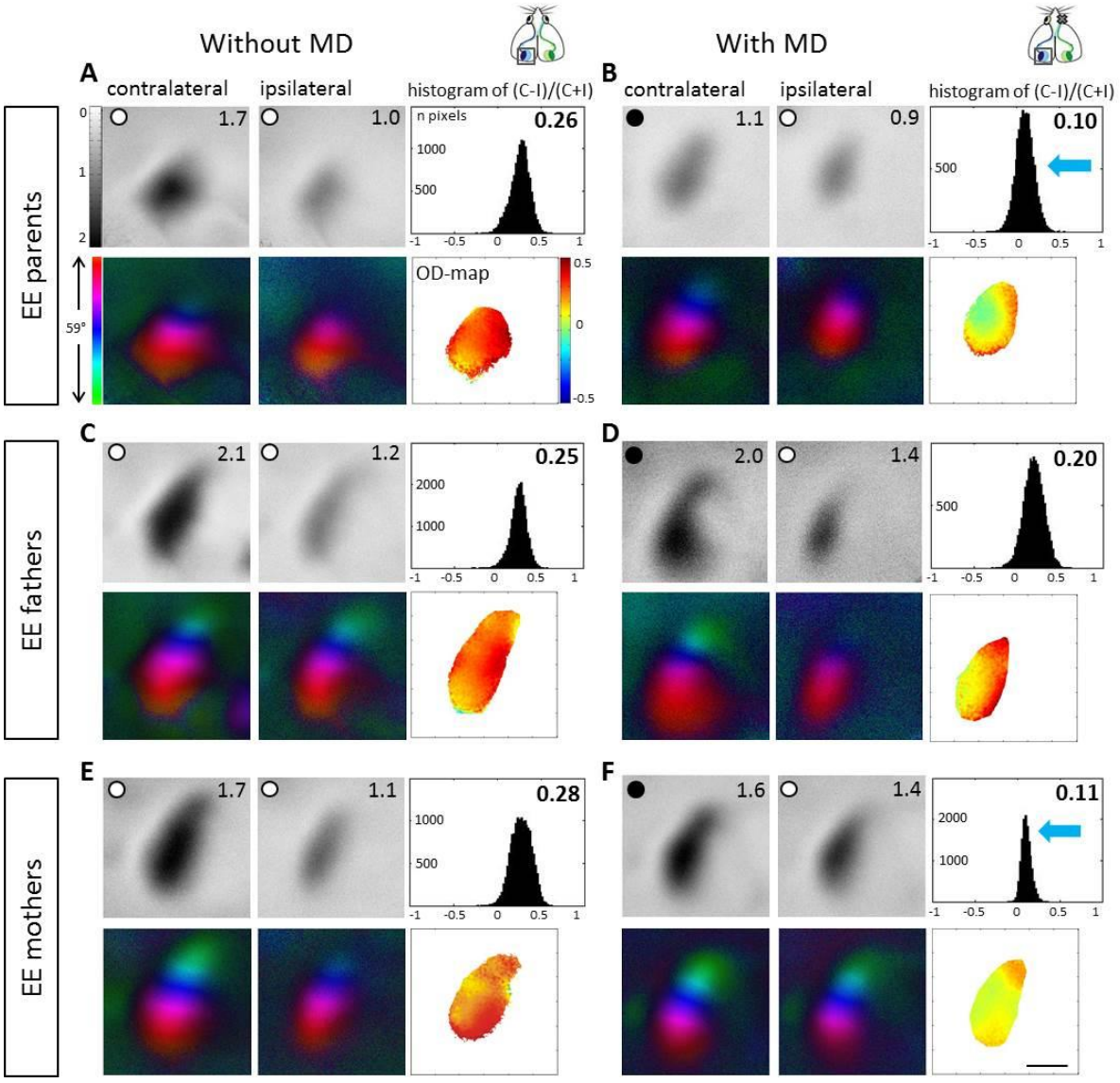


Figure 88: Examples of optical imaging recorded maps from mice born in SC from EE-parents, EE-father and EE-mothers with or without MD. Activity and polar maps of the binocular part of V1 and ODI values of the left hemisphere measured with optical imaging of intrinsic signals in mice born in SC from EE-parents (**A, B**), EE-fathers (**C, D**) and EE-mothers (**E, F**). Mice without MD (**A, C and E**) showed a contralateral dominance with stronger activities after stimulation of the contralateral eye, positive ODI values and warm colors in the 2-dimesional OD-map. After 7 days of MD mice born in SC from EE-parents or EE-mothers (**B, F**) an OD-shift was observed, both eyes activated V1 equally strong, lower ODI values, colder color in OD-maps and a shift of the histogram to the left (blue arrows) was observed, while in mice born from EE-fathers the V1 remained dominated by the contralateral eye (**C**) like in mice without MD. Scale bar: 1 mm.

Further quantitative analysis of the ODIs for every group revealed that non-EE offspring of EE-parents displayed an OD-shift after MD implying that the positive effect of EE on OD-plasticity can be transferred to the next generation. Non-EE mice from EE-fathers did not show an OD-shift after MD whereas non-EE mice from EE-mother did, suggesting that the

responsible parent is the mother for transmitting the OD-plasticity to the next generation. Specifically, non-EE mice from EE-parents had an ODI of 0.26±0.02 without MD (n=4, PD131-205) which decreased significantly to 0.13±0.01 after 7 days of MD (n=7, PD130-261; p=0.0002, t-test). The non-EE offspring of EE-fathers displayed an ODI of 0.22±0.03 without MD (n=6, PD127-188) which was not significantly changed after MD (0.16±0.02; n=5, PD132-194; p=0.177, t-test). Non-EE offspring of EE-mothers showed an OD-shift after MD: ODI reduced from 0.25±0.01 in mice without MD (n=5, PD142-171) to 0.12±0.03 in mice with MD (n=4, PD142-177; p=0.0024, t-test; Figure 89A). Differences between mice of all groups without MD were not significant (p>0.05, ANOVA). Additionally, the difference in ODIs between mice from EE-parents and EE-mother after MD was also not significant (p>0.05, t-test).

V1-responses, after stimulation of each eye, were further quantified to investigate changes after the 7 days of MD. For non-enriched offspring of EE-parents V1 activation after contralateral eye stimulation was 2.02±0.13 in mice without MD, whereas after MD degreased significantly to 1.39±0.11 (p=0.0068, t-test). In contrast, open (ipsilateral) eye responses remained unchanged between MD and no MD mice of the same group (noMD/MD: 1.20±0.09/1.21±0.10, p=0.897, t-test). In mice born in SC from EE-fathers and SC-mother the activation of V1 after contralateral eye stimulation did not change significantly in mice with or without MD (with/without MD: 1.36±0.28/1.67±0.17; p=0.358, t-test). Similarly V1-activation after ipsilateral eye stimulation remained unchanged in the same group of mice with or without MD (with/without MD: 1.04±0.18/1.06±0.13; p=0.924, t-test). At last SC offspring of EE-mothers showed a V1-activation after contralateral eye stimulation of 1.50±0.09 without MD and 1.69±0.16 with MD. The difference on V1-activation between MD and noMD mice was not significantly different (p=0.307, t-test). The V1-activation after ipsilateral eye stimulation of mice born in SCs from EE-mothers was 1.00±0.10 without MD and increased to 1.34±0.12 after MD but the increase was not significant (p=0.054, t-test; Figure 89B). Together our data suggest that the positive effect of EE on OD-plasticity of adult mice can be transmitted to the next generation of non-enriched mice, and the responsible parent is the mother.

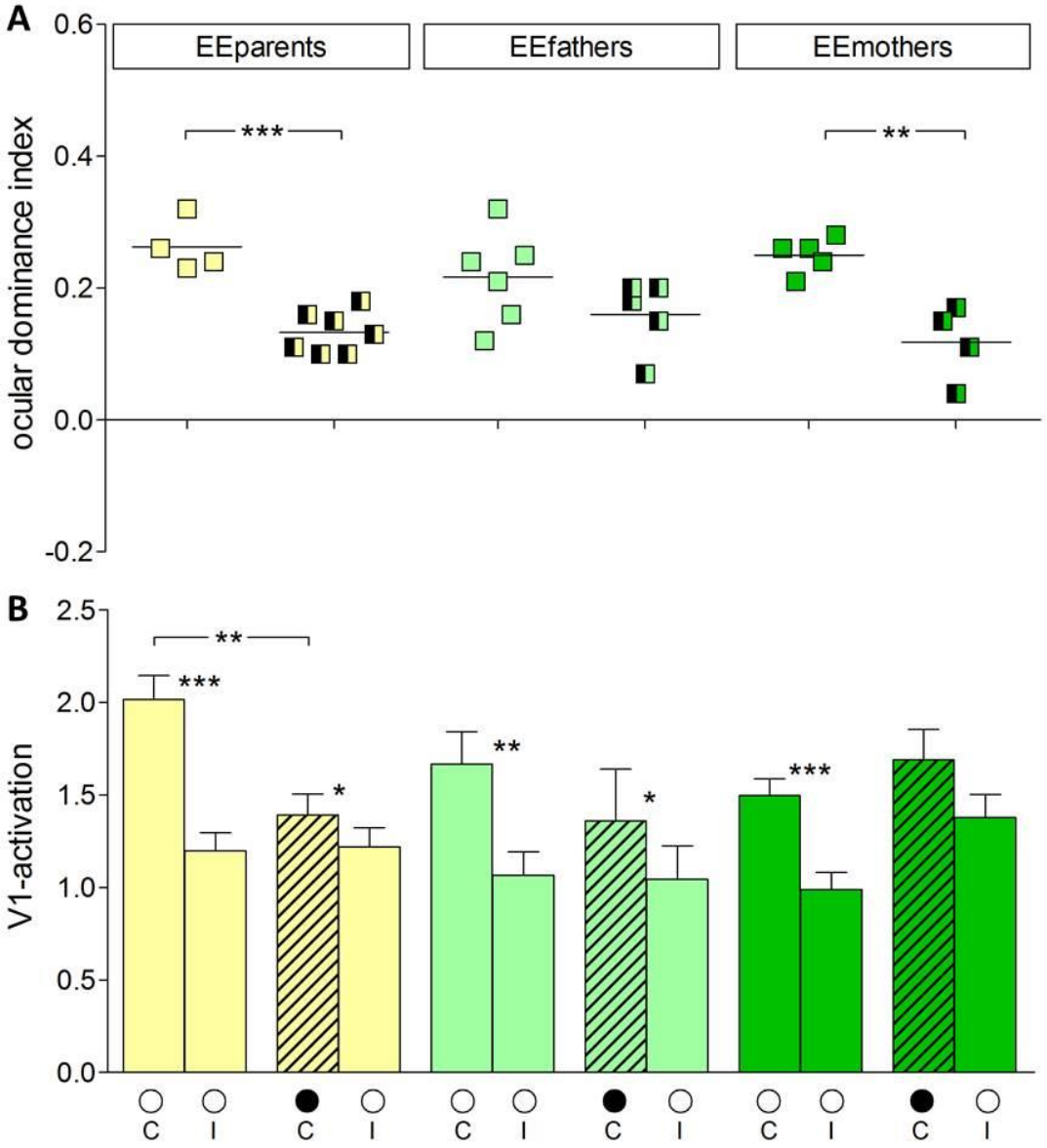


Figure 89: ODIs and V1-activation of mice born in SCs from EE-parents, EE-fathers or EE-mothers. A. Optically imaged ODIs without (filled boxes) and with MD (half-filled boxes). Symbols represent ODI-values of individuals; means are marked by horizontal lines. Offspring of EE-parents (yellow) and of EE-mothers (green) showed an OD-shift after MD, while offspring of EE-fathers (light green) did not. **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye without and after MD (black filled circle indicates MD eye). In mice born in SCs from EE parents the observed OD-shift was mediated by decreased deprived eye (contralateral) responses in V1. There were no significant changes in the other groups in V1-responses. In EE-mothers group after MD an increase in open eye responses (ipsilateral) in V1 was observed but was not significant (p=0.054, t-test).

Standard cage mice of this age (>PD110) did not show OD-plasticity (Lehmann and Löwel, 2008) while EE-mice older that >PD110 display a strong OD-shift towards the open eye. The OD-shift in EE-mice is mainly mediated by reduced closed eye responses in V1 (juvenile-like shift; Greifzu et al., 2014). Comparison of the data from mice born and raised in SC from EE-

parents, EE-fathers or EE-mothers with previously published data of adult mice born and raised in EE (Greifzu et al., 2014) or in SC (Lehmann and Löwel, 2008) revealed that the observed OD-shift in the EE-parents group is similar to the same age EE-mice (Figure 90). In both groups a change in the OD is observed after MD and quantification of the V1-responses showed that after MD, closed eye stimulation resulted in a weaker activation of V1. Thus, we can conclude that OD-plasticity observed in adult offspring of EEparents and EEmothers is similar to the one observed in EE mice of the same age, while SC mice of similar age do not show OD-plasticity any more.

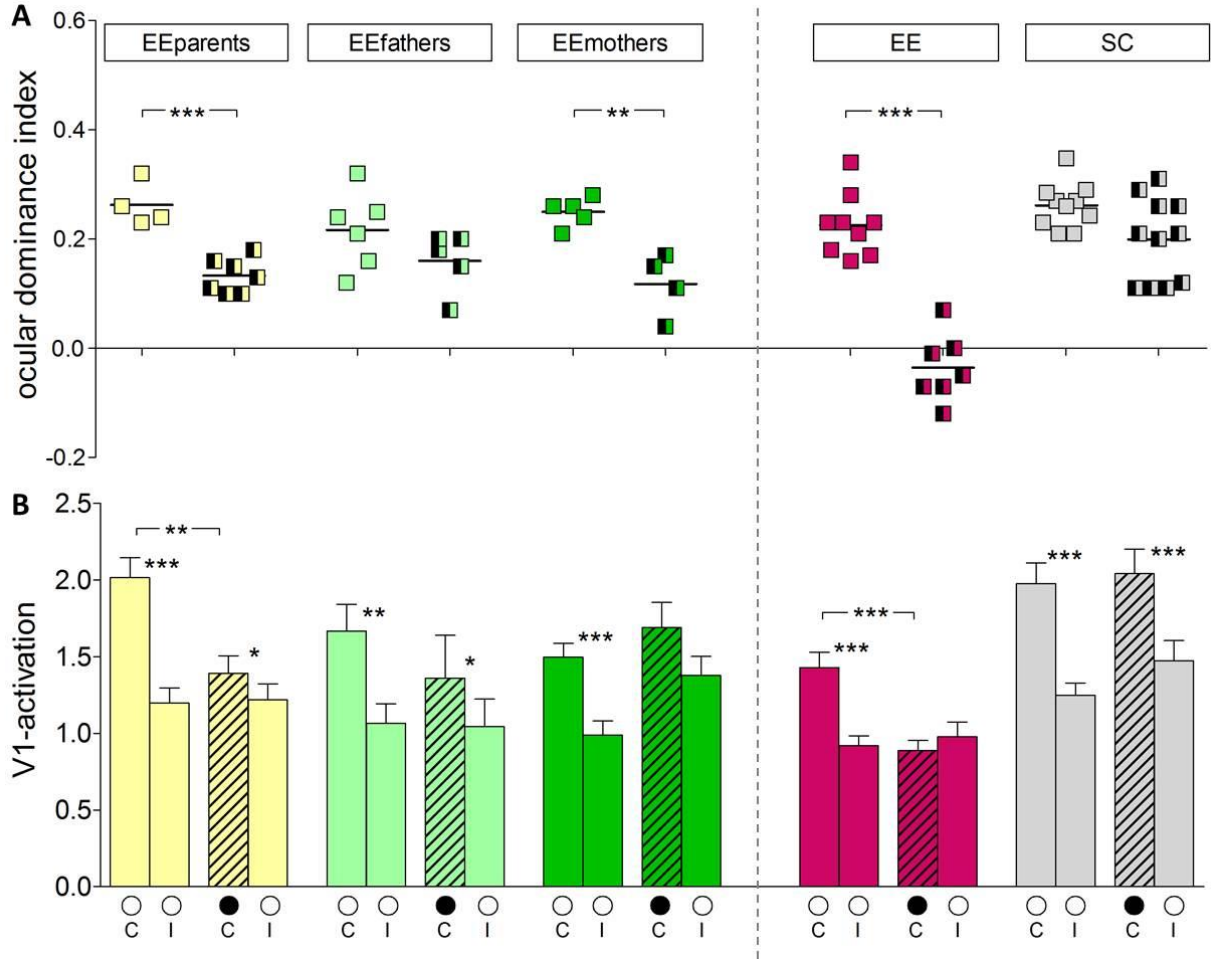


Figure 90: ODIs and V1-activation of mice born in SCs from EE-parents, EE-fathers or EE-mothers. A. Optically imaged ODIs without (filled boxes) and with MD (half-filled boxes). Symbols represent ODI-values of individuals; means are marked by horizontal lines. Offspring of EE-parents (yellow) and of EE-mothers (green) showed an OD-shift after MD, while offspring of EE-fathers (light green) did not. Mice born and raised in EE (purple) showed a strong OD-shift after 7 days of MD (Greifzu et al., 2014). Same age SC-mice did not show any change in the OD (Lehmann and Löwel, 2008) **B.** V1-activation elicited by stimulation of the contralateral (C) or ipsilateral (I) eye without and after MD (black filled circle indicates MD eye). In mice born in SCs from EE parents the observed OD-shift was mediated by decreased deprived eye (contralateral) responses in V1 and is similar to the one observed in mice born and raised exclusively in EE.

4. Discussion

4.1. Summary of the results

Neuronal plasticity is crucial for proper development and function of the central nervous system. As the brain matures, its ability to change declines, together with that the ability to learn, memorize or recover from various brain injuries. One of the approaches shown to improve brain plasticity is enriched environment (EE). The work presented in my dissertation investigated the influence of EE on ocular dominance (OD) plasticity of mice. There are couple major differences between housing conditions of mice. In particular, EE-mice are housed in a bigger cage, in larger groups (up to 16 animals per cage) with a variety of stimulatory objects like running wheels, maze, ladder and tunnel. Thus, EE provides optimal environment for enhanced exploration, social interaction, cognitive stimulation and voluntary physical training (van Praag et al., 2000). In a recent study from our laboratory we showed that raising mice in EE prolonged OD-plasticity into adulthood up to PD196 (oldest mouse tested; Greifzu et al., 2014). Additionally, EE-housing could restore OD-plasticity in adult mice transferred from standard cages (SCs) to EE when they were older than PD110, whereas OD-plasticity was abolished at this age, when mice were housed in SC. The OD-plasticity in EE-mice was "juvenile-like", as changes were mainly mediated by reduction of deprived eye responses in V1 (Greifzu et al., 2014). Moreover, we examined if EE could prolong OD-plasticity further into older age (up to PD809) and if OD-plasticity continues to be "juvenile-like". We found that OD-plasticity was preserved for life in mice housed in EE. Interestingly, EE-mice above PD400 did not show "juvenile OD-plasticity", but revealed adult like OD-plasticity mediated by increased open eye responses in V1. Further, behavioural tests reviled that visual abilities were unaffected in EE raised old mice.

Typical "juvenile OD-plasticity" of SC-mice is characterized by strong OD-shifts upon 3-4 days of monocular deprivation (MD; Espinosa and Stryker, 2010). I showed, here, that 4 days of MD can induce such a strong OD-shift not only in EE mice during the critical period but also during adulthood (up to PD200). Critical period EE-mice similarly to SC-mice (Sato and Stryker, 2008) exhibited a strong OD-shift that was mainly mediated by a decrease in closed eye responses in V1. Interestingly, an OD-shift in older EE-mice in contrast to critical period EE-mice was a result of an increase in open eye responses in V1, normally only observed after 7 days of MD in adult SC-mice (Sato and Stryker, 2008). To understand this difference I performed extra experiments, which revealed that that old EE mice had a significant OD-shift already after 2 days of MD, and this shift is getting stronger if the MD persists. This finding suggests that EE-raised mice might have different mechanisms that allow the visual cortex plasticity to occur and understanding it seek further investigations.

To better understand the effects of EE on visual cortex plasticity I investigated one of the main components of EE, which is the running wheel (RW). To test the effect of physical exercise on OD-plasticity mice were raised in a SC with a RW. We showed that RW-raised mice continued to display OD-plasticity into adulthood, unlike mice without a RW. Additionally, running for 7 days was already sufficient to restore OD-plasticity in adult SC-

raised mice (up to PD242). The OD-shift of RW-mice was mediated by decreased deprived eye responses in V1 (Kalogeraki et al., 2014), similar to the critical period SC-mice after 4 days of MD (Sato and Stryker, 2008). Moreover, we found that physical exercise preserved OD-plasticity in old mice after a cortical lesion in S1 with a therapeutic effect on mice OD-plasticity after a localized cortical stroke.

Although EE-raised mice displayed OD-plasticity into adulthood, it was unknown whether this effect of EE on OD-plasticity is reversible when mice are transferred to SCs. After transferring EE-raised mice in SCs, I found that OD-plasticity of EE-mice rapidly declines. Within 1 week of SC housing the OD-plasticity was already abolished. To start to elucidate the mechanism responsible for this fast loss of plasticity I performed pharmacological treatment with fluoxetine as well as tested the therapeutic effect of RW. Although, fluoxetine did not restore OD-plasticity in these mice, transferring the mice from EE to a SC with a RW preserved OD-plasticity. Striking influence of EE on adult mice plasticity prompted us to investigate the impact of EE housing on future non-EE offspring. In particular, I found that OD-plasticity can be passed on to the next generation of non-enriched mice. Only offspring of EE-mothers and SC-father showed an OD-shift after MD suggesting maternal involvement.

My dissertation emphasizes the importance of the environment not only on healthy brain plasticity but also in the recovery of diseased brain after stroke. We could successfully indicate that minimal level of physical exercise keeps the brain “fitter”. Most importantly, old mice with no previous running experience benefit significantly from short running regime, similar to mice with life-long access to running. However, a continuous enrichment is necessary to keep the brain younger and more plastic. On the other hand, a more restricted environment leads to plasticity loss. Finally, we can suggest that the parent’s environment and the level of enrichment greatly impact the OD-plasticity of future generations.

4.2. The positive effect of EE on OD-plasticity

Conventionally, mice are raised in SCs with no access to stimulatory objects and possibilities of extensive social interactions (housed either alone or in a group up to 4 animals). As a result, visual cortical plasticity rapidly declines with age and is usually absent beyond PD110 (Espinosa and Stryker, 2012; Levelt and Hübener, 2012). In contrast, mice housed in larger and more stimulating EE-cages, get the opportunity for more social interactions, physical exercise and cognitive stimulation. EE housing is known to promote plasticity mechanisms in rats (Sale et al., 2004; Sale et al., 2007; Baroncelli et al., 2010b). Studies in adult rats showed that 2 to 3 weeks of EE-housing promotes OD-plasticity in V1 after MD (Sale et al., 2007; Baroncelli et al., 2010b; Baroncelli et al., 2012; Scali et al., 2012). We showed for the first time that mice raised from birth in EE has a great influence on adult mice OD-plasticity (Greifzu et al., 2014). In our study, EE-raised mice above PD110 continued to show a strong OD-shift compared to SC-raised mice of a similar age when measured with optical imaging of intrinsic signals (Lehmann and Löwel, 2008). Interestingly, the OD-shift observed in adult EE-raised mice was juvenile-like: the OD-shift was mediated by reduced V1 responses after deprived eye stimulation as observed in juvenile SC-mice during the critical period (Hofer et al., 2006a; Heimel et al., 2007; Sato and Stryker, 2008). Our data suggest that EE can prolong

the sensitive phase for OD-plasticity into late adulthood, and that this OD-plasticity is similar to the one observed in critical period mice after 4 days of MD ("juvenile-like" OD-plasticity). In addition, we showed that EE-housing can also restore OD-plasticity in adult mice that were raised in a SC and then transferred to EE when they were above PD110.

EE modifies brain circuitry and its molecular composition: increased levels of BDNF, serotonin and IGF as well as reduced extracellular GABA have been reported in EE housed rodents (Baroncelli et al., 2010b; Sale et al., 2014). In agreement with these findings our patch-clamp experiments revealed that GABAergic inhibition was significantly reduced in V1 slices of adult EE mice and the GABA/AMPA ratio was like in 4-wk-old SC-raised animal. It has been previously shown that diazepam (a positive allosteric modulator of $GABA_A$ receptor) treatment prevented the restoration of OD-plasticity (Baroncelli et al., 2010b). Unlike in Baroncelli et al. (2010b) study, treatment of our EE- mice with diazepam only partially reduced OD-plasticity. This discrepancy might arise from the different species used (rats vs mice), different experimental design or conditions (in present study mice were raised from birth in EE whereas they housed their rats for 3 weeks in EE cages). To further elucidate the mechanisms responsible for EE-effect to preserve OD-plasticity, we quantified the parvalbumin (PV)-positive inhibitory interneurons in V1 using immunostaining. Specifically, we did not find a difference in number of PV-positive cells between SC- and EE-mice. PV-positive interneurons are thought to play a crucial role in OD-plasticity (reviewed in Espinosa and Stryker (2012)) but they represent only 39% of GABAergic inhibitory interneurons in the mouse visual cortex (Gonchar et al., 2007). Thus, other GABAergic interneurons, like calretinin or somatostatin could also be responsible for changes in the GABAergic inhibition, which needs to be further investigated. Summarizing, our data suggest that a reduction in the inhibition is involved in the prolonged OD-plasticity, however, future study needs to be done to fully understand its mechanisms.

Remodeling of the extracellular matrix and specifically the degradation of perineuronal nets (PNNs) has been shown to play a role in prolongation of OD-plasticity in adult rats (Pizzorusso et al., 2002; Harauzov et al., 2010). Reduced PNNs density in the visual cortex has been reported for EE housed rats (Sale et al., 2007; Scali et al., 2012). To this end, we measured the number of WFA-stained PNNs in EE-mice's V1. We found the same number of WFA-stained PNNs in both SC and EE mice in contrast to what was described before (Sale et al., 2007; Scali et al., 2012). These opposite results can be explained if we take under consideration the differences in the experimental design. In the previous studies rats were housed in EE only for a short period of 2 to 3 weeks with the enrichment starting when the animals were already adult (Sale et al., 2007; Scali et al., 2012), whereas in our study we used mice born and raised in EE for at least 5 months before the experiment started (Greifzu et al., 2014). It is possible that through the time the PNN density in our EE-mice might have developed normal levels (like in SC-mice), while in the other studies PNNs density was reduced in response to the short EE exposure.

Our observation for the effect of EE-housing on OD-plasticity raised two key questions. Firstly, does OD-plasticity persist beyond the age of PD200 or disappears at some point during the mice lifespan. Secondly, if this OD-plasticity will still be juvenile-like. Given that

adult EE-mice after 7 days of MD showed OD-plasticity mediated by reduced deprived eye responses in V1 which is similar to the critical period SC-mice after 4 days of MD, it is important to understand whether the critical period in EE-mice persists longer or whether the critical period remains open for the whole life time of the mice. Using optical imaging of intrinsic signals, we showed that, EE-mice display a lifelong OD-plasticity (PD809 oldest mouse tested). Additionally, we found that mice transferred from SC- to EE-cages beyond PD110 regained OD-plasticity until PD922 (oldest animal tested). We conclude that, OD-plasticity does not disappear during mouse's lifespan if the animal has the chance to experience enriched housing conditions, even beyond PD110.

OD-plasticity in SC-mice is age dependent and decreases gradually in mature animals (Lehmann and Löwel, 2008). There are striking differences between OD-plasticity observed in juvenile and adult mice (for review see Espinosa and Stryker (2012)). We observed before that mice raised in EE showed "juvenile-like" OD-plasticity during adulthood (up to PD200) after 7 days of MD, which is mainly mediated by reduction in closed eye responses in V1 (Greifzu et al., 2014). This type of changes is typical for critical period SC raised mice after 4 days of MD (PD28-PD40) (Frenkel and Bear; Hofer et al., 2006b; Sato and Stryker, 2008). In older EE-mice (>PD400) the observed OD-shift was mediated by increased open eye potentiation in V1, similar to the adult type of OD-plasticity that SC-mice display after 40 days of age (Sato and Stryker, 2008). We hypothesize that a switch from juvenile to adult OD-plasticity must take place at some point between PD200 and PD400 in EE-mice, thus EE prolonged the sensitive phase for juvenile-like OD-plasticity by a factor of about 10 compared to SC-mice. Further studies are required to address this hypothesis.

Although EE promotes visual cortex plasticity, it was unknown whether it affects the sensory performance. Previous studies in rodents revealed that EE accelerated the increase of visual acuity that occurs during early postnatal life (Prusky et al., 2000c; Cancedda et al., 2004; Landi et al., 2007). This early increase in visual acuity is possibly due to an accelerated retinal development and earlier eye opening of EE-mice (Cancedda et al., 2004; Sale et al., 2004; Landi et al., 2007; Sale et al., 2007). By using two different behavioural tests (optomotor system and visual water task) the visual performance of old EE-and SC-mice (PD690) were investigated. We did not observe significant differences in the visual acuity or in orientation discrimination measured with the cortex-dependent visual water task (VWT) (Prusky et al., 2000b; Prusky and Douglas, 2003). Further, the spatial frequency thresholds of the optomotor reflex, measured in the virtual-reality optomotor setup were unaltered (Prusky et al., 2004). Although, our visual acuity values measured with the VWT and the spatial frequency thresholds values of the optomotor reflex were similar to previously published data of age-matched SC-mice (Lehmann et al., 2012). Therefore, our results indicate that the visual acuity and other visual parameters are not higher in adult EE-mice compared to SC-mice. Furthermore, we found no significant differences between old SC- and EE-mice (~PD700) in orientation discrimination ability with the VWT. Importantly, our data resemble a previous study that measured the orientation discrimination in SC- mice using the same method (Pielecka-Fortuna et al., 2014). Additional studies in young SC-animals with other behavioural tests revealed orientation discrimination thresholds in a range similar to what we

measured (Reuter, 1987; Andermann et al., 2011). Summarizing, our data indicate that EE-housing had no obvious effect on basic visual abilities of old mice.

4.3. Four days of MD resulted in OD-plasticity in old EE mice

Critical period is a sensitive phase during early life, where brief alterations in visual experience can induce cortical plasticity. Given that EE has such a powerful impact on OD-plasticity of adult mice, it was important to test whether EE influences OD-plasticity during the critical period. We found that 4 days of MD can induce a dramatic OD-shift in critical period EE-mice mediated by reduction of closed eye responses in V1. The observed OD-plasticity was similar to previously published data from SC-mice during the critical period (Frenkel and Bear; Gordon and Stryker, 1996; Hofer et al., 2006a). We indicated that OD-plasticity during the critical period was not affected by the housing conditions as SC and EE-raised mice share the same characteristic for OD-plasticity. Further, the OD-shift after 4 days of MD in EE-mice during the critical period was comparably strong to the older EE-mice after 7 days of MD (Greifzu et al., 2014). In both cases, the OD-shift was due to reduced closed eye responses in V1 confirming the hypothesis that old EE-mice showed “juvenile-like” OD-plasticity.

OD-plasticity in SCs mice is gradually decreasing as the animal matures. Several manipulations have been proposed to extend the sensitive period for OD-plasticity in adults. Although EE housing has been proposed as an effective manipulation to promote plasticity in rats (Sale et al., 2004; Sale et al., 2007; Baroncelli et al., 2010b) and mice (Greifzu et al., 2014) it is still unclear whether EE extends the critical period or simply increases the levels of adult OD-plasticity. Critical period plasticity is open for a limited duration of time and is quantitatively and qualitative different from adult OD-plasticity (for review see Espinosa and Stryker (2012)). One of the characteristic of juvenile plasticity is that 4 days of MD could result in an OD-shift. If the OD-plasticity of old EE-mice is indeed “juvenile-like” then 4 days of MD would be enough to induce a significant OD-shift in these mice. To this end, we performed MD for 4 days in young (PD80-101) and old (PD121-183) mice born and raised in EE. We found that 4 days MD caused an OD-shift in both young and old EE-mice. Although the OD-shift was present was not as strong as after 7 days of MD is same age groups, (Greifzu et al., 2014) suggesting that longer period of MD is shifting the OD even more. Our data are different from what was observed before for young SC-mice, where 7 days of MD are required to induce OD-plasticity (Sato and Stryker, 2008), while in SC-mice older than PD110 MD could not induce OD-plasticity (Lehmann and Löwel, 2008). Thus the surrounding environment has an influence on OD-plasticity and mice raised in EE are more prone to plasticity changes even when they are old. Interestingly, the observed OD-shift in both age groups of EE-mice after 4 days of MD is due to increases in open eye responses in V1.

It is evident that 4 days MD resulted in an OD-shift in EE-mice after the critical period, however the mechanism might be different from previously described for similar age EE-mice after 7 days MD (Greifzu et al., 2014). We observed that after 4 days of MD the potentiation of the open eye was significantly increased and V1 was equally dominated by both eyes.

However, MD for a longer period (7 days) resulted in equal domination of V1 due to decreased deprived eye responses. Additional chronic experiments with old EE-mice 2 and 4 days after MD showed that the OD-shift is present already after 2 days of MD. When the MD persisted (after 4 days) the ODI decreased even more due to increased open eye responses in V1.

In a recent study, the initial responses of neurons in V1 after MD were investigated in mice (Kuhlman et al., 2013). Specifically, it was shown that the most immediate change in cortical responsiveness after MD is a binocular disinhibition of pyramidal neurons resulting from a rapid loss of excitatory input to PV cells. When this occurs, vision no longer drives strong inhibitory responses and the evoked firing rates of excitatory neurons increase back to normal levels, despite continued MD (Hengen et al., 2013; Kuhlman et al., 2013)(for review see Trachtenberg (2015)). Thus, it is possible that the initial increase in open eye potentiation that we observed in old EE-mice shortly after MD was driven by a reduction in firing rates of the inhibitory cells which resulted in a higher firing rate of excitatory neurons. However, a longer period of MD (7 days) might restore the inhibition levels and through a disinhibitory mechanism the activities of pyramidal binocular neurons might get back to normal. Nevertheless, additional experiments are needed to test this hypothesis. In particular, using *in vivo* 2-photon imaging the changes in activation of pyramidal neurons and inhibitory interneurons over the days of MD can be monitored. Analysis of relative changes in activity in pyramidal and in inhibitory interneurons after MD may help us to understand the underlying mechanism after MD in old EE-mice.

4.4. Which component of EE is responsible for preservation of OD-plasticity?

EE exerts its effect on neuronal plasticity by providing the mice with a combination of motor, social and cognitive stimulating factors (Sale et al., 2014). The impact of each one of the diverse components of EE on cortical plasticity is of a particular interest. In a recent study the components of the EE were tested seperatly in rats regarding their effect on recovery from amplyopia (Baroncelli et al., 2012). They observed that both physical exercise and visual enrichment promoted the recovery from long-term amblyopia in rat V1, whereas social interaction had no effect (Baroncelli et al., 2012). The activation of the primary motor cortex due to physical activity might lead to the activation of cross-modal plasticity in V1. In support of this hypothesis, a recent study in awake mice running on a treadmill showed that the visually evoked firing rate of V1 neurons was two- to three-fold increased (Niell and Stryker, 2010; Fu et al., 2014) and also enhanced visual performance (Bennett et al., 2013). Additionally, forced visual exposure to temporally coherent visual stimuli for 6 hours per day was also shown to increase OD-plasticity in 3-month-old SC-raised mice (Matthies et al., 2013). Moreover, enhanced sensory and motor input in EE promoted physiological maturation and consolidation of visual cortical circuits in dark-reared rats and thus even without visual experience (Bartoletti et al., 2004). However, it was not yet investigated whether physical exercise alone can alter the timing of the sensitive phase for OD-plasticity.

We showed that raising mice in SCs equipped with a RW prolonged the sensitive phase for OD-plasticity into late adulthood (PD242 oldest mouse tested). In contrast, similar age mice raised in a SC without a RW did not show OD-plasticity. In addition, the preserved OD-shift in the RW-mice was mediated by a reduction of deprived eye responses in V1. Those reductions after deprived eye stimulation were previously only observed after 4 days of MD in 4-week-old mice (Hofer et al., 2006a; Heimel et al., 2007; Sato and Stryker, 2008) and after 7 days of MD in adult mice raised in an EE (Greifzu et al., 2014). Thus, daily voluntary running in mice seems to preserve a juvenile brain and has similar effects on prolonging OD-plasticity as the complete EE. While voluntary physical exercise seems to mimic the effect of EE on OD-plasticity, the OD-shift observed in RW-mice was not as pronounced as in adult EE-mice (Greifzu et al., 2014). Together these results suggest that running alone can prolong OD-plasticity in mice, but the additional EE-components are necessary to increase the plasticity-promoting effect.

Additionally, we showed that short-term running had the same effect on OD-plasticity as long-term running: 7 days of running during the MD period was sufficient to restore a juvenile-like OD-plasticity in SC-raised mice. However, short term running for 4 hours per day promoted recovery from long-term MD in mice only when was combined with visual stimulation (Kaneko and Stryker, 2014). This study differs from ours as in our case the mice had full access to a RW for 7 days and no particular visual stimulus was presented during running. We can conclude that a minimum amount of running is necessary to prolong OD-plasticity in adulthood and if this is not possible a combined strategy might give the same result.

Baseline visual abilities and their experience-dependent increase after MD were also tested in mice raised with or without RW. As expected, we did not find any significant differences between the two groups and the values were comparable to previously published data of SC-raised C57Bl/6 mice (Prusky et al., 2006). Moreover, the V1 activation and the quality of the retinotopic maps were similar between mice with and without RW. Together these data indicate that physical exercise had no effect either on the spatial frequency and the contrast sensitivity thresholds of the optomotor reflex or the quality of the retinotopic maps and the strength of V1-activation.

Physical exercise seems to be a powerful tool to maintain and restore plasticity into older age but the cellular mechanisms underlying this effect are not yet clear. It has been showed that locomotion activates vasoactive intestinal peptide (VIP)-positive interneurons and optogenetic activation of these interneurons mimics the effects of locomotion in stationary awake mice (Fu et al., 2014). Running has been proposed to act via disinhibitory circuits reducing inhibition on layer 2/3 pyramidal neurons (Fu et al., 2014; Fu et al., 2015) and decreased GABA release has been reported for rats after 3 weeks of running (Baroncelli et al., 2012). Thus, extended running experience may reduce the inhibitory drive onto pyramidal cells and thus promote cortical plasticity. However, it is likely that other molecules and mechanisms that are known to be affected by EE-housing and involved in regulating OD-plasticity are also involved in the effect of running, e.g. BDNF, PNNs, IGF, serotonin and histone acetylation

(for review see Sale et al. (2014)). Additional studies are needed to reveal the underlying mechanisms promoting cortical plasticity after running.

4.5. Therapeutic effect of running after stroke

It was previously shown that a small localized cortical lesion in neighboring S1 prevented OD-plasticity in V1 and also prevented the improvements of visual capabilities after MD in 3 months old mice. Anti-inflammatory treatment with ibuprofen rescued the experience-enabled enhancement of visual abilities of the open eye but not the OD-plasticity (Greifzu et al., 2011). Additionally, we showed previously that EE housing prevented the loss of OD-plasticity in adult mice after a localized stroke in S1 (Greifzu et al., 2014). Here we tested whether physical exercise alone is already beneficial for the brain regarding OD-plasticity after a PT-induced localized cortical lesion. We tested the effect of running by the means of running wheel on OD-plasticity after stroke in two different experimental paradigms: in mice born and raised in RW-cages, thus experiencing running throughout their life to investigate whether running prevents the loss of OD-plasticity after stroke and in mice transferred to a RW-cage for 14 days soon after the induction of the lesion to investigate the therapeutic effect of physical exercise on OD-plasticity after stroke. We found that in both cases, OD-plasticity was present after a PT-lesion in S1, indicating that physical exercise preserved and restored OD-plasticity in adult mice. Moreover, we observed a trend towards decrease in deprived eye responses in V1 after MD for every group suggesting a "juvenile-like" OD-plasticity, but the differences were not significant. Notably, the restoration of OD-plasticity in 14dRW mice seems to be due to the running and not a result of the waiting period after PT and MD. In support of this conclusion are previously published data from SC raised mice where late MD was performed 1 week after the induction of PT-lesion and OD-plasticity was still absent (Greifzu et al., 2011). This indicates that the waiting period of one week before MD had no influence on OD-plasticity after stroke and the rescue of OD-plasticity is specific due to running.

Imaging of the non-lesioned hemisphere in both lifelong running and 14dRW mice showed that OD-plasticity was not affected by stroke. This observation is in line with previous studies showing that the effect of stroke on OD-plasticity was restricted to the affected hemisphere rather than "whole-brain" (Greifzu et al., 2011; Pielecka-Fortuna et al., 2015b). Furthermore the basic visual abilities of the mice and their improvement after MD were tested using the optomotor setup. We found that, after a PT lesion in S1, mice showed significant enhancements in both spatial frequency and contrast sensitivity thresholds of the optomotor reflex after 7 days of MD. In contrast, a PT-lesion in SC raised mice resulted in impaired visual abilities improvements (Greifzu et al., 2011). While EE housing only partially restored the increase in both spatial frequency and contrast sensitivity thresholds after stroke (Greifzu et al., 2014) in our case physical exercise in both lifelong or 14dRW mice fully restored the increase in visual abilities after MD and the final values were similar to non-lesioned mice raised in SCs (Prusky et al., 2006) or in RW cages (Kalogeraki et al., 2014). It was shown that inflammation interferes with the experience-enabled visual abilities enhancements of the open eye as treatment with ibuprofen after the PT-lesion resulted in restoration of MD induced

improvement of visual abilities (Greifzu et al., 2011). Based on that we can speculate that physical exercise reduces the level of inflammation and acts as the anti-inflammatory treatment, but more extensive studies are needed to confirm this hypothesis.

Analysis of the lesion position and size showed no significant difference in localization, length, depth and volume of lesion between lifelong and 14dRW mice and only the lesion diameter was significantly smaller in 14dRW mice. This could be explained by the different pattern of blood vessels among individuals. As the PT method of inducing a lesion depends on the position of the illumination the different pattern of vessels in the illuminated area could result in differences on the lesion size. We did not find a correlation between the lesion size and the OD-shift after MD which is in agreement with observations from Greifzu et al. (2011) study. Moreover, we have recently shown that even a smaller and more distant to V1 lesion, positioned anterior in secondary motor cortex (M2) area, can affect OD-plasticity in mice (Pielecka-Fortuna et al., 2015b). Thus, the smaller diameter of the lesion in 14dRW mice is most probably insignificant and the restoration of OD-plasticity in these mice is specific to physical exercise.

Summarizing our data showed that physical exercise due to running wheels not only could preserve, but also restore OD-plasticity in adult mice after stroke. Additionally, physical exercise in PT mice restored the improvement of spatial frequency and contrast sensitivity thresholds after MD. These data indicate that physical exercise protects from stroke induced impairments, and has subsequently the same effect like EE (Greifzu et al., 2014).

Previous studies pointed out the beneficial effect of physical exercise on stroke. Running wheel exercise increased the number of newborn hippocampal neurons after PT stroke in mice and improved spatiotemporal learning in Morris water maze (Geibig et al., 2012). Moreover mice with middle cerebral artery occlusion (MCAO) showed long-term functional and cognitive improvements after running (Gertz et al., 2006). Physical exercise has also a neuroprotective function: voluntary running 2 to 3 weeks before a MCAO-stroke, reduced cerebral infarct size and sensory-motor deficits in rodents (Wang et al., 2001; Endres et al., 2003). However, how physical exercise contributes on preservation of OD-plasticity after a PT lesion is not yet clear.

It has been shown that stroke affects the balance between excitation and inhibition in the affected neuronal network (Carmichael, 2006) which is responsible for the negative consequences such as impaired plasticity and absent functional recovery. High levels of the neurotransmitter glutamate after ischemic stroke lead to excitotoxicity and neuronal cell death (Lai et al., 2014). Additionally, a lesion induced in V1 led to reduced basal GABAergic transmission measured further away from the lesion border (Imbrosci et al., 2015). It seems that changes in glutamatergic and GABAergic transmission after stroke can lead to negative consequences and thus may interfere with plasticity. We showed that raising mice in an EE not only preserved a low GABAergic inhibition and juvenile OD-plasticity into adulthood, but also protected from stroke induced impairments in V1 after a S1-lesion (Greifzu et al., 2014) suggesting that decreased cortical inhibition is a permissive factor for preserved OD-plasticity in V1 after an S1-lesion. Physical exercise influences many neurotransmitter systems in the brain including glutamatergic (Farmer et al., 2004; Vasuta et al., 2007; Lou et al., 2008) and

GABAergic (Molteni et al., 2002). For example, genes related to the GABA system were downregulated after physical exercise (Molteni et al., 2002). Altogether, these studies implicate that a lower inhibitory tone after running protects V1 from OD-plasticity impairments after a lesion in S1.

Another possible way with which physical exercise may influence the OD-plasticity after stroke is through remodeling of the extracellular matrix by MMPs activity. MMPs play a role in plasticity in the healthy as well as in the diseased brain (reviewed in Wright and Harding (2009)). It has been shown that MMPs activity is a crucial component of OD-plasticity in the healthy brain (Spolidoro et al., 2012). After cerebral ischemia the expression of many MMPs (especially MMP-2 and MMP-9) is increased (Gasche et al., 1999; Heo et al., 1999; Montaner et al., 2001; Rosell et al., 2006). Inhibition of MMPs with a broad-spectrum MMPs inhibitor immediately before a stroke in mice partially rescued experience-dependent barrel cortex plasticity (Cybulska-Klosowicz et al., 2011). Most recently, we reveled that optimal level of MMP-activity is crucial for adult visual cortex plasticity to occur in the healthy and in stroke-affected brains. Specifically, in healthy mice, inhibition of MMPs activity with a broad spectrum inhibitor during 7 days of MD resulted in lost OD-plasticity whereas after stroke single, but not double application of MMPs inhibitor shortly after the induction of the lesion rescued OD-plasticity (Pielecka-Fortuna et al., 2015a). A recent study in rats reported elevated levels of MMP-9 but not MMP-2 after mild treadmill exercise (Nishijima et al., 2015), thus running has an influence the MMPs activity. It would be interesting to test whether MMPs activity is changed after stroke in mice with access to physical exercise, as well as whether treatment with MMPs-inhibitors could change OD-plasticity after stroke in RW-mice.

4.6. Changes of the housing environment affects OD-plasticity

Although EE housing has proven to be beneficial for the brain (for recent review see Sale et al., 2014), it was not clear if continued enrichment is needed to promote a positive effect on OD-plasticity and whether the effect of EE is reversible when the animals are not experiencing enrichment any more. To this end we raised mice in EE, until they were above PD110 and then they were transferred to a normal SC where they receive MD for 7 days after a short period (1 to 12 days) and were then tested for OD-plasticity. We found that OD-plasticity was rapidly lost in these mice: already after 1 week in SC. Thus, continued enrichment is needed to promote OD-plasticity in adult mice and a short period exposure to a more deprived environment is enough to reverse the effect of EE on OD-plasticity.

At molecular level, studies based on gene chip analysis have revealed that a large number of genes related to neuronal structure, synaptic transmission and plasticity, neuronal excitability and neuroprotection change their expression levels in response to EE (Rampon and Tsien, 2000). One group of molecules particular sensitive to experience that are also implicated in plasticity are the neurotransmitters: EE increases acetylcholinesterase activity (Rosenzweig et al., 1962), noradrenaline (Naka et al., 2002) and serotonin levels (Rasmuson et al., 1998). EE elicits a 2-fold enhancement of serotoninergic transmission in the visual cortex and infusion of serotonin synthesis inhibitor not only blocks plasticity in response to MD but also

completely counteracts the effects of EE on GABAergic inhibition and BDNF (Baroncelli et al., 2010b). The central role of serotonin in promoting adult visual cortex plasticity has been demonstrated in rats chronically treated with fluoxetine, a selective serotonin reuptake inhibitor (SSRI) widely used in treatment of depression and various psychiatric disorders. Fluoxetine delivered in the drinking water restored OD-plasticity after MD in adult rats and a complete recovery from amblyopia. Like in EE, fluoxetine treatment reduced intracortical inhibition and increased BDNF expression in visual cortex (Maya-Vetencourt et al., 2008).

We tested whether OD-plasticity could be restored in mice transferred to SCs from EE using fluoxetine. Mice born and raised in EE were transferred to a SC for a period of 3 weeks during which fluoxetine was administrated through drinking water. Contrary to Maya-Vetencourt et al. (2008) we did not observed a change in OD-plasticity after fluoxetine treatment. This difference could be explained based on the differences in animals used and on the experimental conditions. Maya-Vetencourt et al. (2008) used rats for their experiments with an optimal fluoxetine dosage of 0.2 mg/ml drinking water per day. In our experiments we used mice and calculated the dosage based on average body weight and the daily drinking amount reported for C57BL/6 mice. Based on previous reports an optimal dosage of fluoxetine to promote an effect on mice behaviour is 10 mg/kg per day (Branchi et al., 2013). After calculating the daily fluoxetine intake for our mice we found that it was 7.28 mg/kg, which is lower than we initially expected because the mice drunk less. It is possible that the dosage our mice achieved was not enough to promote an effect on OD-plasticity. Further experiments with a higher dosage may be essential to reveal an implication of serotonin in restoration of OD-plasticity. Moreover, there is a time delay of several weeks between the increase of neurotransmitters and the antidepressant effects (Nestler, 1998) implying that the timing of the treatment is also crucial. Possibly in our case the timing of the treatment was not long enough to promote an effect on cortical plasticity and a longer treatment is needed to have an effect. A recent study showed that antidepressant treatment with fluoxetine in a favorable environment would lead to the reduction of depression symptoms while in a stressful environment might lead to worst prognosis, suggesting that the effect of fluoxetine derives as a combination of the treatment and the environment (Branchi et al., 2013). Changing housing conditions by transferring mice from an EE to a SC might act as a stress factor thus fluoxetine treatment might not be able to act beneficial for promoting the OD-plasticity.

In contrast to fluoxetine treatment, transferring mice from EE to a cage with RW was enough to preserve OD-plasticity. The observed OD-shift was similar to our previous data from RW-mice (Kalogeraki et al., 2014) and the OD-shift was mediated by reduced closed eye responses in V1: juvenile-like OD-shift. Thus, a minimum level of enrichment (only a running wheel) was sufficient to preserve OD-plasticity in adult mice. A study comparing the effects of running and fluoxetine treatment in the hippocampus revealed that new cell survival was enhanced by 200% after running only. Both running and fluoxetine also increased the percentage of new cells that became neurons (Marlatt et al., 2010). Furthermore, fluoxetine treated mice are reported to be less active in locomotion (Marlatt et al., 2010). Thus, the response of the brain to physical exercise is much stronger than to antidepressants. This

observation supports our finding were physical exercise was able to preserve OD-plasticity in V1 whereas fluoxetine treatment was not.

Furthermore, enhanced social interaction by housing more rats in a SC, was not capable of recovering amblyopia (Baroncelli et al., 2012). Rosenzweig et al. (1978) already showed before that social grouping alone is insufficient to explain the cerebral effects of EE in rats. Another recent study showed that increased social interaction alone had no positive effect on motor coordination and learning abilities in mice (Madronal et al., 2010). In agreement with the previous studies, we also showed here that housing mice in groups after transferring them to SCs was not sufficient to promote plasticity changes.

All together above data stress the importance of the housing conditions on experimental animals and the outcome of plasticity studies. Even a small change in the housing environment like the addition of a RW could result in plasticity changes. There is a debate on the field regarding the different enrichment protocols and whether all animals in an EE cages are getting the same level of enrichment (Frick and Benoit, 2010). We did not observe higher variability in our experimental paradigm between the EE-mice. It is clear that housing mice in a more restricted environment with less stimulations and no opportunities for physical exercise, as SC resulted in an earlier decline of OD-plasticity.

4.7. The positive effect of EE can be passed to the next generation

Genetic processes work in combination with an organism's environment and experiences to influence development and behaviour. It is evidence that, except the genes transferred to the next generations by germ lines, the experiences of the parents can markedly influence both structure and function in the nervous system of subsequent generations through epigenetic changes. Several studies have reported long lasting epigenetic modifications as DNA methylation and histone acetylation, induced by the environmental enrichment. The alterations in the posttranslational modification patterns caused by EE can be transferred to the next generation and influence the behaviour of the offspring (for review see Arai and Feig (2011)). In a study Arai et al. (2009) showed that even short-term enrichment of the parents enhances long term potentiation (LTP) not only in these enriched mice but also in their non-enriched offspring, even if the offspring never experience an EE. Using a genetic modified mouse model for defective LTP and contextual fear conditioning memory it was shown that both LTP and fear conditioning memory were masked in the offspring of enriched mutant parents (Arai et al., 2009). Additionally Arai et al. (2009) proposed that transmission of this effect to the next generation occurs from the EE-mother to their offspring during embryogenesis (Arai et al., 2009).

Inspired from the Arai et al. (2009) study, we wanted to test this hypothesis in our experimental paradigm. Our aim was to investigate whether OD-plasticity can be transferred from mice that were born and raised in EE to their offspring that were born and raised exclusively in SC. To this end mating of EE-mice took place in an EE cage and few days before delivery (7-10 days) pregnant dams were transferred to a SC. Pups were born and raised in SCs until they were at least110 days old and then tested for OD-plasticity after

induction of MD. We found that non-enriched offspring of EE-parents can maintain OD-plasticity into late adulthood (PD261 oldest mouse tested). SC mice did not show OD-plasticity after MD at this age (Lehmann and Löwel, 2008), whereas EE-mice showed a strong OD-shift at similar age (Greifzu et al., 2014) suggesting that OD-plasticity could be transmitted to the next generation. However the OD-shift observed in non-enriched offspring of EE parents was not as strong as in EE-mice, indicating that continues enrichment is required for stronger OD-shift. Analysis of the V1-activation after each eye stimulation revealed that the observed OD-shift in the offspring was mediated by decreased closed eye responses in V1, similar to what was observed before in EE mice (Greifzu et al., 2014). Together these data suggest that the positive effect of EE on OD-plasticity can be passed on to the next generation.

As both parents were born and raised in EE it was not clear from which parent this effect on OD-plasticity was transmitted to the next generation. Mating between enriched and non-enriched males and females helped us to clarify this point. We found that after mating EE-male with SC-female the offspring did not show OD-plasticity whereas after mating SC-male with EE-female OD-plasticity was observed. The OD-shift found in non-enriched offspring of SC-father and EE-mother was equally strong as the one observed in offspring of EE-parents. Our data clearly demonstrate that mothers have a prominent role in transferring the OD-plasticity to the offspring.

It is plausible to argue that the ability for retaining plasticity into late adulthood is transferred from the mother, as mothers can have greater influence on the pre- and post-natal development of the offspring (Liu et al., 2000). Mothers provide the offspring with nutritional and behavioural support such as licking and grooming which may have long lasting beneficial effects for brain plasticity (Champagne et al., 2003). It is known that mothers raised in EE provide their pups with higher levels of maternal care compare to SC raised mothers, which may also explain the long-lasting plasticity observed in their offspring (Baroncelli et al., 2010a). On the other hand studies from Arai et al. (2009) showed that 4-week old offspring of EE-mice also displayed enhanced synaptic plasticity in the hippocampus even if they never experience EE. In addition the mother's but not the father's environment contributed to the transgenerational transmission of the effect of EE. Enhanced synaptic plasticity was observed also when offspring of EE-mothers were raised from birth by a non-EE foster mother, suggesting that the effect of EE was transmitted to the offspring before birth (Arai et al., 2009). Our data suggest that having only a mother with EE experience is enough to transmit the positive effect of EE on OD-plasticity. However, it is not yet clear whether the OD-plasticity in the offspring is due to epigenetic modifications that is passed on to the next generation or due to maternal care of the EE-mother. Further studies are required to reveal if the observed phenotype is due to the behaviour of the mother after birth or occurs during embryogenesis. Experiments with pups from EE-mother but raised by a foster SC-mother could possibly segregate the behavioural and genetic components involved in the inheritance of ability for retaining plasticity.

4.8. Conclusions

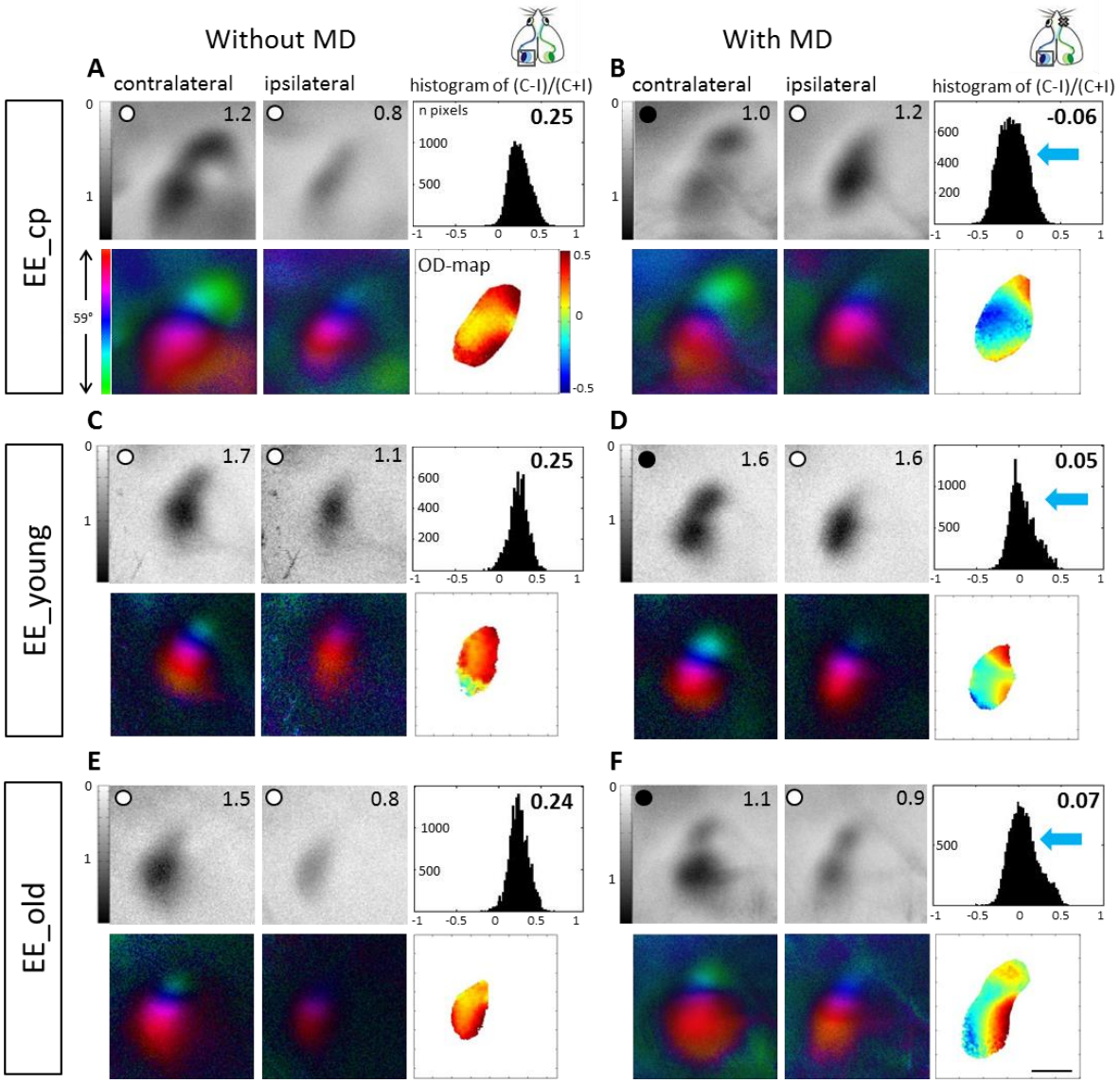
The data presented here provide clear evidence that raising mice in EE has a beneficial effect for brain plasticity and specifically OD-plasticity. Mice raised in EE not only showed a prolongation of the sensitive phase for OD-plasticity but retained a lifelong visual plasticity. The influence of the environment and the experiences of the mice have a great influence on OD-plasticity as a small change in the environment like the addition of a running wheel prolonged OD-plasticity into adulthood. It is important to mention that the effect of physical exercise on visual plasticity is not only present when the mice have access to running throughout their life, but even a short period of exposure to running wheel during adulthood has the same effect, suggesting that is never late to reinduce OD-plasticity. EE and physical exercise alone proved to be beneficial to promote OD-plasticity in mice after stroke and physical exercise after the stroke was able to rescue the negative effects of stroke on OD-plasticity.

Additionally, we found that continued enrichment is necessary to promote OD-plasticity in adulthood as mice transferred from EE to SC rapidly lost OD-plasticity. Addition of a RW in the SC cage could restore OD-plasticity in those mice whereas fluoxetine treatment had no effect. More interestingly we found that the positive effect of EE on OD-plasticity can be passed on the next generation from the mother even if the offspring never experience an EE.

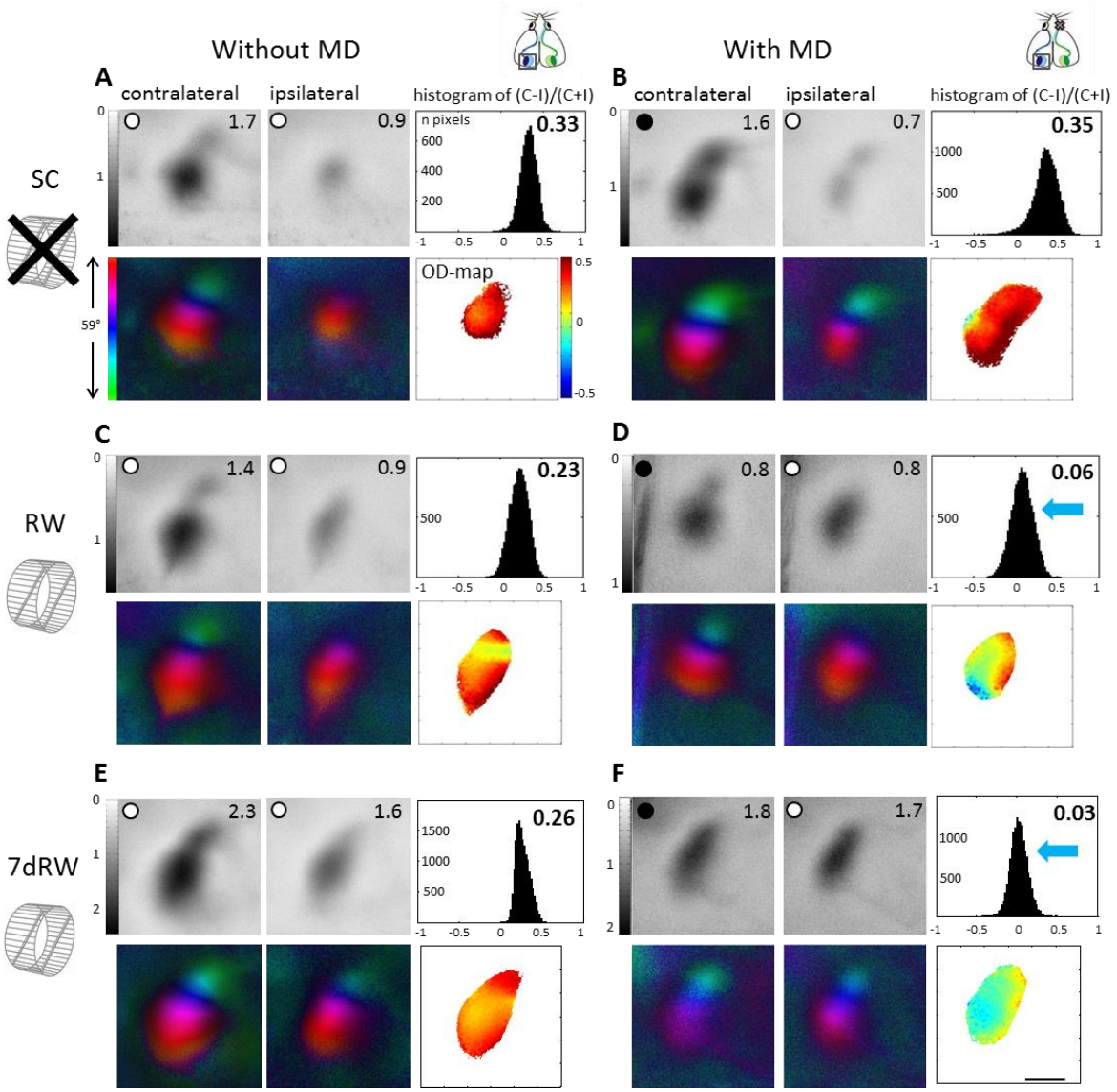
All together data described in my dissertation suggest that the surrounding environment has a great influence on neuronal plasticity. Several approaches have been proposed over the years to prolong neuronal plasticity not only in healthy brain but also after injuries or in neurodegenerative diseases. EE has been proposed as a non-invasive method to influence brain plasticity. Data presented here confirm the previous observations that EE can be used as a noninvasive technique to promote OD-plasticity in adulthood and underlying the potential of physical exercise on prolongation of OD-plasticity as well as its therapeutic role regarding recovery from stroke. Present data opens new avenues to study further the mechanisms underlying plasticity and the influence of the EE on promoting plasticity effects and recovery from brain injuries.

In fact, some of the beneficial components of EE have been already tested for human therapy. In particular, a suitable enrichment, which has already been applied for elderly people, is dancing (Kattenstroth et al., 2013). Beneficial effects of dancing classes included better posture and reaction times, improved cognitive, tactile and motor performance (Kattenstroth et al., 2013). In addition, a clinical trial started to test the effects of EE on rehabilitation after stroke (Janssen et al., 2010). This approach includes different enrichment possibilities for the patient, such as computer, internet, reading, puzzles, board games, and music. Moreover, another paradigm of enrichment used for humans is virtual reality video gaming. Playing video games is an innovative therapy approach used for amblyopia and stroke rehabilitation (for review see Laver et al. (2012)). As suggested above EE is a highly promising tool that can be used as a therapy after a brain damage or neurological diseases, however future research is necessary to fully understand the mechanisms responsible for beneficial effects of EE.

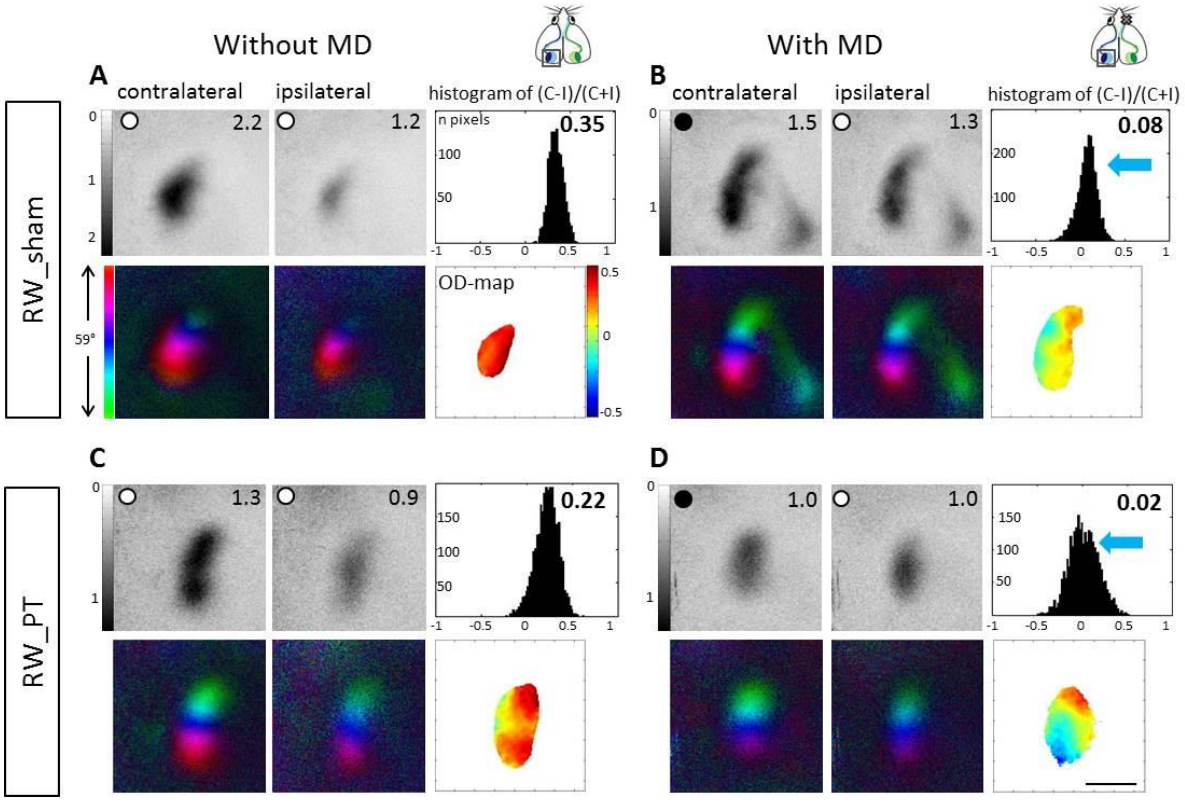
Appendix



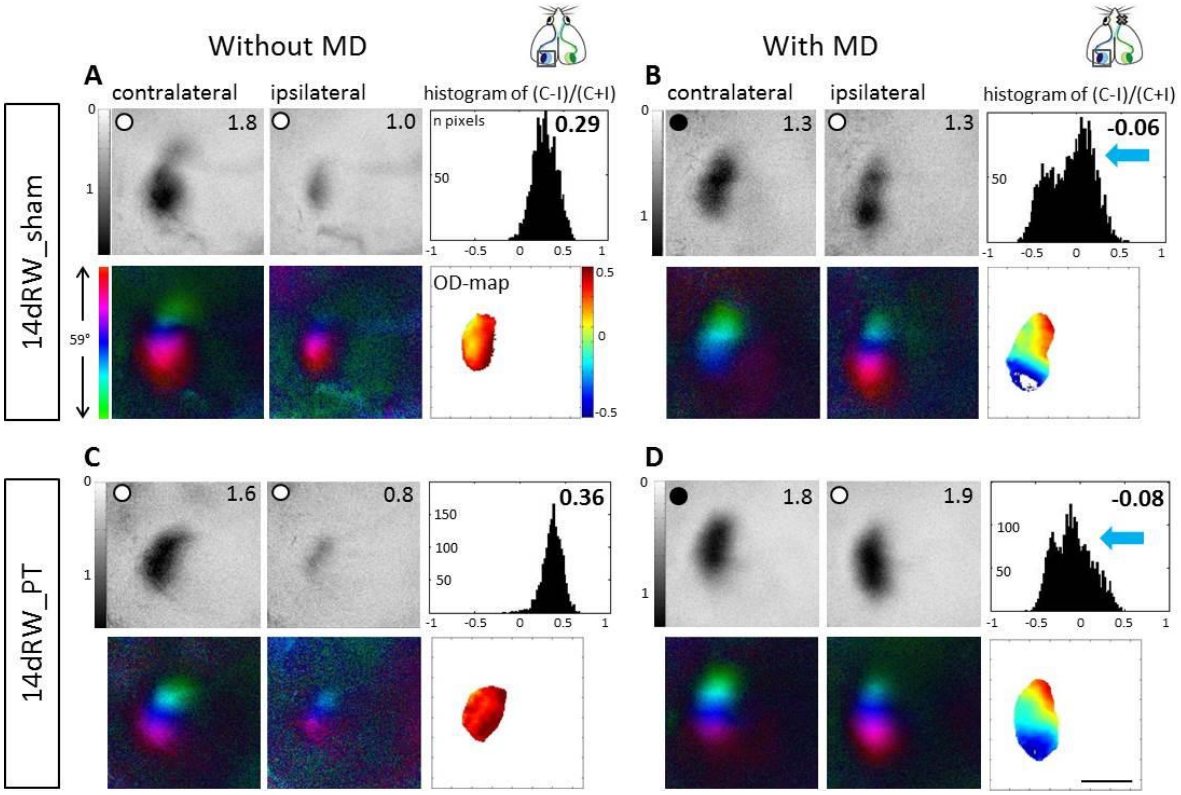
Appendix Figure 1: The OD changed after 4 days of MD in all age groups of EE-mice. More examples of data presented in the results part 3.3.2. Optically recorded activity maps after contralateral and ipsilateral eye stimulation in the binocular region of mouse V1 are presented for critical period EE-mice (PD27-34 **A** and **B**), young EE-mice (PD80-101; **C** and **D**) and old EE-mice (PD121-183; **E** and **F**), without MD (**A**, **C** and **E**) and after 4 days of MD (**B**, **D** and **F**). Grayscale-coded response magnitude maps with the activity value on top right corner, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. Scale bar: 1 mm.



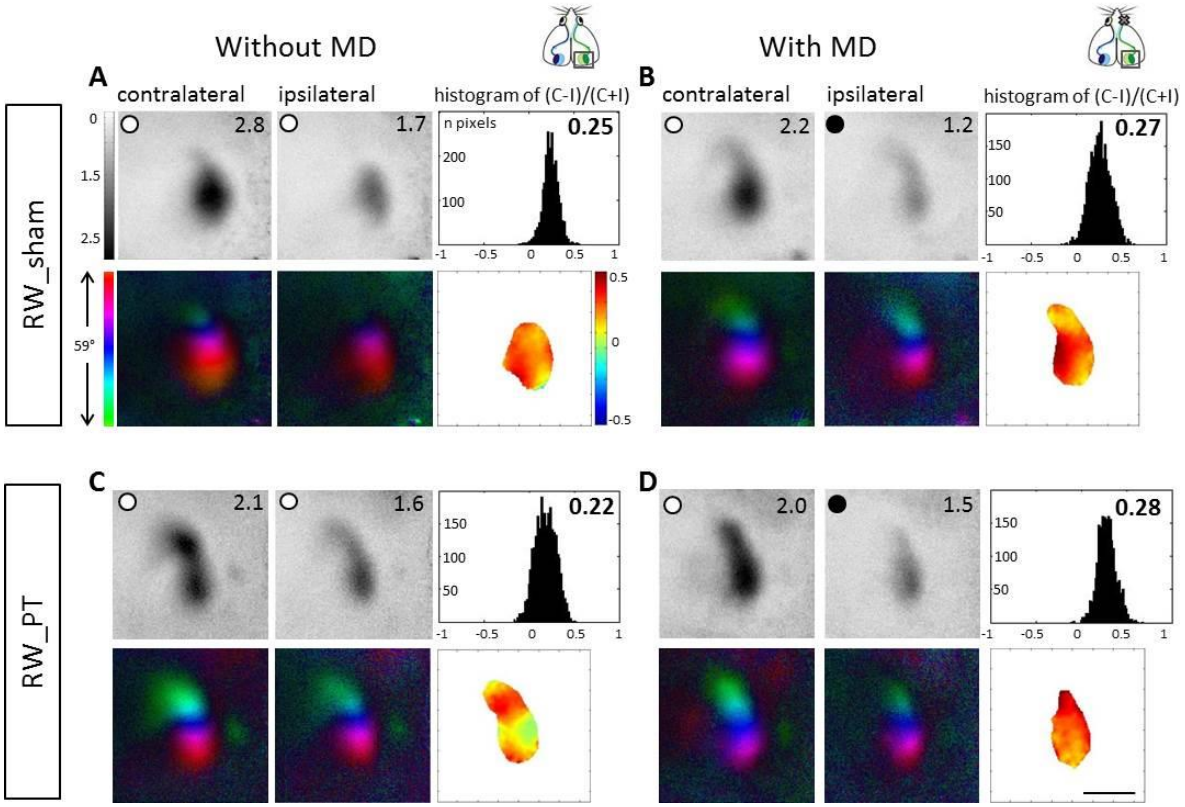
Appendix Figure 2: Running prolonged and restored the sensitive phase for OD-plasticity in mice in adulthood (>PD110). More examples of data presented in the results part 3.4.2. Optically recorded activity maps of the contralateral and ipsilateral eye in the binocular region of mouse primary visual cortex (V1) in mice raised in SCs without a running wheel (**A** and **B**), with a running wheel (**C** and **D**) and with 7 days of running wheel (**E** and **F**), before (**A**, **C** and **E**) and after MD (**B**, **D** and **F**). Grayscale-coded response magnitude maps, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. Scale bar: 1 mm.



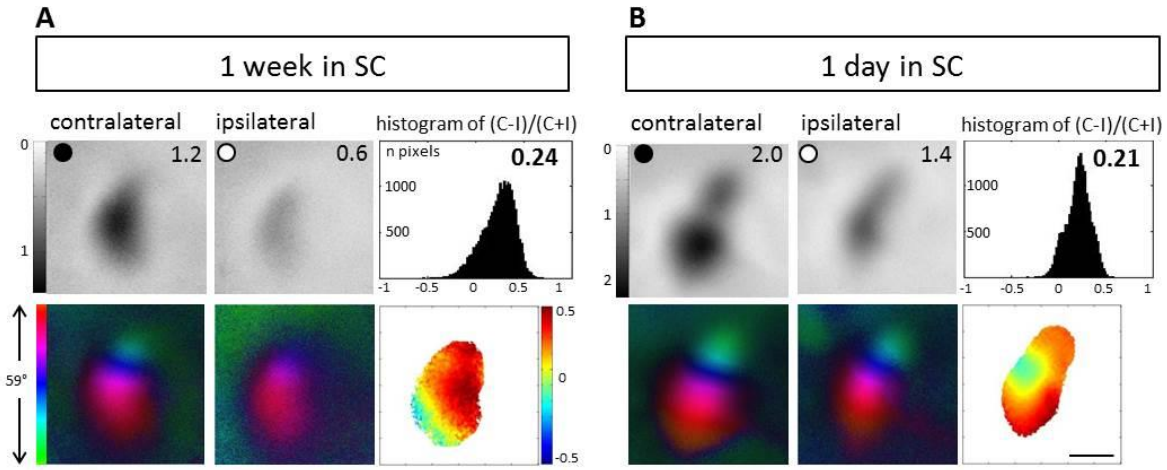
Appendix Figure 3: OD-plasticity is preserved after a localized cortical stroke in S1 when the mice born in a cage with a running wheel. More examples of data presented in the results part 3.5.2. Examples of optical imaging recorded maps from lifelong running groups of the left hemisphere. Activity and polar maps of the binocular part of V1 and ODI values of the left hemisphere measured with optical imaging of intrinsic signals in RW sham (**A, B**) or PT (**C, D**) treated animals. Grayscale-coded response magnitude maps, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. Scale bar: 1 mm.



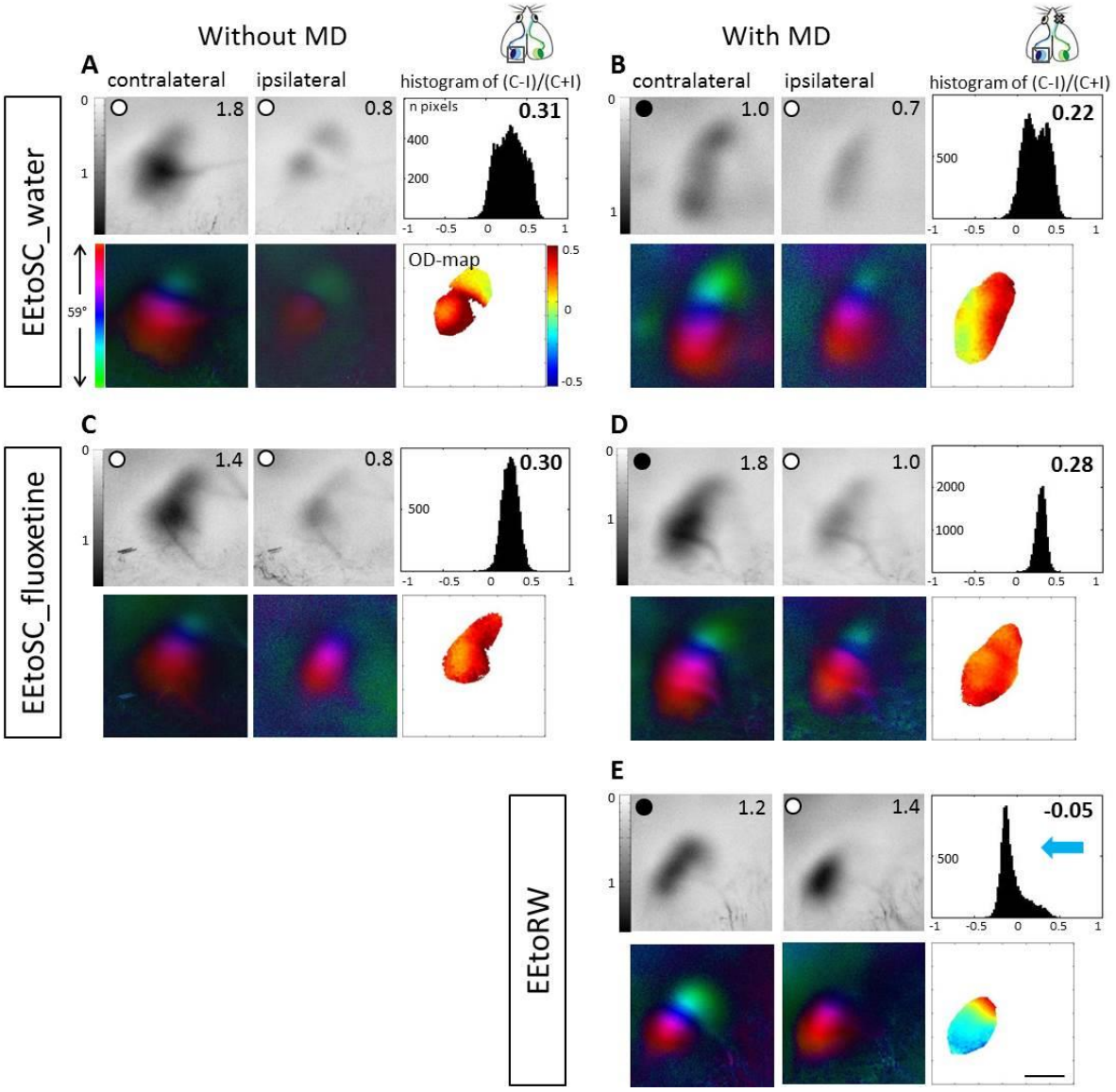
Appendix Figure 4: OD-plasticity is restored in mice with running wheels after the stroke induction. More examples of data presented in the results part 3.3.2. Optical imaged maps recorded from 14dRW groups of the left hemisphere. Activity and polar maps of the binocular part of V1 and ODI values of the left hemisphere 2-dimensional OD-maps and ODI histograms for 14dRW sham (**A, B**) and PT (**C, D**), without (**A, C**) or with MD (**B, D**). Grayscale-coded response magnitude maps, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. Scale bar: 1 mm.



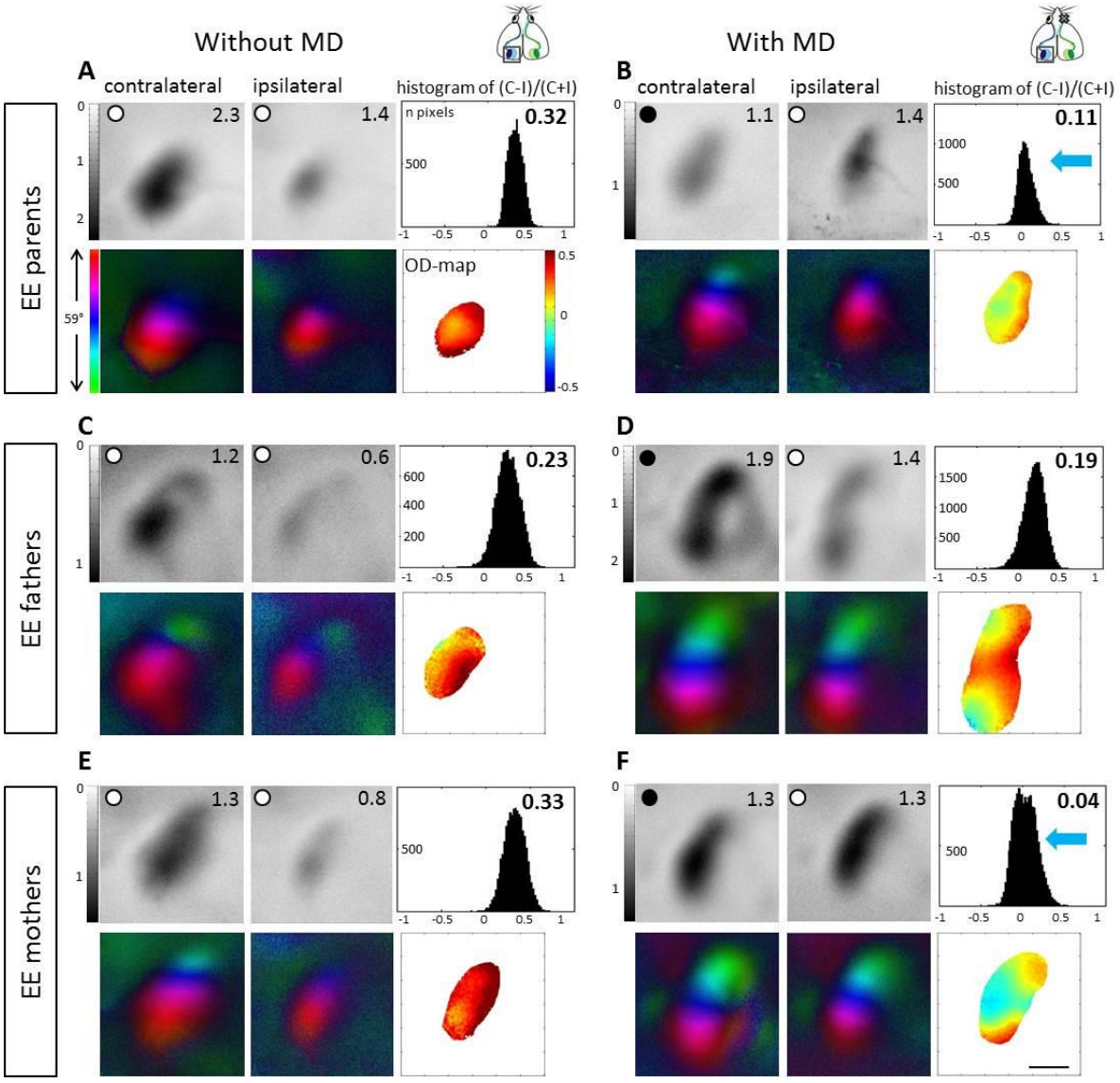
Appendix Figure 5: Examples of optical imaged maps of 14dRW groups of the right hemisphere. More examples of data presented in the results part 3.5.3. Activity and polar maps of the binocular part of V1 and ODI values of the right hemisphere 2-dimensional OD-maps and ODI histograms for 14dRW sham (**A, B**) and PT (**C, D**), without (**A, C**) or with MD (**B, D**). Grayscale-coded response magnitude maps, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. Scale bar: 1 mm.



Appendix Figure 6: OD-plasticity is rapidly lost in EE mice when transferred to SCs. More examples of data presented in the results part 3.6.1.2.Optically imaged maps from EEtoSC mice. Activity and polar maps of the binocular part of the left V1,ODI values, 2-dimensional OD-maps and ODI histograms for EEtoSC mice that received an MD 1 week (A) or 1 day (B) after the transfer from EE to SC. All mice showed a contralateral dominance: activity patches from the contralateral eye were darker than those from the ipsilateral eye, with positive ODI values and warm colors in the OD-map. Scale bar: 1 mm.



Appendix Figure 7: Running wheels but not fluoxetine treatment rescue OD-plasticity in mice transferred from EE to SC. More examples of data presented in the results part 3.6.2.2. Maps acquired from EE-mice transferred to SCs without or with MD after treatment with water, fluoxetine or RW. Optically recorded activity maps after stimulation of the contralateral and ipsilateral eye in the binocular region of mouse primary visual cortex (V1), of EEtoSC mice with water (**A** and **B**), with fluoxetine (**C** and **D**) or with RW (**E**), before (**A** and **C**) and after MD (**B**, **D** and **E**) are illustrated. For every example grayscale-coded response magnitude maps, polar maps, 2-dimensional OD-maps and the histogram of OD-scores including the average OD-index (ODI) are illustrated. Scale bar: 1 mm.



Appendix Figure 8: OD-plasticity can be passed on to the next generation of non-EE mice. More examples of data presented in the results part 3.7.2. Optical imaging recorded maps from mice born in SC from EE-parents, EE-father and EE-mothers with or without MD. Activity and polar maps of the binocular part of V1 and ODI values of the left hemisphere measured with optical imaging of intrinsic signals in mice born in SC from EE-parents (**A, B**), EE-fathers (**C, D**) and EE-mothers (**E, F**). Scale bar: 1 mm.

Abbreviation list

BDNF	brain-derived neurotropic factor
CP	critical period
cyc/deg	cycle per degree
EE	enriched environment
GABA	γ-aminobutyric acid
IGF	insulin-like growth factor
LGN	lateral geniculate nucleus
MD	monocular deprivation
n	number of animals
N_2O	nitrous oxide
mg	milligram
ml	milliliter
mm	millimeter
MMPs	matrix metalloproteinases
O_2	oxygen
OD	ocular dominance
ODI	ocular dominance index
PD	postnatal day
PNNs	perineuronal nets
PT	photothrombosis
PV	parvalbumin
RW	running wheel
S1	primary somatosensory cortex
SC	standard cage
SST	somatostatin
V1	primary visual cortex
VIP	vasoactive intestinal protein
VWT	visual water task

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