Cross-modal changes in primary visual cortex induced by somatosensory manipulation

Dissertation

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by Dipl. Biol. Marcel Isstas

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Gutachter:

Herr Prof. Dr. rer. nat. Jürgen Bolz, Friedrich-Schiller-Universität Jena
Herr Prof. Dr. rer. nat. Hermann Wagner, RWTH Aachen
Frau Prof. Dr. rer. nat. Manuela Nowotny, Friedrich-Schiller-Universität Jena

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Abbrevations

AD	Auditory deprivation
A1	Primary auditory cortex
AMPAr	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor
CCD	Charge-coupled device
CHL	Conductive hearing loss
CN	Cochlear nucleus
CO	Chiasma opticum
cpd	Cycles per degree (cyc/deg)
CPP	3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid
CS	Colliculus superior
DE	Dark exposure
GABA	Gamma-aminobutyric acid
HPLC	High pressure liquid chromatography
IC	Inferior colliculus
ION	Infraorbital nerve
LGN	Lateral geniculate nucleus
LTD	Long term depression
LTP	Long term potentiation
MD	Monocular deprivation
mEPSCS	Miniature excitatory postsynaptic currents
MGN	Medial geniculate nucleus
NLL	Nuclei of the lateral lemniscus
NMDAr	N-methyl-D-aspartate receptors
OD	Ocular dominance
ODP	Ocular dominance plasticity
OI	Optical imaging
PoM	posteriomedial complex
PSD	postsynaptic density

PV	Parvalbumin
RGC	Retinal ganglion cells
s.e.m.	Standard error of the mean
S1	Primary somatosensory cortex
SC	Colliculus superior
SO	Superior olivary complex
SOM	Somatostatin
V1	Primary visual cortex
VA	Visual acuity
VEPs	Visual evoked potentials
VPM	Ventral posterior medial nucleus
VWT	Visual water task
WD	Whisker deprivation

1. Introduction

In mammals different sensory organs had evolved, specialized in sensing a certain aspect of their environment, like eyes for detecting electromagnetic radiation, ears that react to changes in the pressure of the surrounding medium and touch-sensitive cells in the skin, to get information about shape and the texture of objects, for example. All this information is combined and integrated by the brain to create a unified perception of the world, which is necessary for a goal directed modulation of behaviour. For this, the sensory organs project over different structures to the cortex of the brain. Here, there are anatomical separated areas which receive predominantly input from the corresponding sensory organ, known as primary sensory cortices. They represent the outer world in very precise organized and topographic structured manner. From here, information reaches higher order cortices. For a long time, it was thought that the information coming from the different sensory organs are combined on this high-level processing stages and that the primary sensory cortices work in isolation. But newer findings could demonstrate that also the primary sensory cortices are interconnected and that the manipulation of one sense not only influences the information processing in the associated primary sensory cortex, but also in the others. This phenomenon is termed cross modal plasticity.

For example, visual abilities, like visual acuity and contrast sensitivity, are better in mice when auditory input is reduced. This is realized by intracortical connections between the auditory and visual primary sensory cortices, establishing a disinhibitory circuit.

Other studies could show, that an early onset of sensory loss or the congenital lack of one sense causes cross modal changes, resulting in a strong improvement of the abilities of the spared senses. But slightly less is known about cross modal changes that take place in fully adult primary sensory cortices after sensory loss.

Therefore, I investigated the cross-modal changes that take place in the spared primary visual cortex, in fully adult mice after auditory and somatosensory deprivation. Because of this, I will give a short introduction into the sensory systems of the mouse which were important for my work.

1.1 Sensory systems of the mouse

The mouse has become a common modal for parsing how sensory systems work. Lot is known about the development, function, plasticity and the underlying architecture of neural circuits.

1.1.1 The visual system of the mouse

Even though mice are nocturnal animals their visual system shares common features with higher mammals. Visual stimuli are first detected by photosensitive cells located in the eyes retina, the rods and cones. They translate the incoming visual cues to electrophysiological signals which are processed by different cell types of the retina before they are relayed onto the retinal ganglion cells (RGC) whose efferences leave the eye as the optic nerve. On the way to downstream structures, the optic nerves from both eyes meet at the chiasma opticum (CO), where the axons coming from the nasal part of the retina cross over to the contralateral side of the brain, while the axons from the temporal part of the retina remain on the ipsilateral side (Seabrook et al., 2017, Erskine and Herrera, 2014). A larger portion of RGC then project to the lateral geniculate nucleus (LGN), as part of the thalamus, while a smaller one projects to other targets, like the colliculus superior (SC) a structure involved in generating visual evoked reflexes (Erskine and Herrera, 2014). In the LGN eye inputs are separated into two territories. A larger domain receives input from the contralateral eye, while the ipsilateral input forms a smaller patch (Drager, 1974, Jaubert-Miazza et al., 2005). Beside this separation the LGN is organized in a retinotopic manner, which means, that neighboured regions of the retina project to neighboured regions in the LGN (Metin et al., 1983, Grubb and Thompson, 2003). From here, the visual information is transmitted predominantly to layer IV of the primary visual cortex (V1) and then relayed to the other cortical layers and high order visual areas. Mouse visual cortex shows two anatomical separated regions. The medial part receives input from the opposing, the contralateral eye. This is the so-called monocular zone of V1. In the lateral part of V1 the neurons receive inputs from both eyes, while the contralateral innervation on these cells is normally stronger (Gordon and Stryker, 1996). As shown for the LGN, V1 also displays a retinotopic organization, meaning that the upper visual field is represented in the posterior part of V1 whereas the lower visual field is presented in the anterior part. A common technique to visualize this organization of V1 is the method of periodic optical imaging of intrinsic signals. Next to an indirect quantification of neural response strength to visual stimuli, the high temporal

and spatial resolution allows to comprehend the direction of the activity wave evoked by a moving visual stimulus presented to the mouse eye (Kalatsky and Stryker, 2003).

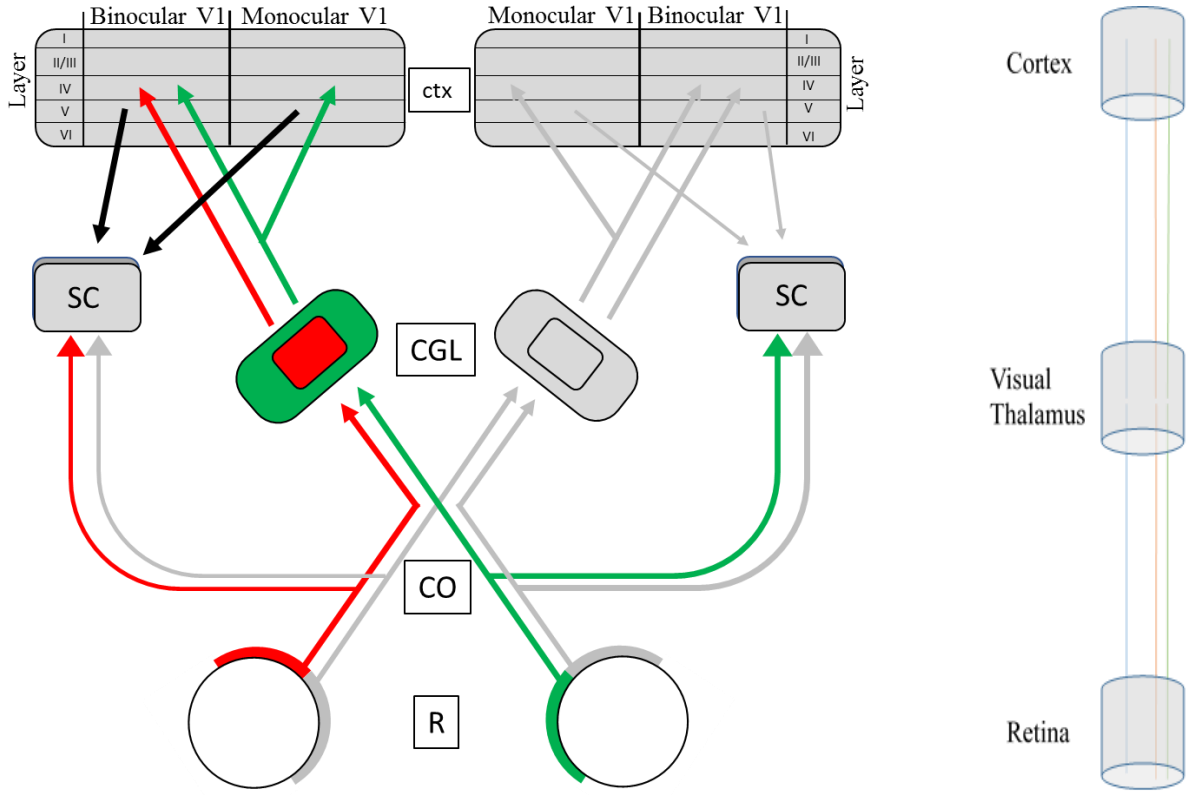

Figure 1: Schematic illustration of the visual pathway in mice. Starting from the retina (R), information is sent over the Chiasma opticum (CO) to the lateral geniculate nucleus (LGN), as part of the thalamus. From here afferents reach Layer IV of the primary visual cortex (ctx). The cortex of one hemisphere gets input from both eyes. In detail, the nasal part of the contralateral eye (green) projects to both parts of primary visual cortex, the binocular and monocular zone, while temporal part of the ipsilateral eye (red) just projects to the binocular part. It also sent fibres to the ipsilateral colliculus superior (SC), like Layer V of primary visual cortex does. Right figure illustrates the retinotopic projection from the Retina, over the visual thalamus to the cortex.

1.1.1.1 Visual abilities of mice

As measured by visual discrimination tasks, like the visual water task (VWT), mice visual acuity ranges around 0.5 cycles per degree (cpd) with a contrast sensitivity of 17% at 0.2 cpd (Prusky et al., 2000, Prusky and Douglas, 2004, Teichert et al., 2018). The data obtained from

the VWT depend on V1, as shown by Prusky and Douglas. When they aspirated V1, they found a significant decrease in visual function (Prusky and Douglas, 2004).

Another example for visual evoked behaviour is the optokinetic reflex (OKR), a head and eye movement, mediated by subcortical structures, including SC, which stabilizes images on the retina (Prusky et al., 2004, Liu et al., 2016). Here, the reflex is detectable with the virtual optomotor system till 0.4 cpd and 13% contrast at 0.2 cpd. Cortex aspiration has no influence on the threshold (Douglas et al., 2005), but an increased V1 activity also improves this reflex (Prusky et al., 2006, Liu et al., 2016).

1.1.2 The auditory system of the mouse

In terrestrial animals sound waves are translated into electrophysiological information by the auditory system, which then provides auditory sensation. The auricle (pinna) collects sound waves and passes them through the meatus acusticus externus to the tympanic membrane. The ossicles (malleus, incus and stapes), building the air-filled middle-ear, transfer the vibrations of the tympanic membrane to the smaller oval window which rests on the inner ear. Here the cochlea, an endolymph-filled labyrinth, is located which contains the sensory epithelium known as the organ of corti with all its inner and outer hair-cells. These cells translate the mechanic vibrations into electrophysiological signals. The frequencies to which the ear of the mouse responds range between 2-100 kHz (Koay et al., 2002). Each frequency activates a different region of the organ of corti. While low frequencies activate the end of the cochlea (C), high frequencies are detected at the beginning, near the oval window. The axon of the hair-cells project as the cochlea nerve (CN) to the cochlear nucleus (CN), the first station of the ascending auditory pathway. Over the superior olivary complex (SO) and the inferior colliculus (IC) the axons reach the medial geniculate nucleus (MGN) of the thalamus. From here the information is transmitted to primary auditory cortex (A1) and from here further on to higher order cortical areas. Similar to the visual system, one finds a tonotopic organized projection, as illustrated in figure 2 (Malmierca and Ryugo, 2012).

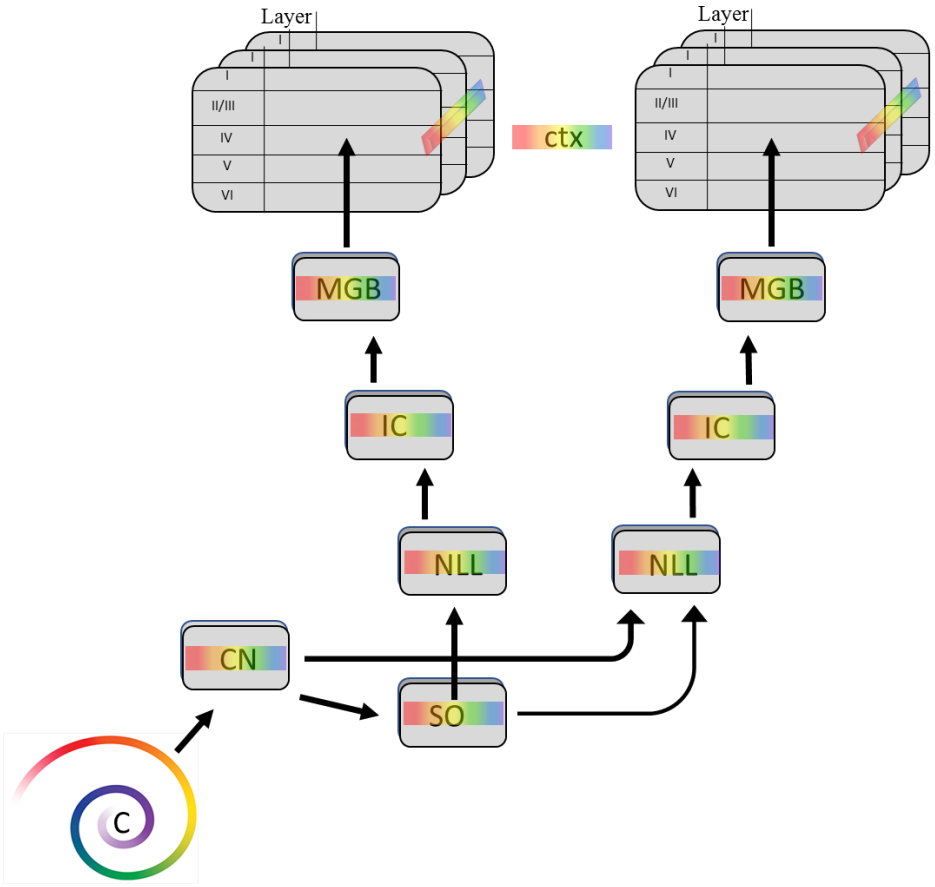

Figure 2: Illustration of the auditory pathway in mice. Information from the cochlea (C) project over the cochlea nucleus (CN) to the superior olivary complex (SO) and from here ipsilateral over the nuclei of the lateral lemniscus (NLL) to the inferior colliculus (IC). This region then project to the medial geniculatum body (MGB), as part of the thalamus and then to Layer 4 of the auditory cortex (ctx). There are also fibres sent from the cochlea nucleus to the nuclei of the lateral lemniscus (NLL) of the contralateral hemisphere, also reaching the cortex over mentioned structures. The tonotop projection is represented by rainbow-colours.

1.1.3 The somatosensory (whisker) system of the mouse

The somatosensory system collects information from inner and outer stimuli. For this, different receptor-types are used, which are located in the skin, the muscles and joints. These receptors are sensitive to thermal, noxious and /or mechanical stimuli. A well-studied system of mechanoreceptors is the mouse whisker-system. Beside the snout, the mouse, as other rodents and carnivore mammals (except bears), got long, hairs with a cylindroid shape. These so-called whiskers or vibrissae are arranged in five rows, while the upper row, consisting of 4 whiskers, is the a-row and the lowest the e-row, with up to seven whiskers (Woolsey et al., 1975). With these whiskers the mice can actively explore their environment, locate objects and collect information about their shape and texture. Each whisker follicle is rooted in a complex structure,

build up by vascular sinuses, muscle spindels for active movements and 100-200 axons (Lee and Woolsey, 1975). The axons of all whiskers build the afferent infraorbital nerve (ION) as part of the trigeminal system and project over different brainstream structures to the posteromedial complex (VPM) and the posteromedial complex (PoM)of the thalamus (Van Der Loos, 1976). From here, afferents predominately end in layer IV of the primary somatosensory cortex (S1) which is organized in patches. Each of these patches, the so called barrels, represents inputs of one main whisker. These barrels are arranged in a somatotopic manner (Welker, 1971), representing the arrangement of the whiskers on the snout.

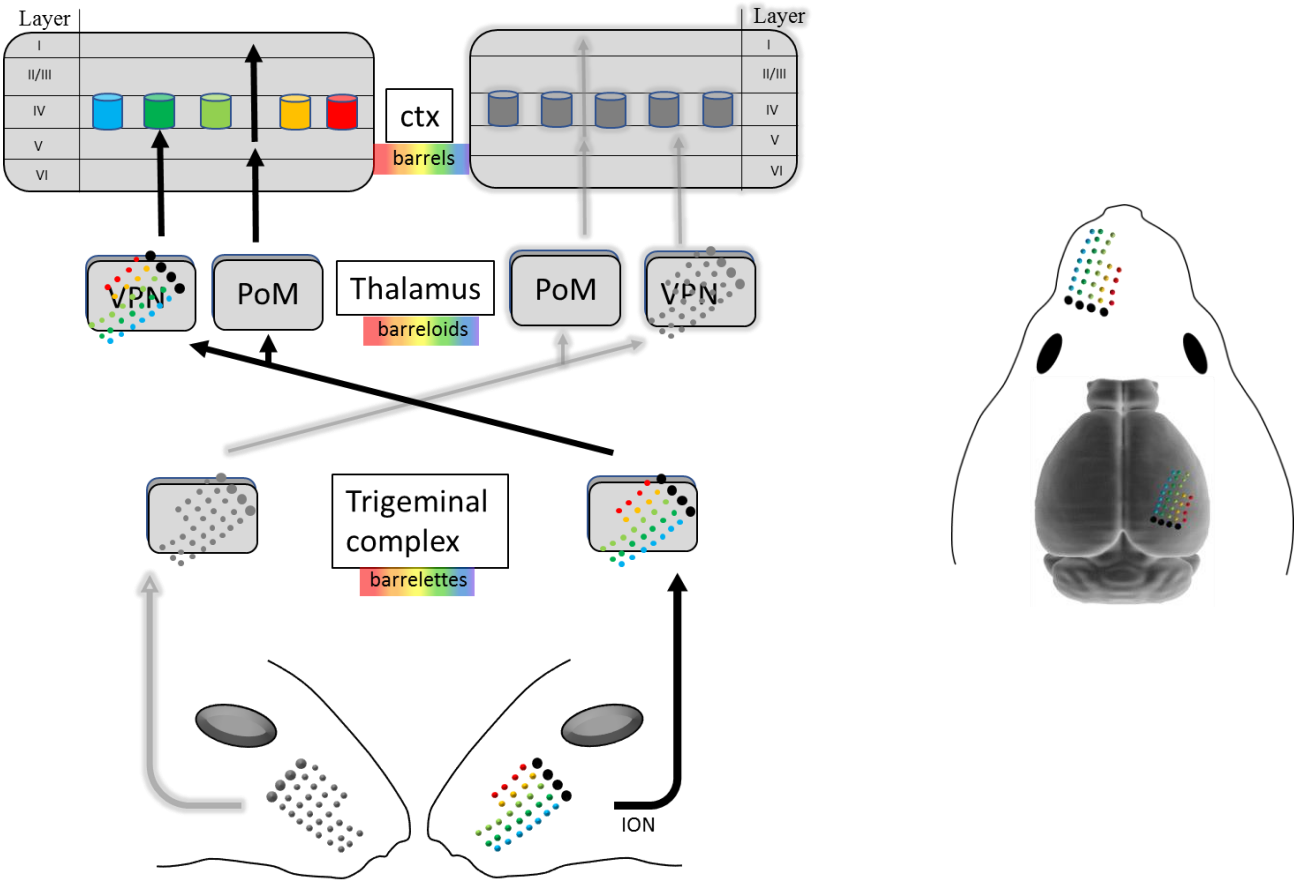

Figure 3: Schematic illustration of the whisker to brain pathway. Whisker project over the infraorbital nerve (ION) to the trigeminal complex and from here to the other hemisphere, reaching to distinct regions of the thalamus, the ventral posteromedial complex (VPM) and the posteromedial complex (PoM). VPM projects to Layer 4 of the barrel cortex, as part of the somatosensory cortex, while PoM projects to Layer 5 and 1. Somatotopic projection is represented by rainbow-colours. Right figure illustrates, that the arrangement of the whiskers is also present in the barrelfield (somatotopic projection).

1.2 Unimodal plasticity in primary sensory cortices

As I investigated, if manipulations of the somatosensory or auditory sense provokes plastic changes in primary visual cortex, I first will give an introduction into unimodal plasticity processes caused by the manipulation of the corresponding sensory organ, known as unimodal plasticity. This is necessary to understand of how I detected cross-modally induced plastic changes in V1.

In general, neuronal circuits of the primary sensory cortices are highly dynamic and synapses are modulated by changes in neuronal activity caused by sensory experience. This so-called neuronal plasticity allows experience-dependent structural and functional reorganization as an adaption to changes of the environment and/or its perception.

1.2.1 Plasticity in mouse primary visual cortex

When Hubel and Wiesel examined the plasticity of cat and monkey visual cortex, they found a change in the responsiveness of neurons in the primary visual cortex to visual stimuli, when one eye was deprived for a while (Hubel and Wiesel, 1998, Hubel and Wiesel, 1970, Hubel et al., 1977, Wiesel and Hubel, 1963). They developed the concept of the so called “critical periods” as they saw, that the alterations caused by the deprivation of one eye (monocular deprivation, MD) in neuronal circuits are age dependent. Only early in life, during brain development, the monocular deprivation leads to profound and permanent changes in V1. Since then the mouse became a famous animal model to investigate experience-dependent plasticity. Also, in mice the neurons of the binocular zone of primary visual cortex are driven by projections coming from both eyes. But most neurons in the binocular zone are more excited by the input of the eye, which is located contralateral to the recorded hemisphere. This phenomena is called ocular dominance (OD) (Frenkel and Bear, 2004, Gordon and Stryker, 1996).

The temporally closure of the dominant eye causes changes in the ocular dominance. This type of experience dependent plasticity is called ocular dominance plasticity. In mouse the strongest shift appears between 28 and 32 days of age, during their critical period. During this time the deprivation of the contralateral eye for 4-7 days leads to a decrease of the deprived eye input on V1 cells (Hofer et al., 2006, Kaneko et al., 2008a, Kaneko et al., 2008b), which is followed by a strengthening of the inputs coming from both eyes. The underlying mechanisms for the

opening and the closure of the critical period are diverse. Hensch et al. could show that the opening of the critical period depends on the development of a proper level of inhibitory transmission provided by the neurotransmitter y-aminobutry-acid (GABA), which is typical for a specific class of neurons, the interneurons. When he reduced GABA-levels by the knock-out of the GAD65-gene, involved in GABA expression and release, the critical period did not open until he compensated the reduced GABA-levels by the administration of Diazepam, a GABA-A receptor agonist (Hensch et al., 1998, Katagiri et al., 2007, Hensch, 2005). The mechanism which then weakens the deprived eye input on neurons in V1 is thought to be Long-Term-Depression (LTD)-like, a mechanism, which leads to a reduction in the efficacy of synapses (Heynen et al., 2003, Frenkel and Bear, 2004). The following strengthening of the input of both eyes is suggested to depend on Long-Term-Potentiation (LTP) and homeostatic plasticity mechanisms, such as synaptic scaling (Mrsic-Flogel et al., 2007, Kaneko et al., 2008b, Ranson et al., 2012). LTP strengthens synapses between neurons, involving the N-methyl-D-aspartate (NMDA) receptor. This kind of ionotropic glutamate receptor is located in the postsynaptic membrane and acts like a coincidence detector, for the simultaneous activation of the pre- and postsynaptic cell, which reacts in the presence of Glutamate and upon an upcoming depolarization (Luscher and Malenka, 2012). While LTD and LTP modify synaptic strength selectively, the process of synaptic scaling includes all synapses and regulates the relative overall synaptic strength, to stabilize the activity of the neuron, by modulating the number of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, also a glutamate receptor, which is responsible for the majority of excitatory synaptic currents (Turrigiano, 2008). After postnatal day (PD) 32 critical period closes, depending on the maturation of inhibitory inputs (Huang et al., 1999, Levelt and Hubener, 2012).

After the critical period it is also possible to induce ocular dominance plasticity by the temporal closure of the contralateral eye, but it shows distinct features from the critical period plasticity. The deprivation time has to be longer, the shift in ocular dominance is of smaller magnitude and is characterized by the potentiation of the ipsilateral eye input, while the contralateral is unaffected (Lehmann and Lowel, 2008, Sato and Stryker, 2008, Sawtell et al., 2003, Hofer et al., 2006, Hofer et al., 2009). Moreover, this form of plasticity is accompanied with a reduction of the inhibitory tone in V1, changing the excitatory/inhibitory balance (Harauzov et al., 2010, van Versendaal et al., 2012). Beyond the 120 days of age a shift of ocular dominance is absence in C57BL/6 J mice, reared under standard conditions (Lehmann and Lowel, 2008). The answer to the question, why beyond PD 120 ocular dominance plasticity is absent, lies in the efforts done to restore it. Lots of interventions that restore ocular dominance plasticity influence the

balance between excitation and inhibition. In detail, exposing animals to an enriched environment, with more sensory-motor interactions, than in standard cages, restored ocular dominance plasticity by decreasing intracortical inhibition (Baroncelli et al., 2010). Also a brief period of dark-exposer (DE) decreases the activation of inhibitory neurons in V1 and restores plasticity (Stodieck et al., 2014, He et al., 2006). Moreover it is shown, that the chronic administration of the antidepressant fluoxetine reduces the cortical inhibition tone and enables experience-dependent plasticity in V1 (Maya Vetencourt et al., 2008, Ruiz-Perera et al., 2015). Furthermore, there are also non neuronal components influencing plasticity in V1. For example, Neurons are embedded in the extracellular matrix (ECM), formed by Chondroitin sulphate proteoglycans (CSPGs). The maturation of the ECM is found to restrict ocular dominance plasticity in rodents, by inhibiting axonal sprouting. Hence, the degeneration of the ECM reactivated cortical plasticity (Pizzorusso et al., 2002).

1.2.2 Plasticity in mouse primary auditory and somatosensory cortex

Also, the auditory and somatosensory cortices can be altered by changes in sensory experience. Presenting an animal a tone with a defined frequency between PD 11 to 15, expands the cortical representation of this frequency. This effect declines with age, like in visual cortex (Zhang et al., 2001, de Villers-Sidani et al., 2007, Barkat et al., 2011), depending on the composition of perineuronal nets, postsynaptic densities, inhibitory transmission (Carulli et al., 2010, van Zundert et al., 2004, Sanes and Kotak, 2011) and Hebbian mechanisms (LTP and LTD) (Chun et al., 2013, Liu et al., 2015b). Also, whisker manipulations alter S1 maps. This can be observed over the whole life-circle in rodents. In young and adult animals the deprivation of a single whisker, a row or more complex deprivation modi leads to rapid map plasticity (Fox, 2002), like an enhancement of spared whiskers response in the surrounding barrels, for example (Wallace and Fox, 1999, Kossut, 1998, Kole et al., 2018). This is thought to depend on Hebbian mechanisms (Chung et al., 2017). As in V1 plastic changes in S1 are associated with alterations of the inhibitory system (Sammons and Keck, 2015).

1.3 Crossmodal plasticity between primary sensory cortices

Until here I described plasticity mechanisms that take place in the primary sensory cortices caused by the manipulation of the corresponding sensory organ. In the following section I will give an overview of the alterations in the spared primary sensory cortices, caused by the deprivation of another sense, termed as cross-modal plasticity.

Primary sensory cortices predominantly process information coming from the corresponding sensory organ. The multisensory integration was thought to take place upstream, in higher cortical areas (Felleman and Van Essen, 1991), but during the last years this view has changed. Many studies could show, that each sensory modality can influence the information processing of the other senses, already on the level of primary sensory cortices. As a substrate for this multimodal interplay between primary sensory cortices several tracing studies in rodents provide evidence that primary sensory cortices are interconnected. It was shown that S1 and A1 directly project to V1 (Henschke et al., 2015, Teichert and Bolz, 2017, Ibrahim et al., 2016, Campi et al., 2010, Masse et al., 2017, Budinger and Scheich, 2009). On the other hand, V1 projects to S1 and weakly to A1. But there is also a reciprocal connection between S1 and A1 (Henschke et al., 2015). Moreover, primary sensory cortices receive subthreshold inputs coming from other sensory modalities (Iurilli et al., 2012, Ibrahim et al., 2016, Campi et al., 2010, Lakatos et al., 2007, Sieben et al., 2013). For example, Iurilli and colleagues could show, that stimulating the whiskers hyperpolarized supragranular pyramidal cells in V1. Furthermore, they found the effect depending on the intracortical connection between S1 and V1 and that the hyperpolarization in V1 is mediated by an increased activity of inhibitory cells in V1 (Iurilli et al., 2012). This idea is supported by in vitro electrophysiological findings. Ibrahim and colleagues demonstrated that Layer 1 and 2/3 inhibitory neurons in V1 receive direct excitatory input from A1 (Ibrahim et al., 2016). These intracortical connections promote the mutual modulation of sensory processing which takes place during perception in the daily life.

Interestingly, losing a sensory modality also leads to changes in the spared primary sensory cortices. For instance, in early onset blind people, the areas linked to vision in seeing individuals now display activation in reaction to auditory stimuli (e.g. speech perception) and/or somatosensation (e.g. Braille reading). Here, the deprived cortex is recruited by the other senses (Sadato et al., 1996, Cohen et al., 1997, Buchel et al., 1998, Roder et al., 1999, Van Boven et al., 2000, Dietrich et al., 2013). Accompanied with this recruitment the remaining senses are often better. Blind people display an enhanced and faster sound source localization (Nilsson and Schenkman, 2016, Van Boven et al., 2000) and a better tactile spatial resolution (Van

Boven et al., 2000), for example. This recruitment is thought to be realized by an unmasking of pre-existing connections coming from other senses (Merabet et al., 2008, Bavelier and Neville, 2002) and not restricted to young individuals. Few studies could show that even in adults the recruitment of the deprived cortex takes place (Campbell and Sharma, 2014, Glick and Sharma, 2017, Buchel et al., 1998)

But the loss of one sense not only leads to a spatial expansion of the spared cortices. There are more alterations affecting both, the spared and deprived cortex. Goel and colleagues could show, that the manipulation of visual experience in form of dark-rearing for one week or enucleation changed AMPA receptor mediated mEPSCs in layer 2/3 in the visual, auditory and somatosensory primary sensory cortices. In visual cortex they found an increase in mEPSC amplitudes, while they were decreased in auditory and somatosensory cortices (Goel et al., 2006, He et al., 2012). These bidirectional changes are in line with homeostatic plasticity mechanisms, described by Turrigiano and Nelson, where a deprivation of inputs increase AMPA receptor function, whereas an increase in activity decrease it (Turrigiano and Nelson, 2004). Here a study could show that visual deprivation sharpens the receptive field in the barrel cortex within two days, for example (Jitsuki et al., 2011). Next to this homeostatic mechanisms some studies found evidence for a strengthening of thalamocortical transmission, accompagnied with a refinement of the spared senses. Visual deprivation improves frequency selectivity and sound discrimination performance of A1 neurons in adult rodents, by the strengthening of thalamocortical synapses, for example (Petrus et al., 2014, Meng et al., 2015, Meng et al., 2017). The seen potentiation of feed-forward excitatory synapses in Layer 4, seem to depend on LTP a mechanism also involved in experience dependent plasticity (Lee and Whitt, 2015). But until now less is known about the cross-modally induced refinement of senses on the behaviour level in fully adult mice.

2. Open questions addressed in this thesis

This work tries to answer following questions:

- **Question 1:**
  Does the deprivation of one sensory modality improve visual performance in fully adult mice on the cortical and behavioural level?

- **Question 2:**
  If so, has the late onset of sensory deprivation (auditory or somatosensory deprivation) also the power to restore ocular dominance plasticity in adult mice, far beyond their critical period?

- **Question 3:**
  If so, does it share common features which are typical for the adult form of ocular dominance plasticity, like reduced GABA levels in V1?

- **Question 4:**
  Is there a time-course of the cross-modally provoked changes in the spared sensory cortex**?**

Question 1: It was shown before, that the deprivation of one sensory modality improves performance of the spared ones in young animals. Different studies show this, on the level of primary sensory cortices, via electrophysiological recordings. I was interested in the question, if cross-modal improvements can also be seen on the behavioural level in fully adult animals. For this, I supervised experiments to determine cortex dependent visual acuity and contrast sensitivity of adult mice, using a psychophysiological test, the visual water task (VWT), before and after somatosensory deprivation, in form of whisker-plucking.

See manuscript 1, entitled: Cross-modal refinement of visual performance after brief somatosensory deprivation in adult mice

Question 2: Adult mice (older than 110 days) display no changes in the ocular dominance in reaction to the occlusion of one eye any more. After seeing that sensory deprivation has a great impact on information processing in the spared cortex, I asked, if somatosensory or auditory deprivation has the power to restore ocular dominance plasticity. For this, I measured, using the method of optical imaging of intrinsic signals, the responsiveness of primary visual cortex to the stimulation of each single eye. After that, the dominant eye was sutured for one week and after reopening the responsiveness of V1 to the stimulation of eye was checked again. Moreover, I systemically administrated drugs to investigate possible mechanisms.

See manuscript 2, entitled: Cross-modal restoration of ocular dominance plasticity in adult mice

Question 3: For further characterization of the effects on the primary visual cortex due to the deprivation of auditory or somatosensory deprivation, GABA levels in V1 were analysed via HPLC and I performed pharmacological interventions combined with the occlusion of the dominant eye and the method of optical imaging of intrinsic signals.

See manuscript 3, entitled: Cross-modal restoration of juvenile-like ocular dominance plasticity after increasing GABAergic inhibition

Question 4: Less is known about the time-course of the cross-modal effects in the spared cortices provoked by the deprivation of one sensory modality. To investigate this, I plucked all main whisker and obtained the responsiveness of V1 in reaction to the stimulation of each eye, using the method of optical imaging of intrinsic signals on different time-points (0, 3 and 7 days) after somatosensory deprivation. Also, in this case HPLC experiments were performed to track changes in neurotransmitter contents in V1. Parallel to these experiments I used an optomotor system to determine if, thresholds of the optokinetic reflex are altered by whisker or auditory deprivation.

See manuscript 4, entitled: Visual deprivation independent shift of ocular dominance induced by cross-modal plasticity

3. Overview of the accepted manuscripts (peer reviewed journals)

Manuscript 1

Cross-modal refinement of visual performance after brief somatosensory deprivation in adult mice

Manuel Teichert[1], **Marcel Isstas[1]**, Steven Wenig[1], Christoph Setz[1], Konrad Lehmann[1] and Jürgen Bolz[1]

[1]University of Jena, Institute for General Zoology and Animal Physiology, 07743 Jena, Germany

European Journal of Neuroscience

2018

DOI: 10.1111/ejn.13798

In this study my colleagues and I investigated the effects of somatosensory deprivation, in form of whisker deprivation, on the visual abilities in adult mice. For this, we first determinate visual acuity and contrast sensitivity in a behavioural two-choice discrimination task, called visual water task. After determining thresholds, we plucked all whiskers in one group and let them intact in controls. Further testing revealed that after 7 till 12 days of whisker deprivation the animals developed a better sight. Acuity and contrast sensitivity increased about 40%. These data were confirmed by experiments with the method of optical imaging of intrinsic signals. Here, we found that the activity evoked in primary visual cortex is stronger to weaker stimuli in deprived mice, compared to the control.

Own contribution:

- Study design: 30%
- Behavioural Tasks: 20%
- Data analysis: 20%

Manuscript 2

Cross-modal restoration of ocular dominance plasticity in adult mice

Manuel Teichert[1*], **Marcel Isstas[1*]**, Yitong Zhang[1] and Jürgen Bolz[1]

[1]Institute of General Zoology and Animal Physiology, 07743 Jena, Germany

[*]**These authors contribute equally to this study**

European Journal of Neuroscience

2018

DOI: 10.1111/ejn.13944

It is well established, that experience dependent ocular dominance plasticity in primary visual cortex declines with age and is absent in animals older than 110 days. After finding, that visual cortex is involved in the improvement of visual abilities in mice of that age and older, we ask ourselves, if somatosensory or auditory deprivation also restores ocular dominance plasticity. To investigate this, we combined somatosensory or auditory deprivation with monocular deprivation. In these animals, ocular dominance shifted in favour of the open eye. Looking for a possible mechanism, we found that the ODI shift is absent in animals, additionally treated with CCP, a NMDA-receptor antagonist, suggesting, that Hebbian mechanisms (i.e. LTP) are involved in the observed effect.

Own contribution

- Study design: 30%
- Mouse preparation: 50%
- Optical imaging recordings: 50%
- Analysis of the data: 50%
- Preparing the manuscript: 30%

Manuscript 3

Cross-modal restoration of juvenile-like ocular dominance plasticity after increasing GABAergic inhibition

Manuel Teichert[1*], **Marcel Isstas[1*]**, Franziska Wieske[2], Christine Winter[2] and Jürgen Bolz[1]

[1]Institute of General Zoology and Animal Physiology, 07743 Jena, Germany

[2]Department of Psychiatry, Technical University Dresden, 01062 Dresden, Germany

***These authors contribute equally to this study**

Neuroscience

DOI: 10.1016/j.neuroscience.2018.09.040

After finding, that whisker and auditory deprivation has the power to restore ocular dominance plasticity in fully adult mice, we asked ourselves if changes in the excitation-inhibition ratio is also responsible for the observed effects, as it well described in literature. Revealed by HPLC analyses we found that the overall GABA content is decreased in primary visual cortex after seven days of whisker removal, which might indicate a reduced inhibition-tone. To underpin the role of inhibition in the restoration of ocular dominance plasticity we tried to abolish the ODI-shift by compensating the reduced GABA-levels by the systemic administration of Diazepam, a positive allosteric modulator of $GABA_A$ receptor. To our surprise this treatment did not abolish the ODI-shift but led to a depression of V1 input through the previously closed eye, the characteristic signature of OD plasticity in juvenile mice during the critical period. Combining sensory deprivation with monocular deprivation and the administration of Diazepam and CCP, prevented the OD-shift, suggesting an Hebbian mechanism (LTD) for the observed. Moreover, the results indicate that there are more and so far unknown mechanisms involved in the cross modally evoked plasticity in the spared primary sensory cortices after sensory deprivation.

Own contribution

- Study design: 30%
- Mouse prepararion: 60%
- Optical imaging recordings: 60%

- Mouse preparation for HPLC experiments: 50%
- Analysis of data: 50%
- Preparing the manuscript: 20%

Manuscript 4

Visual deprivation independent shift of ocular dominance induced by cross-modal plasticity

Manuel Teichert[1+], **Marcel Isstas[1+]**, Lutz Liebmann[2], Christian A. Hübner[2], Franziska Wieske[3], Christine Winter[3], and Jürgen Bolz[1*]

[1] University of Jena, Institute of General Zoology and Animal Physiology, 07743, Jena, Germany

[2]University of Jena, University Hospital Jena, Institute of Human Genetics, 07743, Jena, Germany

[3]Department of Psychiatry, Technical University Dresden, 01062 Dresden, Germany

[+]**These authors contribute equally to this work**

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In this work, I could show, that depriving the whisker leads to a shift in the ODI without manipulating the visual cortex in form of a monocular deprivation. For this I performed chronic imaging experiments immediately after whisker deprivation, again after three days and seven days. On day three after WD, I observed a decreased ODI, mediated by a potentiation of the ipsilateral eye. This effect did not depend on visual input through the contralateral eye, but closing the ipsilateral eye for three days abolished the ODI-shift. This shows, that the potentiation needs visual experience just on the ipsilateral eye. The shift was accompanied by an increasement of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) mediated mEPSC amplitudes, which indicates a strengthening of the excitatory thalamo-cortical synapses in layer IV of V1. Moreover, HPLC experiments show, that the excitatory/inhibitory-ratio in V1 was increased three days after WD, because of higher Glutamat levels. These changes in the balance of excitation and inhibition were previously

shown to restore ocular dominance plasticity. Because of this I administrated Diazepam to rebalance the Glutamat/GABA-ratio. This treatment abolished the ODI-shift after three days of WD.

As the electrophysiological results from above show higher AMPA receptor mediated mEPSC amplitudes, I also tested the role of NMDA receptors, by antagonizing them by the administration of CCP. This treatment also blocked the ODI-shift.

Moreover, I could show that whisker deprivation and auditory deprivation leads to changes of the optokinetic reflex (OKR), a head and eye movement, mediated by subcortical structures, which stabilizes images on the retina. For this I tested the animals in the optomotor system. Both, the thresholds for the presented spatial frequencies and contrast sensitivity improved in the deprived mice, with a peak after three days. The next days the thresholds came down, but persisted after six days above baseline levels. The aspiration of V1 shows, that only the overshoot after three days depends on changes in V1 activity, but not the slight improvement persisting after six days, which leads to the assumption that WD and AD also provoke compensatory mechanisms in the additional visual pathway, which mainly involves subcortical structures. I could show, that these compensatory mechanisms need the NMDA receptors as the effects were abolished by the administration of CPP.

Own contribution

- Study design: 30%
- Mouse preparation: 60%
- Optical imaging recordings: 60%
- HPLC experiments: 10%
- Electrophysiological recordings: 0%
- Analysis of the data: 50%
- Behavioural task: 100%
- Preparing the manuscript: 20%

4. Manuscripts

4.1 Manuscript 1

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FENS Federation of European Neuroscience Societies

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COGNITIVE NEUROSCIENCE

Cross-modal refinement of visual performance after brief somatosensory deprivation in adult mice

Manuel Teichert, Marcel Isstas, Steven Wenig, Christoph Setz, Konrad Lehmann and Jürgen Bolz

Institute for General Zoology and Animal Physiology, University of Jena, Erbertstraße 1, 07743 Jena, Germany

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Keywords: contrast sensitivity, cross-modal plasticity, somatosensory deprivation, visual acuity, visual cortex

Abstract

It is well established that the congenital lack of one sensory modality enhances functionality in the spared senses. However, whether a late onset deprivation of one sense leads to such alterations is largely unknown. Here, we investigated whether a somatosensory deprivation induced by bilateral whisker removal affects visual acuity and contrast sensitivity in fully adult mice. Using the visual cortex-dependent visual water task, we found that a brief somatosensory deprivation markedly improved behavioral visual acuity and contrast sensitivity by about 40%. Determining these attributes of vision using periodic optical imaging of intrinsic signals in the same mice revealed that visual cortex responses elicited by weak visual stimuli were massively increased after somatosensory deprivation. Strikingly, comparison of visual acuity and contrast sensitivity values determined by the visual water task and intrinsic signal imaging revealed that these measurements were almost identical, even at the level of individual animals. In summary, our results suggest that a brief manipulation of somatosensory experience profoundly boosts visual cortex-dependent vision in adults.

Introduction

It has been demonstrated that an early onset sensory deprivation can have striking effects on the spared sensory cortices (Bavelier & Neville, 2002; Lomber *et al.*, 2011). Such changes are broadly referred to as 'cross-modal plasticity' and can lead to a compensatory functional enhancement of the remaining senses (Bavelier & Neville, 2002; Lee & Whitt, 2015). For example, superior visual abilities have been shown for congenitally deaf individuals and experimental animals (Neville & Lawson, 1987; Lomber *et al.*, 2010). Likewise, early onset blind individuals display enhanced auditory functions (Lessard *et al.*, 1998; Roder *et al.*, 1999). Moreover, there is growing evidence that such compensatory changes also take place after a short period of sensory deprivation in juvenile animals. In this regard, it was demonstrated that 1 week of dark exposure (DE) leads to an improved frequency selectivity and discrimination performance of neurons in the auditory cortex (A1; Petrus *et al.*, 2014). In addition, the same treatment refined intra- and inter-laminar connections in A1 (Meng *et al.*, 2017).

However, it remains unclear whether these cortical alterations lead to an improved perception at the behavioral level. In addition, it is poorly understood whether an enhanced processing in the remaining sensory cortices also appears after a very late onset deprivation of other sensory modalities.

To address these issues, we performed a somatosensory deprivation by bilateral whisker deprivation (WD) in fully adult mice far beyond their visual critical period (Hensch, 2005; Lehmann & Lowel, 2008) and investigated its effects on visual cortex function.

Materials and methods

Animals and rearing conditions

C57BL/6J (Jackson labs) mice were raised in transparent standard cages on a 12-h light/dark cycle, with food and water available *ad libitum*. Animal housing in our institution is regularly supervised by veterinaries from the state of Thuringia, Germany. For this study, we used a total of 28 adult male mice (P120-P240). All experimental procedures have been performed according to the German Law on the Protection of Animals and the corresponding European Communities Council Directive of November 24, 1986 (86/609/EEC), and were approved by the Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (Thuringia State Office for Food Safety and Consumer Protection) under the registration numbers 02-050/14 and 02-032/16.

Whisker deprivation (WD)

Whisker deprivation was performed as described previously (He *et al.*, 2012). Briefly, animals were deeply anesthetized with 2%

Correspondence: Jürgen Bolz, as above.
E-mail: jurgen.bolz@uni-jena.de

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isoflurane. Whiskers (macro vibrissae) were plucked bilaterally using fine forceps. Subsequently, mice received an injection of carprofen (4 mg/kg, s.c.) and were returned to their standard cages. Over the following days, whiskers were re-shaved every other day. Control mice were also anesthetized with 2% isoflurane, and whiskers were sham-plucked by gently pulling each vibrissa using fine forceps. Anesthesia was maintained at the same time as for a typical WD surgery (10 min). In addition, control mice received the same dosage of carprofen (4 mg/kg, s.c.).

Visual water task (VWT)

To assess behavioral contrast sensitivity and visual acuity, we used the VWT, a visual cortex-dependent visual discrimination task based on reinforcement learning (Prusky *et al.*, 2000b, 2004). For determining contrast sensitivity and visual acuity, we used a total of 20 mice. Initially, animals were trained to distinguish a vertical sine wave grating with a low spatial frequency (0.1 cyc/deg) and 100% contrast from a grey with the same luminance. Subsequently, their ability to perceive varying contrasts frequencies at a spatial frequency of 0.2 cyc/deg was tested. In another group of mice, we determined visual acuity at 90% contrast. The apparatus is a water-filled trapezoidal-shaped pool, with two monitors placed side by side on the wider end. A midline divider between the two monitors sets the choice point between both visual stimuli. Below the monitor showing the sine wave grating, a submerged platform is placed invisible to the animals. The position of the grating and the platform is changed in a pseudorandom manner during training and testing. Animals were trained and tested 10 times in one session, and two sessions separated by 3 h were run in a single day. The task was performed with off-switched room lights. After achieving 90% accuracy, we determined the contrast or spatial frequency thresholds by reducing the contrast or spatial frequency of the sine wave grating until the level of correct attempts dropped below 70%. All runs after WD and an equal number of runs immediately before WD were used to calculate frequency-of-seeing curves for each animal. Percentage of correct responses was plotted against contrast or spatial frequency, respectively. The values at which the curve dropped below 70% were calculated by interpolation.

Optical imaging of intrinsic signals

Mouse preparation for optical imaging

Animals were initially anesthetized with 4% isoflurane in a mixture of 1 : 1 O_2/N_2O and placed on a heating blanket (37.5 °C) for maintaining body temperature. Subsequently, mice received injections of chlorprothixene (40 μg/mouse i.m.) and carprofen (4 mg/kg, s.c.). The inhalation anesthesia was applied through a plastic mask and maintained at 0.5% during the experiment. The animal was fixed in a stereotaxic frame, and we removed the skin of the left hemisphere to expose the visual cortex. The exposed area was covered with 2.5% agarose in saline and sealed with a glass coverslip. Cortical responses were always recorded through the intact skull.

Imaging of visual cortex

Visual stimuli were presented on a high refresh rate monitor (Hitachi Acuvue HM 4921-D) placed 25 cm in front of the animal. Visual stimulation was adjusted so that it only appeared in the binocular visual field of the recorded hemisphere (−5° to +15° azimuth, −17° to +60° elevation). Using a Dalsa 1M30 CCD camera (Dalsa, Waterloo, Canada) with a 135 × 50 mm tandem lens (Nikon, Inc., Melville, NY), we first recorded images of the surface vascular pattern via illumination with green light (550 ± 2 nm) and, after focusing 600 μm below the pial surface, intrinsic signals were obtained via illumination with red light (610 ± 2 nm). Frames were acquired at a rate of 30 Hz and temporally averaged to 7.5 Hz. The 1024 × 1024 pixel images were spatially averaged to a 512 × 512 resolution.

Determining cortical contrast and spatial frequency tuning

Determination of V1 spatial frequency and contrast tuning was performed using optical imaging of intrinsic signals ($n = 28$) as described previously (Teichert & Bolz, 2017). Briefly, visual stimuli were static sine wave gratings of various contrasts (90, 50, 20, 10 and 5% at 0.2 cyc/deg) and spatial frequencies (0.1, 0.2, 0.3, 0.4 and 0.5 cyc/deg at 100% contrast) reversing after 8 s (temporal frequency: 0.125 Hz). To obtain one amplitude map to one contrast or one spatial frequency stimulus, we stimulated both eyes for 2.5 min. For each condition, we averaged at least two amplitude maps. All data were analyzed using MATLAB.

Data analysis

From the recorded frames, the signal was extracted by Fourier analysis at the stimulation frequency and converted into amplitude and phase maps using custom software (Kalatsky & Stryker, 2003). The magnitude component represents the activation intensity of the visual cortex. All magnitudes are multiplied with 10^4 so that they can be presented in small numbers.

Statistical analysis

Values of contrast sensitivity and visual acuity of control and WD mice at baseline conditions and after 7–12 days obtained in the VWT were compared by a repeated measure two-way ANOVA. V1 contrast and spatial frequency tuning curves of control and WD mice were compared by a two-way ANOVA with contrast or spatial frequency as a repeated measurement factor. Group data were compared by *post hoc* two-tailed student's *t*-tests, with paired *t*-tests for before–after comparisons and unpaired *t*-tests for between-group comparisons. The resulting *P*-values were then Bonferroni corrected. To analyze the correspondence of VWT data with imaging data, a Wilcoxon signed-rank test was used. In the graphs, the levels of significance were set as *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. Data were analyzed using GRAPHPAD PRISM 7.0 and SPSS and are presented as means and standard error of the mean (SEM) or as measurements of individual animals.

Results

WD markedly improves behavioral visual acuity and contrast sensitivity in adult mice

As a first step, we examined whether bilateral WD enhances behaviorally relevant visual acuity using the visual cortex (V1)-dependent visual water task (VWT; Prusky *et al.*, 2000b; Prusky & Douglas, 2004). For this, trained adult mice (> 120 days) were divided into two groups, a control group ($n = 5$) in which mice received a sham surgery and an experimental group ($n = 6$) in which all whiskers

were removed (WD group). We then determined the visual acuity for all mice in both groups during the following 7–12 days.

Figure 1 depicts representative VWT traces of the visual acuity thresholds of two control (Fig. 1a and b) and two WD mice (Fig. 1c and d). It is clearly visible that in control mice the spatial frequency thresholds remained almost unchanged within the 12 days after the sham surgery. However, within the 12 days after WD, there was a massive increase in spatial frequency thresholds. Interestingly, not all WD mice reached their visual acuity maximum at the same time point after WD, but all of them reached this value between 7 and 12 days after this intervention. Hence, in our quantification, we focused on this time period.

Quantification revealed that visual acuity in control mice remained unaltered during the time tested (Fig. 2a, baseline: 0.51 cpd (cycles per degree) ± 0.04 cpd; after 7–12 days: 0.54 cpd ± 0.03 cpd). In contrast, in the WD group, there was a massive increase in visual acuity 7–12 days after WD by 37% (Fig. 2a, baseline: 0.51 cpd ± 0.01 cpd; after 7–12 days: 0.7 cpd ± 0.03 cpd; control vs. WD after 7–12 days: 0.54 cpd ± 0.03 cpd vs. 0.7 cpd ± 0.03 cpd). Statistical analysis using a two-way ANOVA with repeated measures found no effect of WD by itself ($F_{1,9} = 4.445$, $P = 0.064$), but highly significant effects of time ($F_{1,9} = 44.096$, $P < 0.001$), and an interaction of the two ($F_{1,9} = 21.35$, $P = 0.001$). *Post hoc* comparison revealed differences between visual acuity values of control and WD mice after 7–12 days ($P = 0.012$, Bonferroni-corrected unpaired *t*-test) and between baseline visual acuity values of WD mice and values obtained after 7–12 days ($P = 0.0018$, Bonferroni-corrected paired *t*-test).

Next, we investigated whether WD also affects behavioral contrast sensitivity using the VWT in a separate group of mice. As it has been described that contrast sensitivity of mice peaks around 0.2 cpd (Prusky & Douglas, 2004), contrast values were determined at this spatial frequency. Quantification revealed that animals of the control group ($n = 4$) remained at the same level over the whole time period tested (Fig. 2b, baseline: 17.5% ± 1.93%; after 7–12 days: 18.75% ± 0.95%). However, WD mice ($n = 5$) dramatically decreased their visible contrast thresholds 7–12 days after WD by about 40% indicating a marked improvement of contrast sensitivity in these animals (Fig. 2b, baseline: 17.2% ± 1.28%; after 7–12 days: 12.2% ± 1.2%; control vs. WD animals after 7–12 days: 18.75% ± 0.95% vs. 12.2% ± 1.2%). Statistical analysis using a two-way ANOVA with repeated measures showed no immediate effects of WD ($F_{1,7} = 4.056$, $P = 0.084$) and time ($F_{1,7} = 3.995$, $P = 0.086$), but a significant interaction of the two ($F_{1,7} = 11.098$, $P = 0.013$). This indicates that changes over time depended on the specific treatment. We therefore conducted *post hoc* comparisons and indeed found significant differences in contrast threshold values of control and WD mice after 7–12 days ($P = 0.009$, Bonferroni-corrected unpaired *t*-test) and between baseline contrast thresholds of WD mice and values obtained after 7–12 days ($P = 0.015$, Bonferroni-corrected paired *t*-test). Taken together, these data indicate that a somatosensory deprivation by WD dramatically boosts visual cortex-dependent visual performance in adult mice.

WD improves V1 spatial frequency tuning

Next, we determined cortical spatial frequency tuning using intrinsic imaging in the same control and WD mice whose visual acuity was previously measured in the VWT. The imaging experiments were performed within 4 days after the completion of the VWT tests, thus, 12–16 days after WD. We measured V1 responsiveness to visual stimuli of increasing spatial frequencies (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 cpd). The evoked amplitude values for each condition were fitted by a linear regression, and the spatial frequency at the null

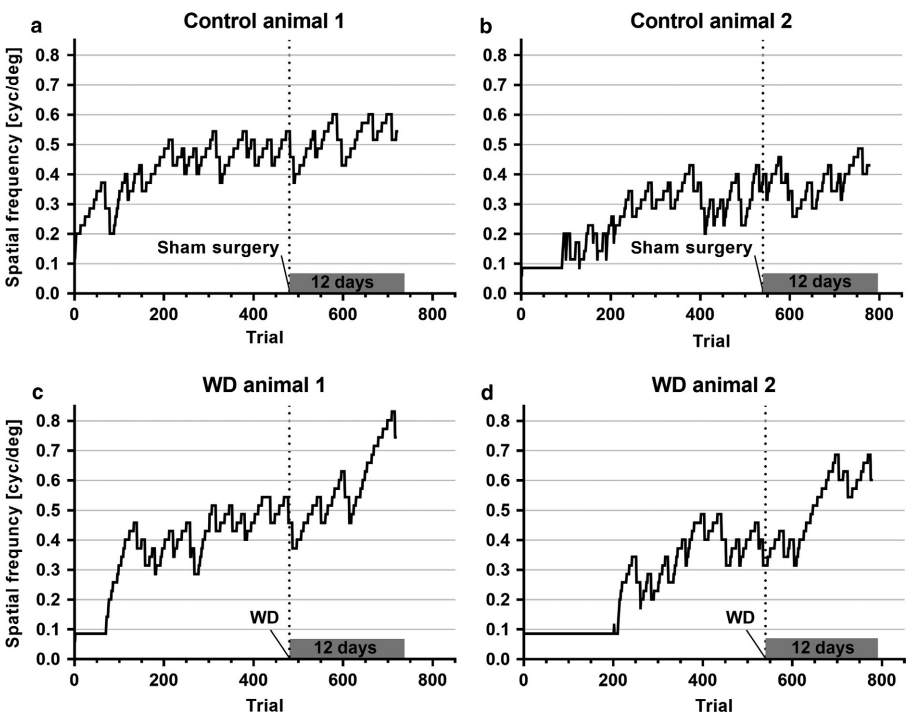

FIG. 1. Behaviorally relevant visual acuity markedly increased after WD. (a, b) Representative VWT traces of two control animals. Visual acuity only slightly increased after sham surgery. (c, d) Representative VWT traces of two WD animals. Visual acuity dramatically increased during the 12 days after WD.

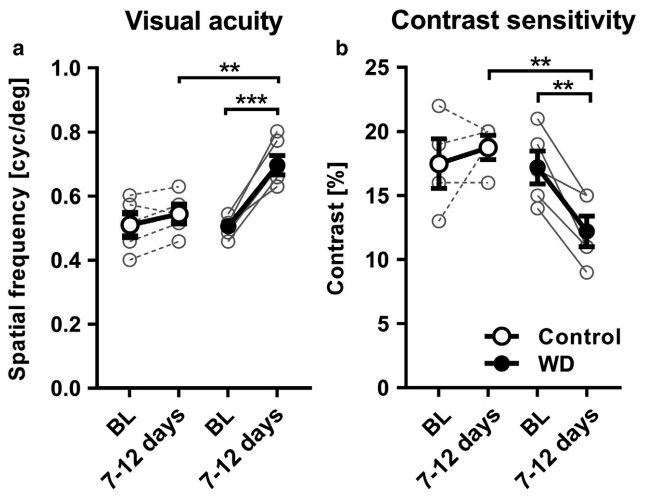

FIG. 2. Somatosensory deprivation boosts behavioral contrast sensitivity and visual acuity. (a) Visual water task measurements revealed that visual acuity of control mice ($n = 5$) remained unchanged. In contrast, after 7–12 days, WD mice ($n = 6$) showed a markedly increased visual acuity. (b) Contrast sensitivity of control animals ($n = 4$) remained at a stable contrast level, whereas in WD mice ($n = 5$) visible contrast was markedly decreased 7–12 days after WD. Bright open circles represent measurements of individual animals. Bold open and closed circles present mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$, paired and unpaired *t*-test, BL, Baseline.

response point was used as a proxy for visual acuity (Heimel *et al.*, 2007, 2010).

Figure 3a schematically illustrates the presented visual stimuli and the elicited V1 amplitude maps of one representative control (middle row) and one WD animal (lower row). It is clearly visible that especially the activity spots evoked by visual stimuli of higher spatial frequency were always darker in WD mice. Quantification showed that in control and WD mice V1 amplitudes (average in the ROI) evoked by all spatial frequency stimuli were always higher (Fig. 3b, control vs. WD; 0.1 cpd: $0.75 \times 10^{-4} \pm 0.08 \times 10^{-4}$ vs. $0.83 \times 10^{-4} \pm 0.06 \times 10^{-4}$; 0.2 cpd: $0.56 \times 10^{-4} \pm 0.06 \times 10^{-4}$ vs. $0.69 \times 10^{-4} \pm 0.06 \times 10^{-4}$; 0.3 cpd: $0.35 \times 10^{-4} \pm 0.04 \times 10^{-4}$ vs. $0.53 \times 10^{-4} \pm 0.05 \times 10^{-4}$; 0.4 cpd: $0.22 \times 10^{-4} \pm 0.05 \times 10^{-4}$ vs. $0.4 \times 10^{-4} \pm 0.04 \times 10^{-4}$; 0.5 cpd: $0.067 \times 10^{-4} \pm 0.03 \times 10^{-4}$ vs. $0.135 \times 10^{-4} \pm 0.05 \times 10^{-4}$). Statistical analysis revealed significant influences of the group ($F_{1,9} = 6.6$, $P = 0.03$, two-way ANOVA with spatial frequency as a repeated measurement factor), but no interactions with the spatial frequency could be detected ($F = 0.86$, $P = 0.495$). The intersection of the cortical visual acuity tuning curve with the null response was shifted to the right in WD animals (Fig. 3b). Thus, in WD mice, V1 cortical visual acuity was markedly increased by about 30% (Fig. 3c, Visual acuity of control and WD mice after 12–16 days: 0.53 cpd ± 0.03 cpd vs. 0.69 cpd ± 0.02 cpd, $P = 0.004$, unpaired *t*-test). These results indicate that a somatosensory deprivation leads to an improved cortical spatial frequency tuning in adult mice.

It has been described that prolonged training near the individual visual threshold can improve vision, a phenomenon broadly referred to as perceptual learning (Schoups *et al.*, 2001; Wang *et al.*, 2016). Hence, we further investigated whether WD acted to facilitate perceptual learning and thereby sharpened visual acuity in the VWT experiments. For this, using intrinsic imaging, we determined cortical spatial frequency tuning in control ($n = 4$) and WD mice ($n = 4$), which were naïve to VWT conditions, 12 days after WD. Quantification showed that VWT naïve WD mice, too, had a markedly increased cortical visual acuity (Fig. 3d, control vs. WD after 12 days: 0.5 cpd ± 0.037 cpd vs. 0.725 cpd ± 0.029 cpd, $P = 0.0031$, unpaired *t*-test). Thus, these results suggest that a short somatosensory deprivation induces a substantial improvement of vision that is independent from perceptual learning.

WD improves V1 contrast tuning

As a next step, we determined V1 contrast sensitivity in the same mice previously tested for their contrast thresholds in the VWT, again using intrinsic signal imaging. Visual stimuli were sine wave gratings of five different contrasts (90, 50, 20, 10 and 5%) with a spatial frequency of 0.2 cyc/deg. To obtain contrast–response tuning curves, the amplitude values of each condition were fitted by a Naka–Rushton equation (Naka & Rushton, 1966; Albrecht & Hamilton, 1982; Heimel *et al.*, 2010). From these curves, we determined the C_{50} as the contrast of the half maximum response.

Figure 4a depicts the presented visual stimuli with varying contrasts and the corresponding evoked V1 amplitude maps of one representative control (middle row) and one WD (lower row) animal. The activity patches evoked by visual stimulation with lower contrasts were always darker after WD. Quantification revealed that visual stimuli of high contrasts evoked a similar activation of V1 in both groups (Fig. 4b, control vs. WD; 90% contrast: 1.03 ± 0.07 vs. 1.06 ± 0.06; 50% contrast: 1.05 ± 0.09 vs. 1.01 ± 0.03). However, low-contrast stimuli evoked always stronger V1 responses in WD mice (control vs. WD; 20% contrast: $0.6 \times 10^{-4} \pm 0.06 \times 10^{-4}$ vs. $0.92 \times 10^{-4} \pm 0.02$; 10% contrast: $0.27 \times 10^{-4} \pm 0.04 \times 10^{-4}$ vs. $0.48 \times 10^{-4} \pm 0.03$). Statistical analysis showed that group had a significant influence ($F_{1,7} = 7.634$, $P = 0.028$, two-way ANOVA with contrast as a repeated measurement factor). In addition, there was a significant interaction between contrast values and group ($F_{4,28} = 5.243$, $P = 0.03$) indicating that visually evoked V1 responses were differentially influenced by WD across the contrast range. Indeed, the average-contrast curve of the WD animals was left shifted compared to the controls (Fig. 4b). Hence, the mean C_{50} was markedly decreased in WD animals by almost 60% (Fig. 4c, C_{50} of control animals and WD animals after 12–16 days: 17.38% ± 0.67% vs. 10.59% ± 0.35%, $P < 0.0001$, unpaired *t*-test).

Next, we tested whether a reduction in apparent contrast was responsible for these results. For this, we scaled the contrast of the WD response curve so that it fitted with the control mice measurements. Indeed, multiplying the contrast by a factor of 1.6 provided a nearly perfect match (Fig. 4d, root mean square error, 0.022). This calculation confirmed that the decreased C_{50} of WD animals was caused by a reduction in the perceived contrast confirming the behavioral VWT data at the neuronal level. In summary, our data strongly suggest that a somatosensory deprivation improves V1 contrast tuning.

Close match of behavior and visually driven V1 activity

Having determined behavioral and cortical visual acuity in the same mice allowed us to compare both measurements at the level of individual animals. Strikingly, the visual acuity values obtained in the behavioral task (VWT) and with optical imaging were almost identical, even at the level of individual animals (Fig. 5a and b, $n = 11$, $P = 0.24$, Wilcoxon signed-rank test). Next, we compared the contrast sensitivity thresholds obtained by the behavioral experiments (VWT) and the C_{50} values obtained by optical imaging in the same WD and control mice. Again, we found that these values were

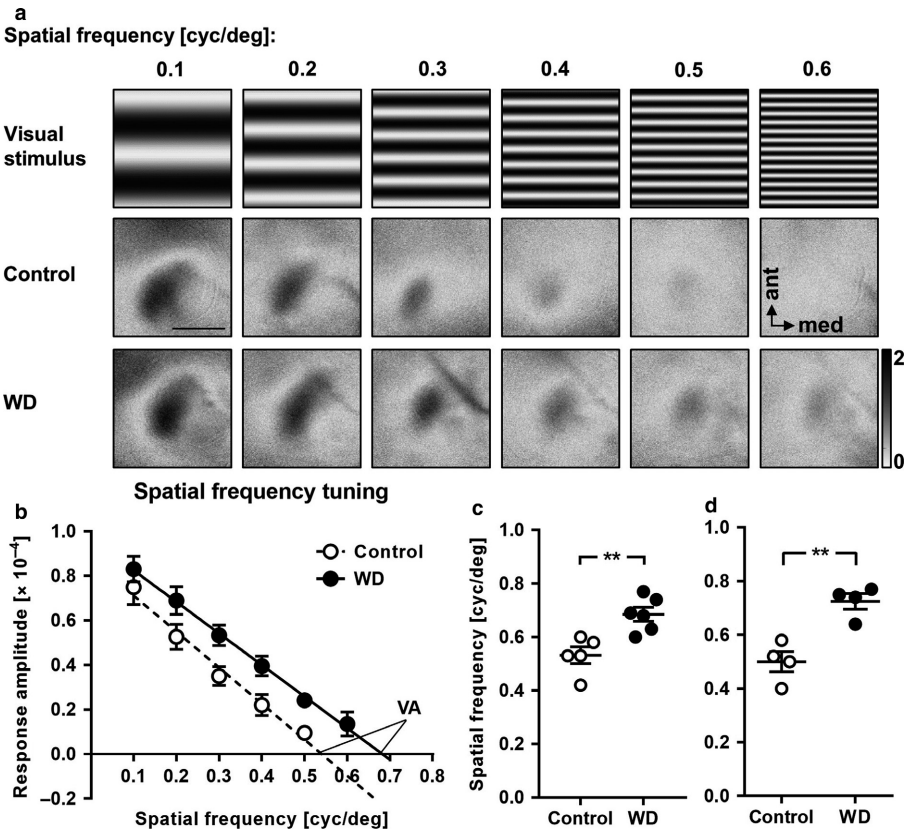

FIG. 3. Intrinsic signal imaging revealed an improved V1 spatial frequency tuning after WD. (a) Schematically illustrated visual stimuli of decreasing spatial frequencies together with the related evoked V1 amplitude maps of one representative control and one WD animal. (b) Average cortical visual acuity (VA) tuning curve was right shifted 12–16 days after WD (n = 6) compared to controls (n = 5). Data are presented as mean ± SEM and means were fitted by a linear regression. In these animals, we previously determined visual acuity in the VWT. (c) Cortical visual acuity of single animals averaged in b. (d) Increased cortical visual acuity in WD mice (n = 4) compared to controls (n = 4) 12 days after WD without previous VWT training. Open and closed circles represent measurements of individual animals. Scatter plots show mean ± SEM, $^{**}P < 0.01$, unpaired t-test. Scale bar: 1 mm.

almost identical (Fig. 5b and c, n = 9, P = 0.093, Wilcoxon signed-rank test) suggesting that the C_{50} obtained by optical imaging of intrinsic signals represents the behaviorally relevant population response of V1 for contrast sensitivity.

These data demonstrate a close match between V1 responsiveness to visual stimuli of different contrasts or spatial frequencies and visually mediated behavior, confirming the high reliability of the imaging method used in this study.

Discussion

Here, we could demonstrate that WD massively enhanced behaviorally relevant visual acuity and contrast sensitivity and V1 spatial frequency and contrast tuning. Thus, our results strongly suggest that a brief deprivation of one sensory modality initiates mechanisms improving perceptual functions in the remaining senses, even in fully adult mice.

Determination of visual abilities by behavioral tests and intrinsic signal imaging

Both the VWT and intrinsic imaging have been shown to provide reliable values for contrast sensitivity and visual acuity (Prusky *et al.*, 2000b; Prusky & Douglas, 2004; Heimel *et al.*, 2007, 2010; Lehmann *et al.*, 2012; Teichert & Bolz, 2017). The data reported in these studies are perfectly in line with values of untreated control mice found in this study. Moreover, we show that contrast and acuity thresholds, both in control and WD mice determined by VWT and periodic optical imaging in the same mice, were practically identical, even at the level of individual animals (Fig. 5). These results demonstrate a strong match between V1 activity and visual cortex-dependent behavior. Thus, V1 has the capacity to extract behavioral relevant information from visual stimuli.

Cross-modal improvements of vision

It has been shown that congenitally deaf cats and humans display enhanced visual abilities (Neville & Lawson, 1987; Lomber *et al.*, 2010). In accordance with these studies, our data show that such compensatory visual alterations can be also induced in the adult visual cortex by a short somatosensory deprivation. Notably, we found cortical contrast sensitivity and visual acuity to be improved by about 40% after WD in both behavioral tasks and intrinsic imaging experiments (Figs 1–4). It has been previously shown that rearing mice in an enriched environment also leads to improved behavioral visual abilities in juvenile mice, which lasted into adulthood, as measured by the visual water task (Prusky *et al.*, 2000a; Cancedda *et al.*, 2004; Sale *et al.*, 2004). For example, visual acuity was improved by about 18% in adult mice after raising them in an enriched environment (Prusky *et al.*, 2000a), which reflects approximately half of the visual acuity enhancement after WD. However, in contrast to the results of this study, visual performance did not

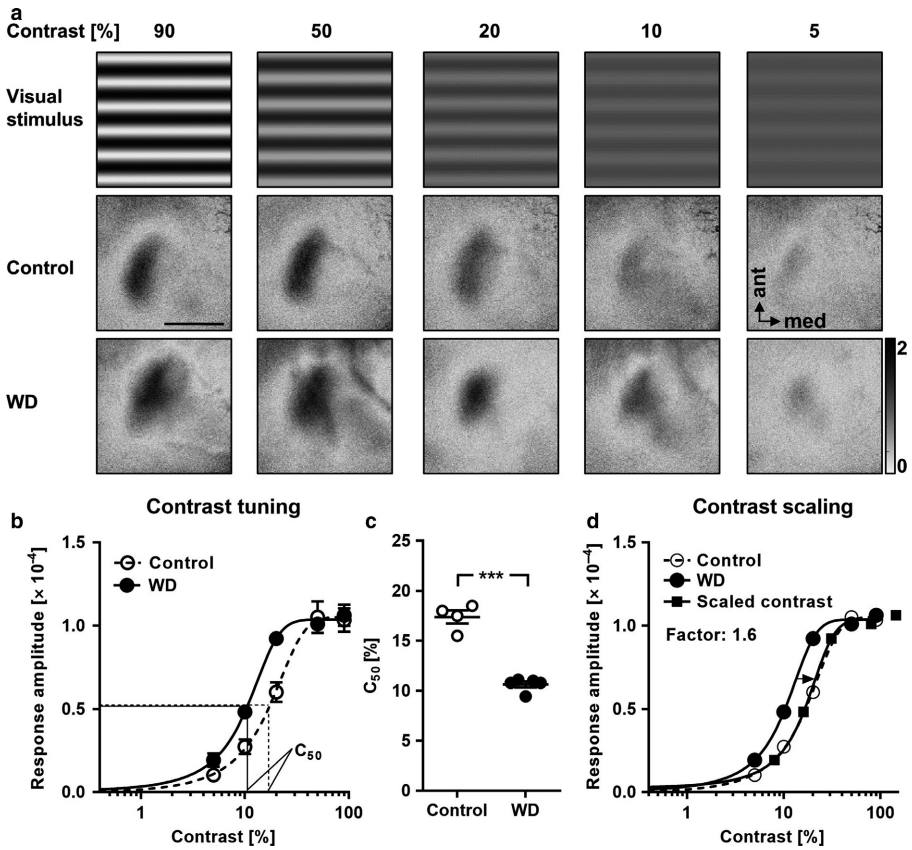

FIG. 4. Intrinsic signal imaging revealed an improved V1 contrast tuning after WD. (a) Schematically illustrated visual stimuli of decreasing contrast together with the corresponding evoked V1 amplitude maps of one representative control and one WD animal. (b) Average-contrast tuning curve was left shifted in WD mice ($n = 5$) compared to controls ($n = 4$). Data are presented as mean ± SEM, and means were fitted by a Naka–Rushton equation. (c) C_{50} contrast was dramatically reduced in WD mice. Open and closed circles represent measurements of individual animals. Scatter plot presents mean ± SEM. (d) Best fit of WD contrast curve with control curve was obtained by scaling the contrast with the factor 1.6 (root mean square error, 0.022). ***$P < 0.001$, unpaired t-test. Scale bar: 1 mm.

improve if the animals were already adult when exposed to enriched environmental conditions (Sale *et al.*, 2007). Another experimental paradigm which has been shown to cause superior visual performance is prolonged training near the individual visual threshold (Hager & Dringenberg, 2010; Sale *et al.*, 2011; Wang *et al.*, 2016). This phenomenon is broadly referred to as visual perceptual learning and can lead to marked improvements of visual acuity and contrast sensitivity in adult mice (Wang *et al.*, 2016) and humans (Polat *et al.*, 2004; Zhou *et al.*, 2006). Our data, however, argue against perceptual learning, as WD mice which were not trained in the VWT also displayed an increased visual acuity, comparable to the acuity values of VWT-WD-mice. Rather, our data indicate that only the loss of whisker input caused compensatory enhancements of behavioral visual abilities suggesting cross-modal plasticity as a potential underlying mechanism.

Potentially, the behavioral visual sharpening effect after WD could be explained by at least two different, but not mutually exclusive cross-modal mechanisms: cross-modal recruitment of the deprived sensory cortex or compensatory plasticity in the spared sensory cortex (Lee & Whitt, 2015). In the experimental paradigm used here (WD), cross-modal recruitment would imply that the deprived somatosensory cortex becomes driven by the spared visual cortex. Hence, the deprived (somatosensory) cortex could act to partially mediate the improved (visual) abilities with the spared sense, as described previously (Cohen *et al.*, 1997; Lomber *et al.*, 2010). While it was originally thought that such changes only appear after congenital or very early loss of one sensory modality (Sadato *et al.*, 2002), there is increasing evidence that, indeed, these regulations can also take place in the adult cortex (Merabet *et al.*, 2008). However, as we did not map the somatosensory cortex after visual stimulation, we cannot make a statement about whether cross-modal recruitment took place in this study.

Instead, using intrinsic signal optical imaging, we found that after WD the spared V1 showed an improved contrast and spatial frequency tuning. This strongly suggests compensatory plasticity within the visual cortex which led to a refinement of V1 processing (Lee & Whitt, 2015). Indeed, previous evidence suggested that functional improvements of the spared sense can be attributed to adaptive plasticity in the spared sensory cortex (Sterr *et al.*, 1998a, b). The observed improvements of V1 contrast tuning after WD can be explained best by the model of contrast gain control (Fig. 4; Soma *et al.*, 2013), as multiplying the tuning curve of WD mice with the factor 1.6 provided an almost perfect match with the contrast tuning curve of control mice. These results suggest that changes in the mean contrast tuning curve can be explained by a reduction in the apparent contrast (Heimel *et al.*, 2010). Notably, these imaging data confirm the results obtained in the VWT, where the apparent contrast was measured directly. Moreover, changes in the apparent contrast are described to provoke changes in visual acuity (Heimel *et al.*, 2010). In particular, enhanced contrast sensitivity is often accompanied by enhanced visual acuity (Heimel *et al.*, 2010). Hence, the improved visual acuity in WD mice

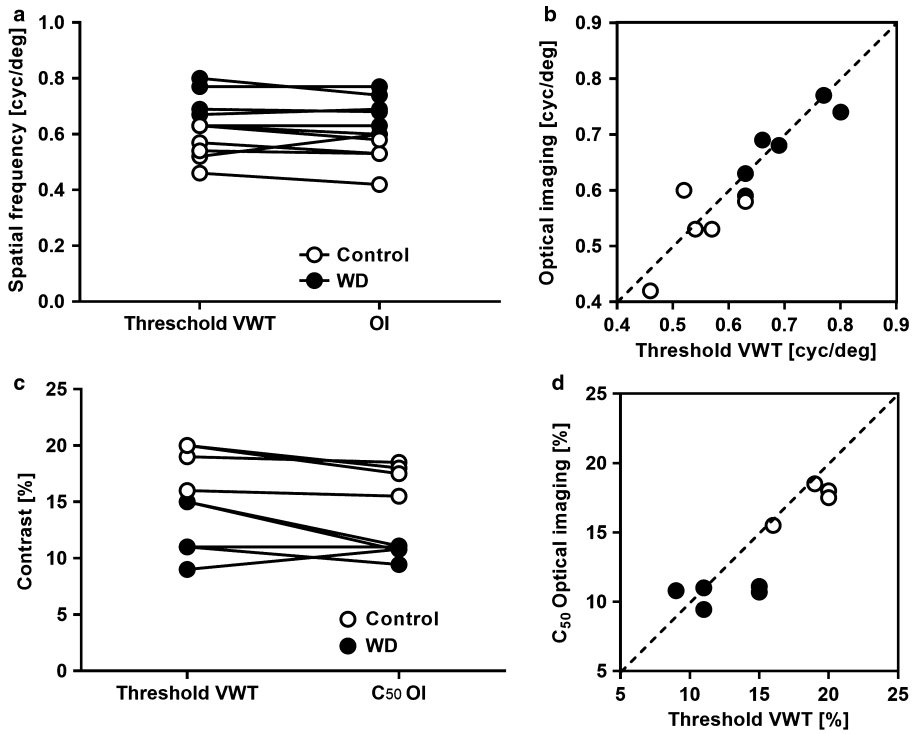

FIG. 5. High reliability of determination of mouse visual perception using intrinsic imaging. (a, b) Individual visual acuity values determined by VWT and intrinsic signal imaging were almost identical ($n = 11$, $P > 0.05$, Wilcoxon signed-rank test). (c, d) Contrast values determined in the VTW and the C_{50} values determined by intrinsic signal imaging were also almost identical ($n = 9$, $P > 0.05$, Wilcoxon signed-rank test). Open and closed circles represent measurements of individual animals. The dashed lines in b and d represent the angle bisectors.

observed in this study might be a result of the enhanced contrast tuning.

Potential mechanisms underlying cross-modal refinements of vision

A recent study demonstrated that 1 week of visual deprivation strengthens thalamo-cortical synapses in A1 in juvenile and adult mice (Petrus *et al.*, 2014). This effect was shown to be accompanied by an increased sensitivity and frequency tuning of A1 neurons (Petrus *et al.*, 2014) and refined intracortical circuits in the spared A1 (Meng *et al.*, 2017). Likewise, visual deprivation sharpened the functional whisker-barrel map at layer 2/3 in the barrel cortex. This effect was accompanied by an increased extracellular serotonin concentration in the spared somatosensory cortex (Jitsuki *et al.*, 2011). Hence, the refinement of V1 processing observed in this study might be also the result of a strengthening of the thalamo-cortical input to V1 along with an increase in the serotonergic tone. Moreover, 1 week of depriving vision by dark exposure was shown to homeostatically reduce AMPA receptor-mediated synaptic transmission in the spared primary somatosensory cortex (S1) and A1 (Goel *et al.*, 2006). These cross-modal changes appeared in a multiplicative manner and thus followed the rules for 'synaptic scaling' (Turrigiano *et al.*, 1998). Thus, it was speculated that scaling down of S1 and A1 synapses may additionally act to sharpen receptive field properties of these spared cortices (He *et al.*, 2012). According to the above-mentioned studies, it is possible that an initial strengthening of thalamo-cortical synapses in the spared primary sensory cortex is followed by a homeostatic adjustment of cortical activity.

Although the above-mentioned studies investigated the effects of a visual impairment on other sensory cortices, we have recently demonstrated the reverse effect, that is, an moderate and acute increase in visual perception upon auditory deprivation (Teichert & Bolz, 2017). This was probably mediated by a disinhibition within the visual cortex, as we could demonstrate reduced activity of inhibitory neurons in the visual cortex after auditory deprivation. Hence, it appears feasible that a similar mechanism takes place also after a prolonged somatosensory deprivation in this study. Thus, as the cortical inhibitory tone is highly relevant for cortical plasticity in juvenile and adult mice (Espinosa & Stryker, 2012; Levelt & Hubener, 2012), the inhibitory neurotransmitter GABA might also be involved in mediating cross-modal plasticity. But as the effects observed in this study are much stronger and extend over a much larger period of time, it is likely that further influences like those discussed above contribute to the sharpening of vision. It will be necessary to precisely investigate the time course of events taking place in the spared cortex to elucidate the underlying mechanisms.

Conclusion

To the best of our knowledge, we could show here for the first time that a late onset sensory deprivation massively enhanced behavioral performance mediated by the spared sense in fully adult mice. These data make it reasonable to conclude that compensatory changes in the spared sense have the potential not only to refine intracortical connections but also to alter cortex-dependent behavior.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

JB and MT designed the study; JB, KL and MT wrote the paper; MT and SW prepared figures; MT, MI, SW, CS, KL analyzed data.

Data accessibility

Data will be shared with the research community upon request. Please contact the corresponding author to access the data related to this manuscript.

Abbreviations

A1, Primary auditory cortex; CCD, Charge-coupled devise; Cpd, Cycles per degree; DE, Dark exposure; P, Postnatal day; S1, Primary somatosensory cortex; V1, Primary visual cortex; VWT, Visual water task; WD, Whisker deprivation.

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4.2 Manuscript 2

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RESEARCH REPORT

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Cross-modal restoration of ocular dominance plasticity in adult mice

Manuel Teichert* | **Marcel Isstas*** | **Yitong Zhang** | **Jürgen Bolz**

Institute of General Zoology and Animal Physiology, Jena, Germany

Correspondence
Jürgen Bolz, Universität Jena, Institut für Allgemeine Zoologie und Tierphysiologie, Erberstraße 1, 07743 Jena, Germany.
Email: jurgen.bolz@uni-jena.de

Abstract

The temporal closure of one eye in juvenile and young adult mice induces a shift of the ocular dominance (OD) of neurons in the binocular visual cortex. However, OD plasticity typically declines with age and is completely absent in matured mice beyond postnatal day (PD) 110. As it has been shown that the deprivation of one sensory input can induce neuronal alterations in non-deprived sensory cortices, we here investigated whether cross-modal interactions have the potential to reinstall OD plasticity in matured mice. Strikingly, using intrinsic signal imaging we could demonstrate that both whisker deprivation and auditory deprivation for only one week reinstated OD plasticity in fully adult mice. These OD shifts were always mediated by an increase of V1 responsiveness to visual stimulation of the open eye, a characteristic feature of OD plasticity normally only found in young adult mice. Moreover, systemic administration of the competitive NMDA receptor antagonist CPP completely abolished cross-modally induced OD plasticity. Taken together, we demonstrate here for the first time that the deprivation of non-visual senses has the potential to rejuvenate the adult visual cortex.

KEYWORDS

auditory deprivation, cross-modal plasticity, monocular deprivation, ocular dominance plasticity, whisker deprivation

1 | INTRODUCTION

The ability of the brain to undergo plastic changes due to experiential alteration typically declines with aging. In sensory systems, the capacity for experience-dependent cortical plasticity is typically restricted to well-defined time windows soon after birth (Hensch, 2005a). For example, a short monocular deprivation (MD) within this so-called critical period shifts the neuronal responses in the binocular primary visual cortex (V1) away from the deprived eye (Fagiolini, Pizzorusso, Berardi, Domenici, & Maffei, 1994; Gordon & Stryker, 1996; Hubel & Wiesel, 1970). Specifically, this "juvenile" ocular dominance (OD) shift is predominantly mediated by a decrease in V1-activation elicited by visual stimulation of the previously closed eye (Gordon & Stryker, 1996). In contrast, in young adult mice, with an age up to 2–3 month, prolonged MD can still initiate an OD shift, which is mainly caused by an increased V1 responsiveness to open eye stimulation ("adult" plasticity) (Hofer, Mrsic-Flogel, Bonhoeffer, & Hubener, 2006; Sato & Stryker, 2008). However, OD plasticity is completely absent in fully adult mice beyond a postnatal day (PD) 110 for animals raised in standard cages (Lehmann & Lowel, 2008).

Abbreviations: A1, Primary auditory cortex; AD, Auditory deprivation; CPP, (R,S)-3-(2-carbooxypiperazin-4-yl)propyl-1-phosphonic; GABA, Gamma-Aminobutyric acid; MD, Monocular deprivation; NMDA, N-methyl-D-aspartate; ODI, Ocular dominance index; OD, Ocular dominance; PD, Postnatal day; PV, Parvalbumin; Sal., Saline; SST, Somatostatin; V1, Primary visual cortex; WD, Whisker deprivation.

*These authors contributed equally to this study.

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It has been demonstrated that the deprivation of one sensory modality can cross-modally induce compensatory cortical changes in a spared sensory cortex, even in adult mice. For instance, one week of visual deprivation strengthens thalamocortical synapses in A1 (Petrus et al., 2014). This effect was shown to be accompanied by a refinement of intracortical circuits in A1 (Meng, Kao, Lee, & Kanold, 2017) and an increased sensitivity and frequency tuning of A1 neurons (Petrus et al., 2014). Furthermore, we could recently demonstrate that 7–12 days of whisker deprivation (WD) profoundly refines visual performance and V1 spatial frequency and contrast tuning in fully adult mice (Teichert, Isstas et al., 2017). These findings suggest massive neuronal changes in the spared sensory cortices after the deprivation of another sense. We therefore wondered whether this so-called cross-modal plasticity also has the potential to restore OD plasticity in adult mice far beyond their sensory critical periods (>PD 120). To this end, we performed either a WD or an auditory deprivation (AD) combined with MD for 7 days in mice of this age and investigated the effects of these interventions on visual plasticity using chronic Fourier-based intrinsic signal imaging (Kalatsky & Stryker, 2003; Kaneko, Stellwagen, Malenka, & Stryker, 2008).

2 | MATERIALS AND METHODS

2.1 | Animals and rearing conditions

C57BL/6J (Jackson labs) mice were raised in a group of 2–3 in transparent standard cages (16.5 × 22.5 cm) on a 12 hr light/dark cycle, with food and water available ad libitum. Between the chronic experiments each animal was housed alone in one standard cage. In general, the environment in the cage was minimally enriched with cotton rolls and nest material. In our mouse facility the light intensity was about 150–170 lux. Animal housing in our institution is regularly supervised by veterinaries from the state of Thuringia, Germany. For the present study, we used a total of 44 adult male mice (PD 120–240). All experimental procedures have been performed according to the German Law on the Protection of Animals and the corresponding European Communities Council Directive 2010 (2010/63/EU), and were approved by the Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (Thuringia State Office for Food Safety and Consumer Protection) under the registration number 02-032/16.

2.2 | Whisker deprivation (WD) and auditory deprivation (AD)

WD and AD were always performed immediately before the first imaging session. WD was performed as described previously (He, Petrus, Gammon, & Lee, 2012; Teichert, Isstas et al., 2017). Briefly, animals were deeply anesthetized with 2% isoflurane in a mixture of 1:1 O_2/N_2O applied through a plastic mask. The eyes of the animal were protected with silicon oil. Whiskers (macro vibrissae) were plucked bilaterally using fine forceps. Subsequently, mice received an injection of carprofen (4 mg/kg, s.c.) for pain prevention and were returned to their standard cages. Over the following days whiskers were reshaved every other day and received a daily administration of carprofen (4 mg/kg, s.c.).

AD was always induced by bilateral malleus removal as described previously (Teichert & Bolz, 2017; Teichert, Liebmann, Hubner, & Bolz, 2017). Briefly, animals were deeply anesthetized with 2% isoflurane in a mixture of 1:1 O_2/N_2O applied through a plastic mask. In addition, mice received a subcutaneous injection of carprofen (4 mg/kg, s.c.) for pain prevention. The eyes of the animal were protected with silicon oil. The tympanic membrane was punctured and the malleus was removed under visual control through this opening using fine sterilized forceps. Great care was taken to avoid any destruction of the stapes and the oval window which is visible through the hearing canal (see (Tucci, Cant, & Durham, 1999)). Over the following days animals received a daily administration of carprofen (4 mg/kg, s.c.).

2.3 | Monocular deprivation (MD)

MD was always performed after the first imaging session, thus, during the same anesthesia like AD and WD. For this, we increased the isoflurane concentration to 2% in a mixture of 1:1 N_2O and O_2. Lid margins of the right eye were trimmed and an antibiotic ointment was applied. Subsequently the right eye was sutured. After MD animals received one injection of carprofen (4 mg/kg, s.c.) and were returned to their standard cages. All animals were checked daily to ensure that the sutured eye remained closed during the MD time. Over the following days animals received a daily administration of carprofen (4 mg/kg, s.c.).

2.4 | CPP and saline injections

To investigate the role of the N-methyl-D-aspartate (NMDA)-receptor on OD plasticity, we administrated the competitive NMDA receptor (R,S)-3-(2-carbooxypiperazin-4-yl)propyl-1-phosphonic (CPP) (Abcam). CPP was diluted in saline and injected intraperitoneally (i.p.) every 24 hr at a dose of 12–15 mg/kg in a volume of 0.12 ml (Sato & Stryker, 2008; Villarreal, Do, Haddad, & Derrick, 2002). In the control group, we daily injected the same volume of saline (i.p.).

2.5 | Optical imaging of intrinsic signals

2.5.1 | Mouse preparation for optical imaging

As described previously (Teichert & Bolz, 2017; Teichert, Isstas et al., 2017), animals were initially anesthetized with

4% isoflurane in a mixture of 1:1 O_2/N_2O and placed on a heating blanket (37.5°C) for maintaining body temperature. Afterward, mice received injections of chlorprothixene (40 µg/mouse i.m.) and carprofen (4 mg/kg, s.c.). The inhalation anesthesia was applied through a plastic mask and maintained at 0.5% during the experiment. The animal was fixed in a stereotaxic frame and we removed the skin of the left hemisphere to expose the visual cortex. The exposed area was covered with 2.5% agarose in saline and sealed with a glass coverslip. Cortical responses were always recorded through the intact skull.

2.5.2 | Mouse preparation for repeated imaging experiments

Repeated intrinsic imaging in the same mice was performed as previously described (Kaneko et al., 2008). Briefly, after the first imaging session the skin was resutured and animals were returned to their standard cages. During the subsequent days animals received a daily injection of carprofen (4 mg/kg, s.c.). Before the next imaging session, the skin was reopened and imaging was performed as described above.

2.5.3 | Imaging of visual cortex

Responses of mouse visual cortex were recorded as originally described by Kalatsky and Stryker (2003). In brief, the method uses a periodic stimulus that is presented to the animal for some time and cortical responses are extracted by Fourier analysis. In our case, the visual stimulus was a drifting horizontal light bar of 2° width, 100% contrast and with a temporal frequency of 0.125 Hz. The stimulus was presented on a high refresh rate monitor (Hitachi Acuvue HM 4921-D) placed 25 cm in front of the animal. Visual stimulation was adjusted so that it only appeared in the binocular visual field of the recorded hemisphere (−5° to +15° azimuth, −17° to +60° elevation). The stimulus was presented alternately to both eyes for 5 min. During the visual stimulation of one eye, the other one was covered by an aluminum foil scrap. Thus, the stimulus was repeated for about 35 times during one presentation.

2.5.4 | CCD camera recording procedure

Using a Dalsa 1M30 CCD camera (Dalsa, Waterloo, Canada) with a 135 × 50 mm tandem lens (Nikon, Inc., Melville, NY, USA), we first recorded images of the surface vascular pattern via illumination with green light (550 ± 2 nm) and, after focusing 600 µm below the pial surface, intrinsic signals were obtained via illumination with red light (610 ± 2 nm). Frames were acquired at a rate of 30 Hz and temporally averaged to 7.5 Hz. The 1,024 × 1,024 pixel images were spatially averaged to a 512 × 512 resolution. We always imaged the left hemisphere of the animals.

2.5.5 | Data analysis

From the recorded frames the signal was extracted by Fourier analysis at the stimulation frequency and converted into amplitude and phase maps using custom software (Kalatsky & Stryker, 2003). In detail, from a pair of the upward and downward maps, a map with absolute retinotopy and an average amplitude map were computed. For data analysis we used the MATLAB standard as described previously (Cang, Kalatsky, Lowel, & Stryker, 2005). The amplitude component represents the activation intensity of the visual cortex. As high levels of neuronal activity decrease oxygen levels supplied by hemoglobin and as deoxyhemoglobin absorbs more red light (610 ± 2 nm), the reflected light intensity decreases in active cortical regions. Because the reflectance changes are very small (less than 0.1%) all amplitudes are multiplied with 10^4 so that they can be presented as small positive numbers. Thus, the obtained values are dimensionless. Amplitude maps were obtained by averaging the response amplitudes of individual pixels from maps to upward and downward moving bars. The ocular dominance index was computed a (C−I)/(C+I) with C and I representing the peak response amplitudes of V1 elicited by contralateral eye and ipsilateral eye stimulation, as described previously (Cang et al., 2005; Kaneko et al., 2008). To each condition, we took at least three magnitudes of V1 responsiveness and averaged them for data presentation.

2.6 | Statistical analysis

Optical imaging data were analyzed by a repeated-measures two-way ANOVA. Group comparison was carried out by paired *t* tests followed by Bonferroni correction. In the graphs, the levels of significance were set as *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$. Data were analyzed using GRAPHPAD PRISM 7.0 and are presented as means and standard error of the mean (*SEM*) and as measurements of individual animals.

3 | RESULTS

3.1 | Both whisker deprivation and auditory deprivation restore ocular dominance plasticity in fully adult mice

Here we investigated whether a deprivation of a non-visual sensory input (auditory and somatosensory) can restore ocular dominance plasticity in fully adult mice (PD 120–240). For this, we measured V1 responsiveness evoked by visual stimulation of the contra and ipsilateral eye chronically at 0 days and 7 days after either WD or AD using Fourier-based intrinsic signal imaging (Kalatsky & Stryker, 2003).

This method enables non-invasive measurements of cortical activity over large brain areas with a high spatial resolution (Bonhoeffer & Hubener, 2016; Teichert & Bolz, 2017) and its reliability has been extensively validated by electrophysiological recordings (Kalatsky, Polley, Merzenich, Schreiner, & Stryker, 2005; Kaneko et al., 2008).

As a first step we examined whether chronic intrinsic signal imaging per se affects visually evoked V1 activity in adult untreated control mice. As depicted in Figure 1 a (upper row) the V1 activity patches evoked by visual stimulation of the contra and ipsilateral eye at 0 days and after 7 days did not change, with the input of the contralateral eye always dominating the input to the binocular region of V1. This shows that repeated intrinsic signal imaging does not affect the normal input strength to V1. Next, we confirmed that MD for 7 days does also not change the ocular dominance in mice of this age (Lehmann & Lowel, 2008) as the strength of V1 amplitude maps obtained after visual stimulation was practically identical to the strength of V1 maps in untreated mice at 0 and 7 days (Figure 1a, lower row).

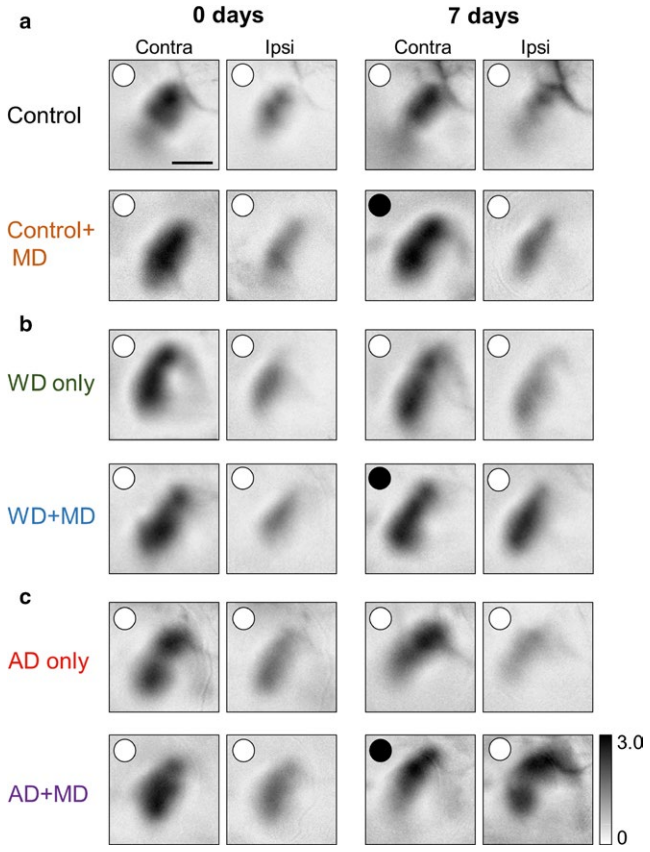

FIGURE 1 Representative examples of V1 activity maps recorded by chronic intrinsic optical imaging after contra or ipsilateral eye stimulation at day 0 and after 7 days. (a) Upper row: In normal control mice, contralateral eye stimulation always evoked stronger V1 responses than stimulation of the ipsilateral eye. Lower row: MD in control mice older than 120 days has no effect on contra or ipsilateral evoked V1 responses. (b) Upper row: WD alone does not change contra or ipsilateral evoked responses. Lower row: In contrast, WD combined with MD leads to a strong increase in ipsilateral (open) eye responses after 7 days, the characteristic feature of OD plasticity in young adult mice. (c) Upper row: In mice, after AD induced by malleus removal, V1 responses to contra and ipsilateral eye stimulation did not change after one week. Lower row: However, MD in AD mice leads to strongly increased V1 responses to ipsilateral (open) eye stimulation after 7 days. Thus, deprivation in both senses, auditory and somatosensory, leads to cross-modally induced restoration of OD plasticity. White circles: open eye, black circles: closed eye, Scale bar: 1 mm, grayscale (0–3) represents fractional change in reflectance $\times 10^{-4}$ (dimensionless)

As a next step we investigated potential cross-modal effects of bilateral WD on the ocular dominance in the binocular zone of V1. For this, we performed WD and measured V1 amplitudes elicited by contra or ipsilateral eye stimulation immediately after WD (0 days) and again after 7 days. As depicted in Figure 1b (upper row) WD alone, without MD, did not affect the intensity of V1 activity patches evoked by contra or ipsilateral eye input. However, if we combined WD with 7 days of MD (WD+MD), the V1 patches evoked by ipsilateral (open) eye stimulation were always darker after 7 days compared to the first imaging session, whereas the V1 maps obtained after visual stimulation of the contralateral (closed) eye remained equally dark after this time (Figure 1b, lower row). This suggests a cross-modal alteration of ocular dominance in these animals.

Knowing that a bilateral WD can reinstate ocular dominance plasticity in the spared V1 we next examined whether the deprivation of another non-visual sensory input, an AD, leads to similar effects. To induce an AD, we bilaterally removed the malleus from the ossicle chain as described previously (Teichert & Bolz, 2017; Teichert, Liebmann et al., 2017). This treatment causes conductive hearing loss (CHL), which massively reduces sound-evoked activity of the primary auditory cortex (A1) (Teichert & Bolz, 2017; Teichert, Liebmann et al., 2017). After this, we performed the first imaging session (day 0) followed by a second imaging session 7 days later.

Figure 1c (upper row) depicts representative V1 maps obtained after visual stimulation of either the contra or ipsilateral eye at 0 and 7 days after this treatment. Like WD, AD alone did also not change the strength of the V1 activity patches between 0 and 7 days after AD. However, after combined AD and MD (AD+MD), V1 activity spots elicited by ipsilateral (open) eye stimulation were always darker after 7 days (Figure 1c, lower row) indication that AD can also reinstate ocular dominance plasticity in fully adult mice, as demonstrated for WD.

Quantification and statistical analysis (repeated-measures two-way ANOVA, Table 1) revealed that neither no treatment nor MD alone led to changes of V1 activity elicited by visual stimulation of the contra or ipsilateral eye after 7 days (Control ($n = 5$): contra: 2.84 ± 0.11 ($\times 10^{-4}$) vs. 2.79 ± 0.19 ($\times 10^{-4}$), $p = 1$, ipsi: 2.08 ± 0.12 ($\times 10^{-4}$) vs. 1.86 ± 0.14

TABLE 1 Repeated-measures two-way ANOVA of data obtained by chronic intrinsic signal imaging

	Group		Time		Interaction	
Control	$F_{1,8}$	p	$F_{1,8}$	p	$F_{1,8}$	p
Contra	2.343	0.164	0	0.991	0.22	0.652
Ipsi	3.403	0.102	0.571	0.472	1.484	0.258
ODI	0.318	0.588	6.713	0.032*	2.79	0.133
WD	$F_{1,10}$	p	$F_{1,10}$	p	$F_{1,10}$	p
Contra	0.027	0.872	4.545	0.059	0.685	0.427
Ipsi	1.09	0.321	7.772	0.019*	18.021	0.002**
ODI	4.265	0.066	43.896	<0.001***	29.149	<0.001***
AD	$F_{1,9}$	p	$F_{1,9}$	p	$F_{1,9}$	p
Contra	1.083	0.325	0.32	0.585	0.451	0.519
Ipsi	15.164	0.004**	9.851	0.012*	17.017	0.003**
ODI	6.219	0.034*	35.398	<0.001***	25.589	0.001**
WD & CPP	$F_{1,9}$	p	$F_{1,9}$	p	$F_{1,9}$	p
Contra	2.16	0.176	0.34	0.574	0.009	0.925
Ipsi	0	0.992	21.006	0.001**	10.56	0.01*
ODI	2.966	0.119	33.532	<0.001***	32.203	<0.001***

*: $p < 0.05$, **: $p < 0.01$, ***:$p < 0.001$.

($\times 10^{-4}$), $p = 0.24$; Control+MD ($n = 5$): contra: 2.50 ± 0.14 ($\times 10^{-4}$) vs. 2.55 ± 0.18 ($\times 10^{-4}$), $p = 0.75$, ipsi: 1.71 ± 0.11 ($\times 10^{-4}$) vs. 1.77 ± 0.11 ($\times 10^{-4}$), $p = 1$, Bonferroni-corrected paired t tests, Figure 2a,b As stated in the Material and Methods section, the values of V1 responses are dimensionless.). Thus, in mice of these groups the ocular dominance index (ODI) remained statistically unchanged although the mean ODI in the Control+MD-group was slightly reduced after 7 days of MD (Control ($n = 5$): 0.23 ± 0.024 vs. 0.23 ± 0.021, $p = 0.96$; Control+MD ($n = 5$): 0.23 ± 0.046 vs. 0.2 ± 0.014, $p = 0.12$, Bonferroni-corrected paired t tests, Figure 2c). First, these results indicate that repeated intrinsic signal imaging during one week provides stable and reliable results. Second, these data show that MD does not induce OD shifts in fully adult mice, confirming previous results (Lehmann & Lowel, 2008).

Statistical analysis of the influence of WD on V1-responsiveness and OD showed significant effects of time and interaction (repeated-measures two-way ANOVA, Table 1). Comparison of V1 activity evoked by visual stimulation of the contra or ipsilateral eye and the ODIs at 0 days and 7 days after WD alone also revealed no significant changes ($n = 5$, contra: 3.01 ± 0.08 ($\times 10^{-4}$) vs. 2.84 ± 0.15 ($\times 10^{-4}$), $p = 0.10$, ipsi: 2.05 ± 0.16 ($\times 10^{-4}$) vs. 2.0 ± 0.16 ($\times 10^{-4}$), $p = 0.52$, ODI: 0.25 ± 0.02 vs. 0.23 ± 0.02, $p = 0.64$, Bonferroni-corrected paired t tests, Figure 2d–f, green data points). These data indicate that 7 days of WD per se does not influence the ocular dominance in the binocular region of V1. However, while V1 responses elicited by contralateral eye (closed eye) stimulation did not change after 7 days of WD and MD, too ($n = 7$, 3.15 ± 0.15 ($\times 10^{-4}$) vs. 2.8 ± 0.19 ($\times 10^{-4}$), $p = 0.22$, Bonferroni-corrected paired t test, Figure 2d, blue data points), there was a marked and highly significant increase of V1 responses evoked by sensory stimulation of the ipsilateral (open) eye after this intervention (2.0 ± 0.11 ($\times 10^{-4}$) vs. 2.4 ± 0.12 ($\times 10^{-4}$), $p = 0.0004$, Bonferroni-corrected paired t test, Figure 2e, blue data points). This led to a massive reduction of the ODI after 7 days of WD and MD (0.25 ± 0.03 vs. 0.07 ± 0.04, $p = 0.0010$, Bonferroni-corrected paired t test, Figure 2f, blue data points). These data strongly suggests that bilateral WD can cross-modally reinstate ocular dominance plasticity in matured mice far beyond their sensory critical periods.

Statistical analysis by a repeated-measures two-way ANOVA revealed significant effects of AD on V1-responsiveness and OD (Table 1). In mice, which received an AD the visually elicited V1 activities and the ODI remained stable between 0 days and 7 days ($n = 6$, contra: 2.86 ± 0.11 ($\times 10^{-4}$) vs. 2.97 ± 0.08 ($\times 10^{-4}$), $p = 0.32$, ipsi: 2.0 ± 0.09 ($\times 10^{-4}$) vs. 2.0 ± 0.13 ($\times 10^{-4}$), $p = 0.98$, ODI: 0.22 ± 0.02 vs. 0.21 ± 0.02, $p = 1$, Bonferroni-corrected paired t tests, Figure 2g–i, red data points). These data indicate that deprivation of a non-visual sensory input alone, for 7 days, does not alter ocular dominance in the binocular zone of V1. If we combined an AD with an MD for 7 days, V1 activity evoked by contralateral (closed) eye stimulation did not change during this time ($n = 5$, 3.1 ± 0.14 ($\times 10^{-4}$) vs. 3.08 ± 0.21 ($\times 10^{-4}$), $p = 1$, Bonferroni-corrected paired t test, Figure 2g, purple data points). However, V1 responses evoked by visual stimulation of the ipsilateral

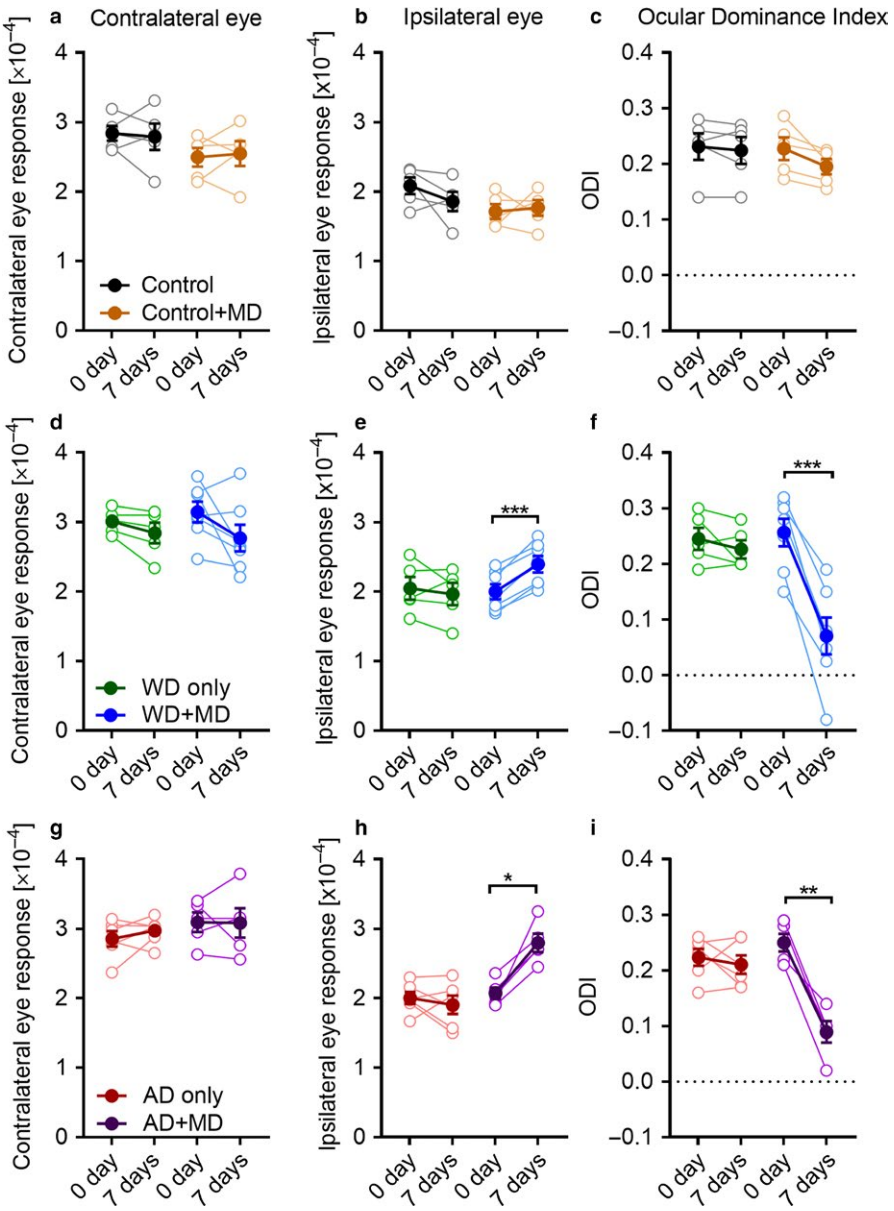

FIGURE 2 Both WD and AD restore ocular dominance plasticity in fully adult mice. (a,b) V1 activity elicited by contra or ipsilateral eye stimulation remained unchanged in control mice ($n = 5$) and mice, which only had MD ($n = 5$, control+MD) between 0 and 7 days. (c) No significant ODI shift in control and control+MD mice. The absence of an ODI shift in control mice with MD confirms that OD plasticity is absent in fully adult mice (d) V1 responses evoked by contralateral eye stimulation were not altered after 7 days of either WD alone ($n = 5$, WD only) or after WD with combined MD ($n = 7$, WD+MD). (e) WD-only mice did not display changes in ipsilateral eye responses in V1, whereas V1 activity after ipsilateral eye stimulation significantly increased after 7 days in WD+MD mice. (f) No ODI shift was observed in WD-only mice. However, there was a strong ODI shift in WD+MD mice indicating a cross-modal restoration of OD plasticity. (g) No alteration of contralateral eye input to V1 in both AD only ($n = 6$) and AD+MD mice ($n = 5$) between 0 and 7 days. (h) V1 activation evoked by the ipsilateral eye remained constant in AD-only mice, but massively increased in AD+MD animals after 7 days. (i) The ODI remained unchanged in AD-only mice but was markedly shifted toward zero in AD+MD mice after 7 days. Thus, deprivation of non-visual senses reinstates visual cortex plasticity. Open circles represent measurements of individual animals. Closed circles represent means of each group ± *SEM*; V1 responses represent fractional change in reflectance $\times 10^{-4}$ (dimensionless); repeated-measures two-way ANOVA followed by Bonferroni-corrected paired *t* tests, $^{**}p < 0.01$, $^{***}p < 0.001$

(open) eye were dramatically increased after 7 days of AD combined with MD (2.07 ± 0.08 ($\times 10^{-4}$) vs. 2.8 ± 0.13 ($\times 10^{-4}$), $p = 0.018$, Figure 2h; purple data points), which resulted in a significant ODI shift toward zero (0.25 ± 0.02 vs. 0.09 ± 0.02, $p = 0.004$, Bonferroni-corrected paired *t* test, Figure 2i; purple data points). These data indicate that AD can also restore ocular dominance plasticity in the adult mouse visual cortex, like shown above for WD.

Taken together, our results indicate that the deprivation of one sensory modality has the potential to enhance neuronal plasticity in a spared sensory cortex.

3.2 | The cross-modally restored ocular dominance plasticity is NMDA receptor-dependent

We found that ODI shifts after either WD or AD combined with MD were always mediated by an increase of V1 activity elicited by visual stimulation of the ipsilateral eye (open eye). It has been reported that V1 activity changes induced by a MD in juvenile and young adult mice depend on N-methyl-D-aspartate (NMDA)-receptor activation (Sato & Stryker, 2008). Specifically, blocking the NMDA receptor by systemic administration of CPP was shown to abolish ocular dominance plasticity in mice during and shortly after the visual critical period (Sato & Stryker, 2008).

To get insights into potential molecular mechanisms that may underlie the observed restoration of visual plasticity after the depriving inputs of a non-visual sensory modality, we daily administered CPP during the 7 days WD and MD period (WD+MD+CPP-group, $n = 5$) to block NMDA receptor activation. In another group of mice we also combined WD with a MD for 7 days and daily administrated saline, as a control (WD+MD+Sal.-group, $n = 6$). Figure 3a illustrates representative V1 maps obtained by chronic intrinsic signal imaging after visual stimulation of either the contra or ipsilateral eye at 0 and 7 days of mice of both groups. It is clearly visible that the activity patch evoked by ipsilateral eye (open eye) stimulation was markedly stronger in mice with WD combined with MD and saline administration, whereas the activity spots elicited by contralateral (closed) eye stimulation remained unchanged after 7 days, confirming that WD can reinstate visual plasticity in the adult V1 (Figure 3a, upper row), as described above. In contrast, in mice of the

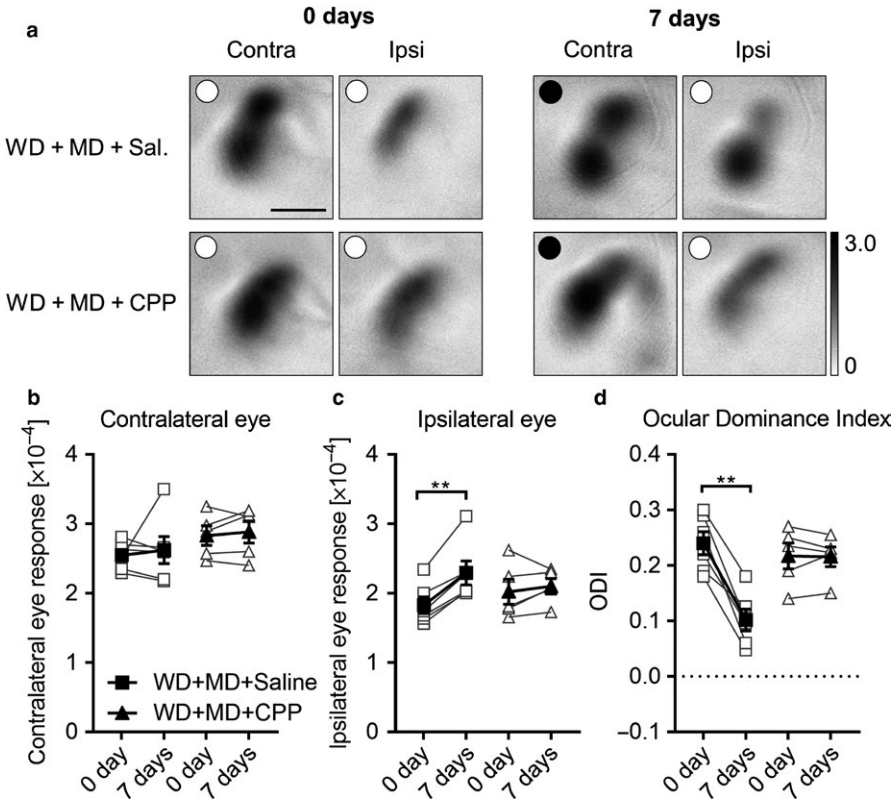

FIGURE 3 Systemic administration of CPP abolishes cross-modally induced ocular dominance plasticity. (a) V1 response maps recorded by chronic intrinsic optical imaging in mice at 0 and 7 days. Upper row: 7 days of WD combined with MD and daily saline administrations (WD+MD+Sal.) led to a strong increase in V1 responses evoked by ipsilateral eye stimulation. Thus, OD plasticity in these animals was restored. Lower row: In mice, after WD combined with MD and daily CPP administrations to block NMDA receptor activation (WD+MD+CPP), V1 responses to contra and ipsilateral eye stimulation did not change after one week. This suggests that blocking the NMDA receptor abolishes cross-modally induced OD plasticity. (b) V1 activity evoked by contralateral eye stimulation remained unchanged in mice of both groups (WD+MD+Sal., n = 6; WD+MD+CPP, n = 5). (c) V1 activity elicited by ipsilateral eye stimulation markedly increased in WD+MD+Sal. mice but was not changed in WD+MD mice that received CPP injection after 7 days. (d) Strong ODI shift in WD+MD+Sal. animals. However, there were no ODI alterations in WD+MD+CPP mice after 7 days. Open squares and triangles represent measurements of individual animals. Closed squares and triangles represent means of each group ± SEM; Repeated-measures two-way ANOVA followed by Bonferroni-corrected paired t tests, **$p < 0.01$, Scale bar: 1 mm, grayscale (0–3) and V1 responses represent fractional change in reflectance $\times 10^{-4}$ (dimensionless)

WD+MD-group which received CPP injections, V1 activity spots remained equally strong after 7 days suggesting an absence of V1 input changes after this treatment.

Statistical analysis by a repeated-measures two-way ANOVA revealed significant effects of CPP treatment on V1-responsiveness and OD (Table 1). Quantification showed that in mice of both groups V1 activation elicited by contralateral (closed) eye stimulation remained stable after 7 days (WD+MD+Sal.: 2.55 ± 0.08 ($\times 10^{-4}$) vs. 2.62 ± 0.2 ($\times 10^{-4}$), $p = 0.58$; WD+MD+CPP: 2.83 ± 0.14 ($\times 10^{-4}$) vs. 2.88 ± 0.16 ($\times 10^{-4}$), $p = 1$, Bonferroni-corrected paired t tests, Figure 2b, black squares and triangles). However, while V1 responses evoked by visual stimulation of the ipsilateral (open) eye significantly increased in WD+MD+Sal. animals after 7 days, it remained unaltered in the WD+MD+CPP-group (WD+MD+Sal.: 1.83 ± 0.11 ($\times 10^{-4}$) vs. 2.30 ± 0.17 ($\times 10^{-4}$), $p = 0.006$; WD+MD+CPP: 2.01 ± 0.18 ($\times 10^{-4}$) vs. 2.10 ± 0.11 ($\times 10^{-4}$), $p = 0.94$, Bonferroni-corrected paired t tests, Figure 2b, black squares and triangles). As a direct consequence, the ODI was shifted toward zero after 7 days in the WD+MD+Sal.-group but was unchanged in WD+MD mice which received CPP injections (WD+MD+Sal.: 0.24 ± 0.02 vs. 0.1 ± 0.02, $p = 0.002$; WD+MD+CPP: 0.22 ± 0.02 vs. 0.22 ± 0.02, $p = 1$, Bonferroni-corrected paired t tests, Figure 2b, black squares and triangles).

Taken together, these data indicate that CPP administration abolishes the restoration of ocular dominance plasticity after the deprivation of a non-visual sense. Hence, our results suggest that cross-modally induced visual plasticity requires NMDA receptor activation.

4 | DISCUSSION

Restoring and enhancing brain plasticity in adults is not only a topic of great scientific interest, but can also have clinical implications. To the best of our knowledge we demonstrate here for the first time that the deprivation of non-visual senses can restore visual cortex plasticity in mice far beyond their visual critical period. The observed OD shifts, both after WD or AD, were always mediated by an increase of V1 responsiveness elicited by open eye stimulations. Other manipulations that have been shown to restore OD plasticity, such as brief dark exposure (He, Hodos, & Quinlan, 2006; Stodieck, Greifzu, Goetze, Schmidt, & Lowel, 2014), visuomotor experience (Tschetter et al., 2013) or repeated MD (Hofer et al., 2006) led to similar alterations of V1 activity. These changes resemble the normal "adult" form of OD plasticity which is typically observed after a prolonged MD period in young adult mice (Ranson, Cheetham, Fox, & Sengpiel, 2012; Sato & Stryker, 2008; Sawtell et al., 2003).

Specifically, a MD in young adult mice for more than 5 days causes potentiation of V1 responses to the input through the open eye (Ranson et al., 2012; Sato & Stryker, 2008; Sawtell et al., 2003). This potentiation has been shown to require visual experience mediated by the open eye (Sawtell et al., 2003). Moreover, systemic administration of the NMDA receptor blocker CPP or cortex-specific genetic deletion of NMDARs prevents open eye response potentiation after MD (Sato & Stryker, 2008; Sawtell et al., 2003). These results indicate that MD induced plastic changes which lead to alterations of V1 activity in young adult mice take place on the visual pathway and, thus, require NMDA receptor activation at least in the visual cortex. In line with these finding we could show that blocking NMDARs by systemic administration of CPP also abolished cross-modally induced OD plasticity. Based on results of the mentioned previous studies (Sato & Stryker, 2008; Sawtell et al., 2003), we believe that cross-modally induced OD plasticity requires NMDAR activation at least in V1. Hence, our results suggest that cross-modal plasticity has the potential to restore a form of plasticity which is normally only present in much younger mice. However, we cannot exclude that the observed OD plasticity requires NMDAR in other brain areas, too. For example, a recent study could demonstrate that MD also leads to OD shifts of neurons in the lateral geniculate nucleus (Jaepel, Hubener, Bonhoeffer, & Rose, 2017). Hence, it might be possible that NMDARs are already required at this early station of the visual pathway.

What might be further potential mechanisms underlying the observed effects? Inhibitory circuits have been implicated to be involved in the restoration of OD plasticity. For instance, reducing the inhibitory tone in V1 by antagonizing $GABA_A$ receptors (Harauzov et al., 2010) or the transplantation of GABAergic progenitors (Isstas, Teichert, Bolz, & Lehmann, 2017; Tang, Stryker, Alvarez-Buylla, & Espinosa, 2014) have been shown to enhance cortical plasticity. Moreover, cross-modal inputs among primary sensory cortices often suppress cortical activity evoked by the primary sense (Ibrahim et al., 2016; Iurilli et al., 2012). For example, auditory and whisker stimulation evoke hyperpolarization in V1 (Iurilli et al., 2012). Thus, the partial loss of somatosensory input after WD may act to reduce the inhibitory drive onto V1 pyramidal cells. Likewise, we could recently demonstrate that AD, as performed in the present study, rapidly leads to both a reduction of the neuronal activity of parvalbumin (PV) and somatostatin (SST) positive inhibitory neurons and an increased excitability of the spared V1 in adult mice (Teichert & Bolz, 2017). In an interesting way, both types of inhibitory neurons have been described to play an important role in cortical plasticity. For example, silencing SST neurons enhances adult plasticity and allows the potentiation of open eye responses (Fu, Kaneko, Tang, Alvarez-Buylla, & Stryker, 2015). Thus, such cross-modal alterations of inhibition might facilitate OD plasticity in adulthood.

Another, but not mutually exclusive, explanation of how the deprivation of a non-visual sensory input provokes OD plasticity, are possible alterations in cortical circuitry. As already mentioned, it has been demonstrated that one week of sensory deprivation strengthens thalamocortical synapses in the spared cortex (Petrus et al., 2014, 2015). However, this cross-modal thalamocortical potentiation requires sensory experience of the spared cortex by its main input (Petrus et al., 2014). Based on these results one could argue that WD or AD combined with MD leads to a potentiation of thalamocortical V1 synapses that relay inputs of the open eye only, as the closed eye provides only little input.

Recently, we could demonstrate marked improvements of V1 spatial frequency and contrast tuning in response to WD (Teichert, Isstas et al., 2017). In the present study we now demonstrate that a sensory deprivation can cross-modally restore cortical plasticity. Hence, our results reveal a massive potential of cross-modal plasticity even well beyond the visual critical period (Hensch, 2005a,b; Lehmann & Lowel, 2008). Thus, partial deprivation of one sense does not only lead to compensatory sensory changes, but can also rapidly recover cortical plasticity in the remaining senses. It has been shown that treatments which restore OD plasticity in adult mice can also promote recovery from long-term MD (He, Ray, Dennis, & Quinlan, 2007). Thus, it is plausible that cross-modally induced plasticity also has the potential to restore vision after amblyopia.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

JB, MT and MI designed the study; JB and MT wrote the manuscript; JB, MT and MI prepared figures; MI, MT and YZ performed experiments; and MT, MI and YZ analyzed data.

DATA ACCESSIBILITY

Data will be shared with the research community upon request. Please contact the corresponding author to access the data related to this manuscript.

ORCID

Jürgen Bolz http://orcid.org/0000-0003-0531-2639

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Cross-modal Restoration of Juvenile-like Ocular Dominance Plasticity after Increasing GABAergic Inhibition

Manuel Teichert, [a†] **Marcel Isstas,** [a†] **Franziska Wieske,** [b] **Christine Winter** [b] **and Jürgen Bolz** [a*]

[a] *Institute of General Zoology and Animal Physiology, 07743 Jena, Germany*

[b] *Department of Psychiatry, Technical University Dresden, 01062 Dresden, Germany*

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[a] *Institute of General Zoology and Animal Physiology, 07743 Jena, Germany*

[b] *Department of Psychiatry, Technical University Dresden, 01062 Dresden, Germany*

Abstract—In juvenile and young adult mice monocular deprivation (MD) shifts the ocular dominance (OD) of binocular neurons in the primary visual cortex (V1) away from the deprived eye. However, OD plasticity is completely absent in mice older than 110 days, but can be reactivated by treatments which decrease GABA levels in V1. Typically, these OD shifts can be prevented by increasing GABAergic transmission with diazepam. We could recently demonstrate that both bilateral whisker and auditory deprivation (WD, AD), can also restore OD plasticity in mice older than 110 days, since MD for 7 days in WD mice caused a potentiation of V1 input through the ipsilateral (open) eye, the characteristic feature of OD plasticity of ''young adult" mice. Here we examined whether WD for 7 days also decreases GABA levels. For this, we performed post mortem HPLC analysis of V1 tissue. Indeed, we found that WD significantly decreased GABA levels in V1. Surprisingly, enhancing GABAergic inhibition by diazepam did not abolish OD shifts in WD mice, as revealed by repeated intrinsic signal imaging. On the contrary, this treatment led to a depression of V1 input through the previously closed contralateral eye, the characteristic signature of OD plasticity in juvenile mice during the critical period. Interestingly, the same result was obtained after AD. Taken together, these results suggest that cross-modally restored OD plasticity does not only depend on reduction of GABA levels in V1, but also requires other, so far unknown mechanisms.

Key words: whisker deprivation, cross-modal plasticity, ocular dominance plasticity, inhibition, diazepam.

INTRODUCTION

The primary visual cortex (V1) of rodents is dominated by the input from the contralateral eye (Drager, 1975, 1978). However, MD for a few days can shift the ocular dominance (OD) away from the closed eye (Wiesel and Hubel, 1963; Gordon and Stryker, 1996). In mice, this effect appears to be strongest between 28 and 32 days of age, the peak of the so-called ''critical period" (Gordon and Stryker, 1996). MD during this period leads to a reduction of V1 inputs from the previously closed eye, the characteristic signature of ''juvenile" OD plasticity (Gordon and Stryker, 1996; Hofer et al., 2006; Kaneko et al., 2008). In young adult mice around 60 days of age, however, the mechanism, that leads to OD shifts, changes, as in these animals MD causes a potentiation of V1 responses to the input through the open eye (Sawtell et al., 2003; Sato and Stryker, 2008; Ranson et al., 2012). These changes are broadly referred to as ''adult" OD plasticity. However, OD plasticity shows an age-dependent decline and is completely absent in mice older than 110 days (Lehmann and Lowel, 2008). It has been suggested that the cortical inhibitory tone, which increases during aging, triggers closing of OD plasticity in fully adult mice (Hensch, 2005; Espinosa and Stryker, 2012; Levelt and Hubener, 2012). Indeed, interventions that can restore OD-plasticity in these older mice typically decrease GABA levels in V1 and thus alter the balance between excitation and inhibition (E/I balance) (Hubener and Bonhoeffer, 2014). These OD shifts, however, can be prevented or at least markedly reduced by artificially strengthening GABAergic inhibition with diazepam, a positive allosteric modulator of GABAA receptors (Maya-Vetencourt et al., 2008; Spolidoro et al., 2011; Greifzu et al., 2014; Stodieck et al., 2014), suggesting that reduc-

*Corresponding author. Address: Universität Jena, Institut für Allgemeine Zoologie und Tierphysiologie, Erberstraße 1, 07743 Jena, Germany. Fax: +49-03641-949102.
E-mail addresses: Manuel.Teichert@uni-jena.de (M. Teichert), Marcel.Isstas@uni-jena.de (M. Isstas), Franziska.Wieske@uniklinikum-dresden.de (F. Wieske), Christine.Winter@charite.de (C. Winter), jurgen.bolz@uni-jena.de (J. Bolz).
† These authors contribute equally to this study.

Abbreviations: A1, primary auditory cortex; AD, auditory deprivation; CPP, (R,S)-3-(2-carbooxypiperazin-4-yl)propyl-1-phosphonic; E/I, excitation/inhibition; GABA, gamma-Aminobutyric acid; HPLC, high-performance liquid chromatography; MD, Monocular deprivation; NMDAR, N-methyl-D-aspartate receptor; OD, ocular dominance; ODI, ocular dominance index; PD, postnatal day; PSD-95, postsynaptic density protein 95; S1, primary somatosensory cortex; V1, primary visual cortex; WD, whisker deprivation.

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ing cortical inhibition is a central hub to restore cortical plasticity in adults.

It has been demonstrated that deprivation of non-visual sensory modalities, such as WD or AD, in fully adult mice lead to compensatory neuronal changes in the spared V1, which are accompanied by improved visual perception at both, the physiological and behavioral level (Teichert and Bolz, 2017b; Teichert et al., 2018b). This type of plasticity is called "cross-modal plasticity" (Bavelier and Neville, 2002; Lee and Whitt, 2015). Moreover, we could recently also show that these sensory deprivations can even restore the "adult" form of OD plasticity in V1 of older mice, since combining either WD or AD, respectively, with MD resulted in a marked increase of V1 responsiveness to open eye stimulation (Teichert et al., 2018a).

Here we investigated whether cross-modally induced OD plasticity is also accompanied by reductions in GABA levels in V1, and, if so, whether OD shifts can be prevented by increasing the inhibitory tone via diazepam administration. Here we found that WD, indeed, cross-modally reduces GABA levels in the spared V1 of fully adult mice, as revealed by post-mortem HPLC analysis. These results indicate that WD decreases inhibition in V1. Using repeated intrinsic signal imaging we found that WD in saline-treated control animals restored the "adult" form of OD plasticity, confirming our recently published finding (Teichert et al., 2018a). However, unexpectedly, when we increased the inhibitory tone in V1 by diazepam administrations, OD plasticity was not prevented. Quite the opposite, this treatment changed the signature of OD shifts from the "adult form" to the "juvenile form", as we found a significant reduction in V1 responses evoked by visual stimulation of the previously closed contralateral eye. Interestingly, we could also show that increasing inhibition in mice after AD also induced "juvenile-like" OD changes, suggesting that this effect is a general feature of cross-modally restored OD plasticity. Moreover, these V1 input changes required NMDA receptor activation, as administration of the NMDA receptor antagonist CPP abolished "juvenile-like" OD shifts, emphasizing the pivotal role of these receptors in cross-modally induced plasticity. To the best of our knowledge, this is the first demonstration that increasing inhibition in the fully adult V1 does not abolish restoration of OD plasticity, but rather leads to a quality change of OD shifts. Taken together, our data suggest that cross-modal induction of cortical plasticity is not only a result of decreased inhibition, but also requires additional, so far unknown mechanisms.

EXPERIMENTAL PROCEDURES

Animals

C57BL/6J (Jackson labs) mice were raised in a group of 2–3 in transparent standard cages (16.5 × 22.5 cm) on a 12-h light/dark cycle, with food and water available *ad libitum*. Between the chronic experiments each animal was housed alone in a standard cage. The environment in the cage was minimally enriched with cotton rolls and nest material. In our mouse facility the light intensity was about 150–170 lux. As demonstrated recently, these rearing conditions are not sufficient to extend OD plasticity into adulthood (Teichert et al., 2018a). Animal housing in our institution is regularly supervised by veterinaries from the state of Thuringia, Germany. For the present study we used a total of 37 fully adult male and female mice (Postnatal day (PD) 120–240). All experimental procedures have been performed according to the German Law on the Protection of Animals and the corresponding European Communities Council Directive 2010 (2010/63/EU), and were approved by the Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (Thuringia State Office for Food Safety and Consumer Protection) under the registration numbers 02-032/16 and 02-050/14. Every effort was made to minimize the number of animals used and their suffering.

High-performance liquid chromatography (HPLC)

WD for subsequent HPLC analysis was performed as described previously (Teichert et al., 2018b; Teichert et al., 2018a) (see below). Micropunches of V1 were taken from 1 mm brain slices at − 3.28 from Bregma from control (n = 5) and WD mice (n = 6, after 7 days) and homogenized by ultrasonication in 20 vol of 0.1 N perchloric acid at 4 °C immediately after collection. A total of 100 ml of the homogenate was added to equal volumes of 1 N sodium hydroxide for measurement of protein content. The remaining homogenate was centrifuged at 17,000*g* and 4 °C for 10 min. Glutamate and GABA levels were determined using methods described previously (Winter et al., 2009). Briefly, amino acids were precolumn-derivatized with o-phthalaldehyde-2-mercaptoethanol using a refrigerated autoinjector and then separated on a HPLC column (ProntoSil C18 ace-EPS) at a flow rate of 0.6 ml/min and a column temperature of 40 °C. The mobile phase was 50 mM sodium acetate (pH 5.7) in a linear gradient from 5% to 21% acetonitrile. Derivatized amino acids were detected by their fluorescence at 450 nm after excitation at 330 nm.

Optical imaging of intrinsic signals

Mouse preparation for optical imaging. Intrinsic signal imaging experiments were performed in a total of 26 animals. In order to measure visually evoked activity of V1 we used Fourier based periodic intrinsic signal imaging (Kalatsky and Stryker, 2003; Isstas et al., 2017; Teichert et al., 2018a). Animals were initially anesthetized with 4% isoflurane in a mixture of 1:1 O_2/N_2O and placed on a heating blanket for maintaining body temperature (37.5 °C). Subsequently, mice received injections of chlorprothixene (20 μg/mouse i.m.) and carprofene (5 mg/kg, s.c.). The animal was fixed in a stereotaxic frame and we removed the skin of the left hemisphere to expose the visual cortex. The exposed area was covered with 2.5% agarose in saline and sealed with a standard microscope glass coverslip. Cortical responses were always recorded through the intact skull. During the

experiment isoflurane inhalation anesthesia was applied through a plastic mask and maintained at 0.5–0.6%.

Mouse preparation for repeated imaging experiments. Repeated intrinsic imaging in the same mice was performed as previously described (Kaneko et al., 2008; Teichert et al., 2018a). Briefly, after the first imaging (0 days) session the skin was re-sutured and animals were returned to their standard cages. During the subsequent days animals received a daily injection of carprofen (5 mg/kg, s.c.) for pain prevention. Before the next imaging session (after 7 days) the previously closed eye and the skin covering the visual cortex was re-opened and imaging was performed as described above (Fig. 1).

Imaging of visual cortex. Responses of mouse primary visual cortex were recorded as described previously (Teichert et al., 2018a). Briefly, the method uses a periodic stimulus that is presented to the animal for some time and cortical responses are extracted by Fourier analysis. In our case, the visual stimulus was a drifting horizontal light bar of 2° width, 100% contrast and with a temporal frequency of 0.125 Hz. The stimulus was presented on a high refresh rate monitor (Hitachi Accuvue HM 4921-D) placed 25 cm in front of the animal. Visual stimulation was adjusted so that it only appeared in the binocular visual field of the recorded hemisphere (-5° to +15° azimuth, -17° to +60° elevation). The stimulus was presented to the contra or ipsilateral, respectively for 5 min and was repeated for about 35 times during one presentation period.

CCD camera recording procedure. Using a Dalsa 1 M30 CCD camera (Dalsa, Waterloo, Canada) with a 135 × 50 mm tandem lens (Nikon, Inc., Melville, NY), we first recorded images of the surface vascular pattern via illumination with green light (550 ± 2 nm) and, after focusing 600 μm below the pial surface, intrinsic signals were obtained via illumination with red light (610 ± 2 nm). Frames were acquired at a rate of 30 Hz and temporally averaged to 7.5 Hz. The 1024 × 1024 pixel images were spatially averaged to a 512 × 512 resolution. We always imaged the left hemisphere of the animals.

Data analysis. From the recorded frames the signal was extracted by Fourier analysis at the stimulation frequency and converted into amplitude and phase maps using custom software (Kalatsky and Stryker, 2003). In detail, from a pair of the upward and downward maps, a map with absolute retinotopy and an average magnitude map were computed. For data analysis we used the MATLAB standard as described previously (Cang et al., 2005; Lehmann and Lowel, 2008). The magnitude component represents the activation intensity of the visual cortex. Since high levels of neuronal activity decrease oxygen levels supplied by hemoglobin and since deoxyhemoglobin absorbs more red light (610 ± 2 nm), the reflected light intensity decreases in active cortical regions. Because the reflectance changes are very small (less than 0.1%) all amplitudes are multiplied with 10^4 so that they can be presented as small positive numbers. Thus, the obtained values are dimensionless. Amplitude maps were obtained by averaging the response amplitudes of individual pixels from maps to upward and downward moving bars. The ocular dominance index (ODI) was computed as $(C - I)/(C + I)$ with C and I representing the peak response amplitudes of V1 elicited by contralateral eye and ipsilateral eye stimulation, as described previously (Cang et al., 2005; Kaneko et al., 2008). To each condition we took at least three magnitudes of V1 responsiveness and averaged them for data presentation.

Whisker deprivation (WD) and auditory deprivation (AD)

WD (n = 11) and AD (n = 9) for imaging experiments were always performed before the first imaging session (Fig. 1) (Teichert et al., 2018a). WD was performed as described previously (He et al., 2012; Teichert et al., 2018a). Briefly, animals were deeply anesthetized with 2% isoflurane in a mixture of 1:1 O_2/N_2O applied through a plastic mask. The eyes of the animal were protected with silicon oil. Whiskers (macro vibrissae) were plucked bilaterally using fine forceps. Subsequently, mice received an injection of carprofene (4 mg/kg, s.c.) for pain prevention and were either imaged or returned to their standard cages for 7 days. Over the following days whiskers were re-shaved every other day, and animals received a daily administration of carprofene (4 mg/kg, s.c.). Control animals (for HPLC experiments) were sham plucked under the same anesthesia regime by gently pulling on each whisker but leaving them intact as described previously (Teichert et al., 2018b). Control mice also received carprofene injections (4 mg/kg, s.c.). AD was always induced by bilateral malleus removal as described previously (Teichert and Bolz, 2017b, a; Teichert et al., 2017). Briefly, animals were deeply anesthetized with 2% isoflurane in a mixture of 1:1 O_2/N_2O applied through a plastic mask. Additionally, mice received a subcutaneous injection of carprofene (4 mg/kg, s.c.) for pain prevention. The eyes of the animal were protected with silicon oil. The tympanic membrane was punctured and the malleus was removed under visual control through this opening using fine sterilized forceps. Great care was taken to avoid any destruction of the stapes and the oval window which is visible through the hearing canal (see Tucci et al., 1999). Over the following days animals received a daily administration of carprofene (4 mg/kg, s.c.).

Monocular deprivation (MD)

MD was always performed after the first imaging session in a total of 26 mice (Fig. 1) (Teichert et al., 2018a). For this, we increased the isoflurane concentration to 2% in a mixture of 1:1 N_2O and O_2. Lid margins of the right eye were trimmed and an antibiotic ointment was applied. Subsequently the right eye was sutured. After MD animals received one injection of carprofene (4 mg/kg, s.c.) and were returned to their standard cages. All animals were checked daily to ensure that the sutured eye

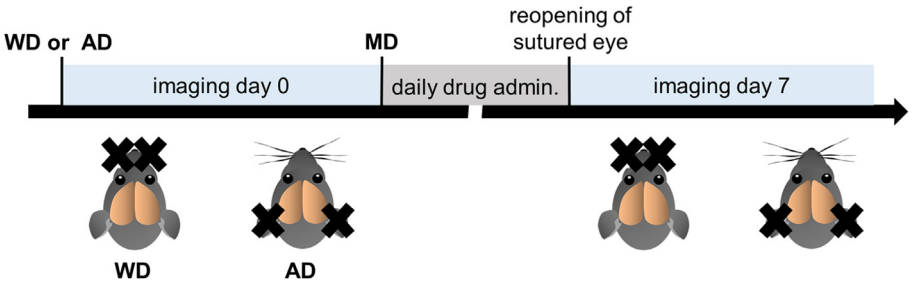

Fig. 1. Experimental time course. WD or AD respectively was always performed before the first imaging session (day 0). Subsequently after the initial imaging session animals were monocularly deprived. During the following days the received daily drug infusions (saline, diazepam, CPP, respectively, or a mixture of CPP and diazepam). The final imaging experiment was performed after 7 days.

remained closed during the MD time. Over the following days animals received a daily administration of carprofene (4 mg/kg, s.c.).

Saline, diazepam and CPP injections

For repeated optical imaging recordings mice of different experimental groups received daily injections of different drugs. The first injection was always performed immediately after the first imaging sessions (Fig. 1). In monocularly deprived control mice in which we also performed WD or AD, respectively, we daily injected saline (i.p., 0.12 ml, $n = 9$). To raise cortical inhibition in monocularly deprived WD and AD mice, we intraperitoneally injected 0.12-ml diazepam solution (in saline, 1 mg/kg, i.p., 0.12 ml, $n = 11$) daily. To investigate the effects the effects of pure diazepam treatment in MD mice, animals received a daily injection of a diazepam solution (in saline, 1 mg/kg, i.p., 0.12 ml, $n = 3$). Furthermore, to block the N-methyl-D-aspartate (NMDA)-receptor in monocularly deprived WD mice which also received diazepam, we daily injected a mixture containing diazepam (1 mg/kg) and (R,S)-3-(2-carbooxypiperazin-4-yl)propyl-1-phosphonic (CPP, Abcam, 12–15 mg/kg, $n = 3$) (Sato and Stryker, 2008; Teichert et al., 2018a) in a volume of 0.12 ml (in saline, i.p.).

Statistical analysis

The normal distribution of the values in each group was analyzed and confirmed by the Kolmogorov–Smirnov test. In addition, the F-test confirmed the equal distribution of values between groups and, thus, allowed us to compare before and after optical imaging values using a parametric paired *t*-test. HPLC data were analyzed by unpaired *t*-tests. In the graphs, the levels of significance were set as: *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. Data were analyzed using GRAPHPAD PRISM 7.0 and are presented as data points of individual animals together with means and standard error of the mean (s.e.m.).

RESULTS

WD cross-modally decreases GABA levels in V1

It has been described that a reduction in GABAergic inhibition and, thus, an alteration of the balance between excitation and inhibition (E/I ratio) in V1, plays a pivotal role for the restoration of cortical plasticity (He et al., 2006; Maya-Vetencourt et al., 2008; Harauzov et al., 2010). Since we could previously demonstrate that bilateral WD reinstalled OD plasticity in fully adult mice (Teichert et al., 2018a), we examined whether this cross-sensory plasticity also leads to changes in the E/I ratio in V1. For this, we quantified glutamate and GABA levels in V1 by post-mortem HPLC analysis. While glutamate levels did not change 7 days after WD ($n = 5$) compared to control mice ($n = 5$, control: 100 ± 2.12%, WD: 96.49 ± 3.53%, $t(8) = 0.852$, $p = 0.42$; unpaired *t*-test, Fig. 2 A), there was a significant reduction in V1 GABA concentration by about 14% ($n = 6$, control: 100 ± 1.90%, WD: 86.20 ± 3.43%, $t(9) = 3.313$, $p = 0.009$; unpaired *t*-test, Fig. 2 B). Hence, the glutamate/GABA ratio was markedly increased 7 days after WD (control: 100.37 ± 1.56, WD: 114.86 ± 1.61, $t(8) = 0.168$, $p = 0.003$; unpaired *t*-test, Fig. 2 C). These data suggest that WD cross-modally decreases GABAergic inhibition in the spared V1 and thereby shifts the E/I balance in favor of excitation.

WD combined with systemic diazepam administration induces juvenile-like OD plasticity

Many studies could demonstrate that OD plasticity which could be restored by interventions decreasing GABA levels in V1 can be prevented by treatments strengthening GABAergic transmission in the cortex. A common tool used for this purpose is allosteric activation of GABA receptors by diazepam (Sale et al., 2007; Maya-Vetencourt et al., 2008; Spolidoro et al., 2011; Greifzu et al., 2014). If administrated systemically, a dosage of 1 mg/kg has been shown to reliably block OD plasticity (Greifzu et al., 2014; Stodieck et al., 2014). Using repeated intrinsic signal imaging, we measured V1 activity driven by visual stimulation of either the contralateral or ipsilateral eye at 0 and 7 days in whisker and monocularly deprived mice which received daily injections of diazepam (1 mg/kg, i.p., WD + MD + Diaz mice, $n = 6$). In control mice we also combined WD with MD for 7 days but these animals were daily treated with saline (WD + MD + Saline mice, $n = 5$).

Fig. 3 A (upper part, black frame) depicts representative V1 activity maps elicited by contra or ipsilateral eye stimulation in control mice before (0 days) and after 7 days of WD combined with MD. Generally, darker activity maps indicate higher visually driven V1 responses. While V1 responsiveness to contralateral

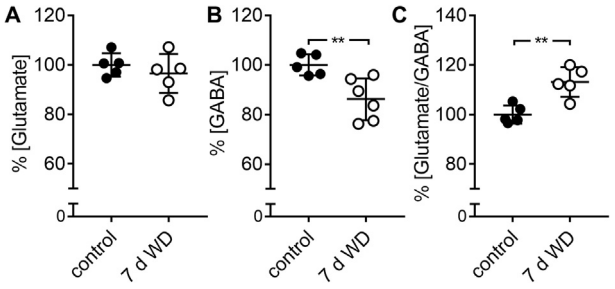

Fig. 2. WD cross-modally decreases GABAergic inhibition in V1. (a) 7 days of WD did not change V1 glutamate levels (n = 5) compared to normal control mice (n = 5), as revealed by post-mortem-HPLC analysis of V1 tissue. (b) Significant reduction of V1 GABA levels at 7 days after WD (n = 6). (c) Hence, there was a significant increase in the glutamate/GABA ratio in WD mice after this time. Open and filled circles represent measurements of individual animals and are presented together with the mean ± s.e.m. **: $p < 0.01$.

(closed) eye stimulation did not change during the time tested, there was a marked increase of V1 input strength evoked by visual stimulation of the ipsilateral (open) eye. These data are in correspondence with our recent findings that WD reinstalled "adult-like" OD plasticity in fully adult mice (Teichert et al., 2018a). Surprisingly, enhancing cortical inhibition with diazepam in WD + MD mice did not abolish OD-plasticity as expected, but instead led to a dramatic reduction of V1 responses mediated by the previously closed contralateral eye, whereas ipsilateral eye driven V1 activity remained unchanged after 7 days (Fig. 3 A, middle part, blue framed). Thus, combining WD and MD together with diazepam administration did also induce OD-plasticity, which was, however, mediated by different V1 input changes than in saline-treated WD mice.

Quantification of V1 activation showed that contralateral (closed) eye input remained unchanged in saline-treated control mice, while ipsilateral (open) eye input significantly increased 7 days after WD and MD (contra: 2.78 ± 0.144 ($\times 10^{-4}$) vs 2.66 ± 0.16 ($\times 10^{-4}$), $t(4) = 1.827 p = 0.147$; ipsi: 1.99 ± 0.14 ($\times 10^{-4}$) vs 2.41 ± 0.14 ($\times 10^{-4}$), $t(4) = 6.647$, $p = 0.0027$, paired t-tests, Fig. 3 B, C). Thus, the ocular dominance index (ODI) significantly decreased from 0.23 ± 0.02 to 0.08 ± 0.03 ($t(4) = 10.8$, $p = 0.0004$, paired t-test, Fig. 3 D). However, in diazepam-treated monocularly deprived WD mice V1 activity evoked by visual stimulation of the previously closed contralateral eye significantly decreased, whereas ipsilateral (open) eye mediated V1 responses remained unchanged (contra: 3.00 ± 0.11 ($\times 10^{-4}$) vs 2.22 ± 0.11 ($\times 10^{-4}$), $t(5) = 4.603$, $p = 0.0058$; ipsi: 1.95 ± 0.12 ($\times 10^{-4}$) vs 1.93 ± 0.05 ($\times 10^{-4}$), $t(5) = 0.257$, $p = 0.808$; paired t-tests, Fig. 3 B, C). Hence, there was a significant reduction of the ODI from 0.26 ± 0.020 to 0.06 ± 0.03 after 7 days ($t(5) = 12.26$, $p = 6.4 \times 10^{-5}$, paired t-test, Fig. 3 D).

The fact that diazepam activates $GABA_A$ receptors and reduces the neuronal activity of postsynaptic pyramidal neurons (Salin and Prince, 1996), raises the possibility that diazepam treatment per se induces a general reduction of V1 responsiveness to visual stimuli, which could in turn explain the above mentioned results. To address this issue, we chronically measured V1 activity evoked by contra and ipsilateral eye stimulation in another group of monocularly deprived mice which only received daily diazepam injection. As illustrated in Fig. 3 A (lower part, green framed), V1 response strength evoked by contra (closed) and ipsilateral (open) eye stimulation remained unchanged after 7 days. Quantification revealed that diazepam treatment alone did not change visually evoked V1 activity elicited by stimulation of the previously closed contralateral and open ipsilateral eye, respectively (contra: 3.30 ± 0.30 ($\times 10^{-4}$) vs 3.30 ± 0.15 ($\times 10^{-4}$), $t(2) = 0.042$, $p = 0.97$; ipsi: 2.05 ± 0.22 ($\times 10^{-4}$) vs 2.00 ± 0.15 ($\times 10^{-4}$), $t(2) = 0.296$, $p = 0.97$; paired t-tests, Fig. 3 B, C). Thus, the ODI remained stable in these mice (0.25 ± 0.02 vs 0.26 ± 0.02, $t(2) = 1.732$, $p = 0.23$; paired t-test, Fig. 3 D). These data indicate that increasing cortical inhibition by diazepam does not decrease visually evoked V1 responses and, does also not reactivate OD plasticity in fully adult mice. Hence, the different V1 input changes in monocularly deprived WD mice and in WD + MD mice which also received diazepam, cannot be explained by the effects of diazepam treatment alone.

OD shifts after MD mediated by a strengthening of V1 input through the ipsilateral (open) eye are characteristically found after MD in young adult mice around postnatal day 60 (Sawtell et al., 2003; Sato and Stryker, 2008; Ranson et al., 2012). Hence, here we confirmed that WD combined with MD (control mice) reinstalls this "adult" form of OD plasticity in fully adult mice, as described previously (Teichert et al., 2018a). However, MD induced OD-shifts which are caused by a reduction of contralateral (closed) eye V1 input are typically present in juvenile mice around postnatal day 28 (Gordon and Stryker, 1996). Thus, our results suggest that the combination of WD, MD and diazepam administration restores "juvenile-like" OD plasticity.

Increasing inhibition after auditory deprivation also reactivates juvenile plasticity

Recently, we could demonstrate that an auditory deprivation (AD), induced by bilateral malleus removal, cross-modally restores "adult-like" OD plasticity in fully adult mice (Teichert et al., 2018a). Hence, we next investigated whether increasing inhibition by diazepam also changes the signature of OD plasticity form "adult" to "juvenile-like" in AD mice, again using repeated intrinsic signal imaging (n = 5, Fig. 4 A). Another group of mice were monocularly and auditorily deprived and received daily injections of saline (n = 4). Quantification of V1 activity revealed that in saline-treated mice V1 responses elicited by contralateral eye stimulation remained unchanged whereas there was a strong increase of V1 activity evoked by visual stimulation of the ipsilateral (open) eye (contra: 3.0 ± 0.12 ($\times 10^{-4}$) vs 2.93 ± 0.08 ($\times 10^{-4}$), $t(3) = 0.66$, $p = 0.55$; ipsi: 2.05 ± 0.15 ($\times 10^{-4}$) vs 2.58 ± 0.11 ($\times 10^{-4}$), $t(3) = 13.48$, $p = 0.0009$; paired t-tests, Fig. 4 B, C). Thus, the average ODI significantly shifted from 0.23 ± 0.01 to 0.09 ± 0.02 ($t(3) = 12.96$, $p = 0.0009$; paired t-test, Fig. 4 D). These data confirm our previous findings that AD reactivates

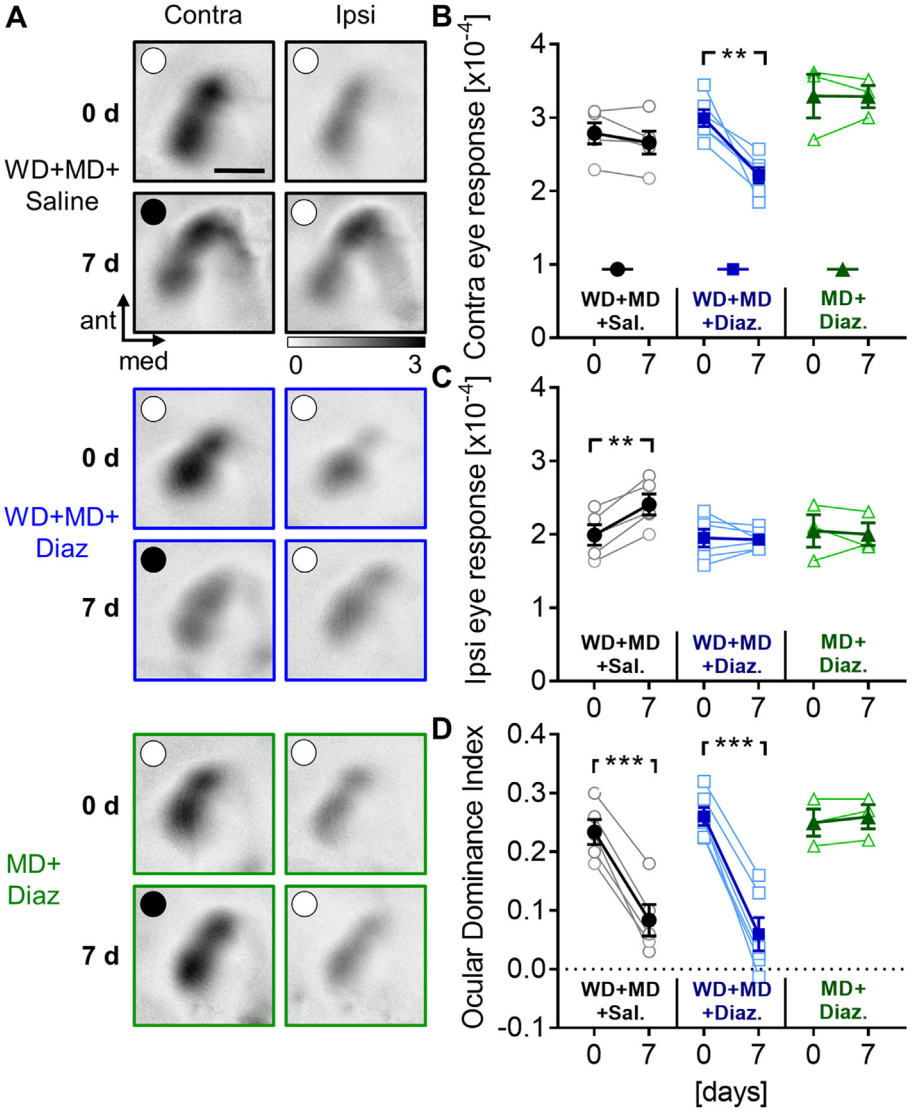

Fig. 3. Increasing cortical inhibition by diazepam in WD mice restores "juvenile-like" OD plasticity. (A) Representative V1 response maps obtained after visual stimulation of either the contralateral or ipsilateral eye before and after MD. Upper part (black framed): in saline-treated control mice (n = 5) combined WD and MD for 7 days induced a strengthening of V1 input through the ipsilateral (open) eye, whereas contralateral (closed) eye input to V1 did not change. Middle part (blue framed): daily administrations of diazepam in monocularly deprived WD mice (n = 6), however, induced a reduction of V1 responses elicited by contralateral (closed) eye stimulation, whereas ipsilateral (open) eye input did not change. Lower part (green framed): Diazepam treatment of mice which were only monocularly deprived did not affect V1 input strength (n = 3). (B, C) Quantification of V1 activity changes described above. (D) We found significant ODI reductions in control mice treated with saline after WD and MD but also after diazepam treatment. However, in mice which only received a MD, increasing inhibition by diazepam did not affect the ODI. Open circles in A: open eye, closed circles in a: closed eye. In B-D: Open circles, squares and triangles represent measurements of individual animals and filled symbols represent means ± s.e.m. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Scale bar: 1 mm.

"adult-like" OD plasticity in fully adult mice. However, increasing inhibition by diazepam in monocularly deprived AD mice induced a loss of V1 input strength through the contralateral (closed) eye, whereas V1 input through the ipsilateral (open) eye did not change (contra: 2.96 ± 0.19 ($\times 10^{-4}$) vs 2.36 ± 0.19 ($\times 10^{-4}$), $t(4) = 12.81$, $p = 0.0002$; ipsi: 0.19 ± 0.22 ($\times 10^{-4}$) vs 2.06 ± 0.20 ($\times 10^{-4}$), $t(4) = 1.108$, $p = 0.33$; paired t-test, Fig. 4 B, C). These differential changes of V1 input led to a significant reduction of the ODI in V1 of these mice (0.27 ± 0.03 vs 0.08 ± 0.03, $t(4) = 10.46$, $p = 0.0005$; paired t-test, Fig. 4 D). These data indicate that increasing cortical inhibition after AD induces "juvenile-like" OD plasticity, like above described for WD mice. Hence, our results suggest that the restoration of "juvenile-like" V1 plasticity after cross-sensory deprivation and increasing inhibition is a general feature of cross-modal plasticity in V1.

OD plasticity after WD and diazepam treatment depends on NMDA receptor activation

We could previously demonstrate that WD-induced OD plasticity requires NMDA receptor activation (Teichert et al., 2018a). Hence, we next examined whether this is also the case for OD shifts induced by WD and diazepam treatment. Therefore, we performed repeated optical imaging experiments in monocularly deprived WD mice which daily received a cocktail containing diazepam (1 mg/kg, i.p.) and CPP (15 mg/kg, i.p. WD + MD + Diaz + CPP mice, n = 3), a competitive NMDA receptor antagonist (Sato and Stryker, 2008). As shown in Fig. 5 A this treatment completely prevented V1 activity changes, which occured in monocularly deprived WD mice treated with diazepam alone. Quantification of V1 activation showed that neither contralateral (closed) nor ipsilateral (open) eye input to V1 changed in these mice (contra: 3.06 ± 0.33 ($\times 10^{-4}$) vs 3.02 ± 0.29 ($\times 10^{-4}$), $t(2) = 0.598$, $p = 0.61$; ipsi: 2.12 ± 0.16 ($\times 10^{-4}$) vs 2.12 ± 0.30, $t(2) = 0.025$, $p = 0.98$; paired t-test, Fig. 5 B, C). As a direct consequence the ODI remained unaltered after 7 days of WD + MD and treatment with diazepam and CPP (0.23 ± 0.03 vs 0.22 ± 0.04, $t(2) = 0.961$, $p = 0.44$, paired t-test, Fig. 5 D). Taken together, these data suggest that "juvenile-like" OD shifts induced by WD + MD and diazepam treatment are mediated by NMDA receptor activation, too. In general, these results underline the pivotal role of NMDA receptors in cross-modally restored OD plasticity in fully adult mice (Teichert et al., 2018a).

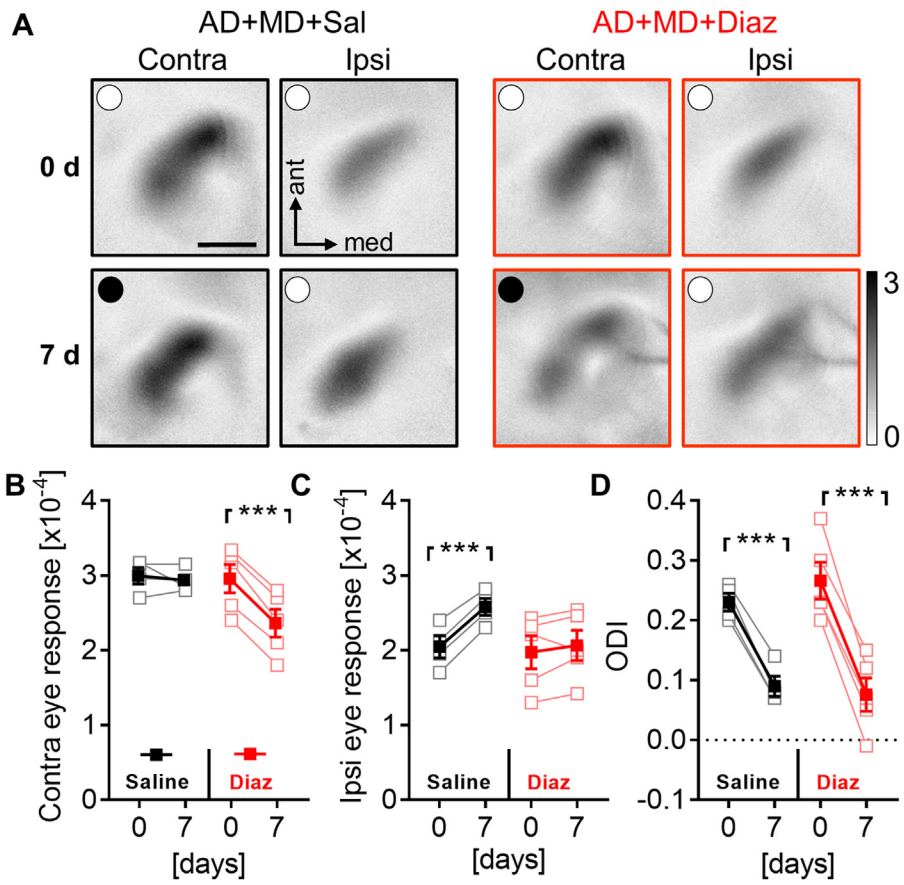

Fig. 4. Increasing inhibition by diazepam after AD cross-modally restores "juvenile-like" OD plasticity in fully adult mice. (A) Representative amplitude maps obtained after visual stimulation of either the contralateral (previously closed) or ipsilateral (open) eye before and after MD in auditorily deprived mice treated either with saline (n = 4) or diazepam (n = 5), respectively. (B) V1 activity elicited by contralateral eye stimulation remained unchanged in saline but significantly decreased in diazepam-treated mice. (C) V1 input through the ipsilateral eye significantly increased in mice which received daily saline injections but did not change in mice after diazepam treatment. (D) In mice of both groups there was a significant reduction of the ODI. Open circles in A: open eye, closed circles in a: closed eye. In B-D: Open circles, squares and triangles represent measurements of individual animals and filled symbols represent means ± s.e.m. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Scale bar: 1 mm.

DISCUSSION

In sensory systems the capacity of the brain to undergo experience-dependent plastic changes is typically restricted to well-defined time windows soon after birth (Wiesel and Hubel, 1963; Hensch, 2005). However, after the end of these critical periods, brain plasticity dramatically declines. Because of its potential translational implications, restoring cortical plasticity in adults is a topic of great scientific interest.

We could recently demonstrate that the deprivation of different non-visual sensory modalities reinstalled OD plasticity in V1 of fully adult mice (Teichert et al., 2018a). Specifically, both WD and AD, combined with MD for 7 days induced OD shifts which were mediated by an increased V1 responsiveness after ipsilateral (open) eye stimulation, whereas contralateral (closed) eye input to V1 did not change (Teichert et al., 2018a). This is the characteristic signature of "adult-like" plasticity, typically found in young adult mice around PD 60 (Fig. 6) (Sato and Stryker, 2008; Ranson et al., 2012). However, here we made the surprising finding that enhancing GABAergic inhibition did not prevent these OD shifts, as suggested by many previous studies (Fig. 6) (Sale et al., 2007; Maya-Vetencourt et al., 2008; Greifzu et al., 2014; Stodieck et al., 2014). Instead, after combining either WD or AD, with diazepam injections, OD shifts were still present but were now mediated by a reduction in contralateral (closed) eye input to V1 but the ipsilateral (open) eye remained unaltered (Figs. 3 and 4), which is the characteristic feature of "juvenile-like" OD plasticity, generally present in very young mice around 30 days of age (Fig. 6) (Gordon and Stryker, 1996; Ranson et al., 2012). These results indicate that mechanisms, which mediate cross-modally induced OD shifts, change after artificially increasing inhibition in the cortex. Thus, our results suggest that the alteration of GABAergic inhibition in V1 is not the only mechanism involved in mediating cross-modally restored V1 plasticity.

To the best of our knowledge, this finding is unique. Previous interventions such as dark exposure (He et al., 2006; Stodieck et al., 2014), environmental enrichment (Sale et al., 2007; Baroncelli et al., 2010; Greifzu et al., 2014), food restriction (Spolidoro et al., 2011) and fluoxetine administrations (Maya-Vetencourt et al., 2008), all restored OD plasticity in V1 of fully adult mice. A common thread of these treatments is that they all cause lower levels of GABAergic inhibition in V1. However, while dark exposure restored the "adult" form of OD plasticity (Stodieck et al., 2014), environmental enrichment and fluoxetine treatment for example reinstalled the "juvenile" form (Fig. 6) (Maya-Vetencourt et al., 2008; Greifzu et al., 2014). Despite these differences, mechanistically, the decrease in inhibition appears to be the central hub, because increasing inhibition by diazepam prevents OD shifts after these interventions (Fig. 6) (Sale et al., 2007; Maya-Vetencourt et al., 2008; Spolidoro et al., 2011; Greifzu et al., 2014; Stodieck et al., 2014).

This idea is further supported by the finding that OD plasticity in fully adult rodents can also be restored by reducing GABA synthesis or antagonizing GABA receptors in the adult V1 (Harauzov et al., 2010). Indeed, in accordance with these findings, we show here that cross-modally induced OD plasticity is also associated

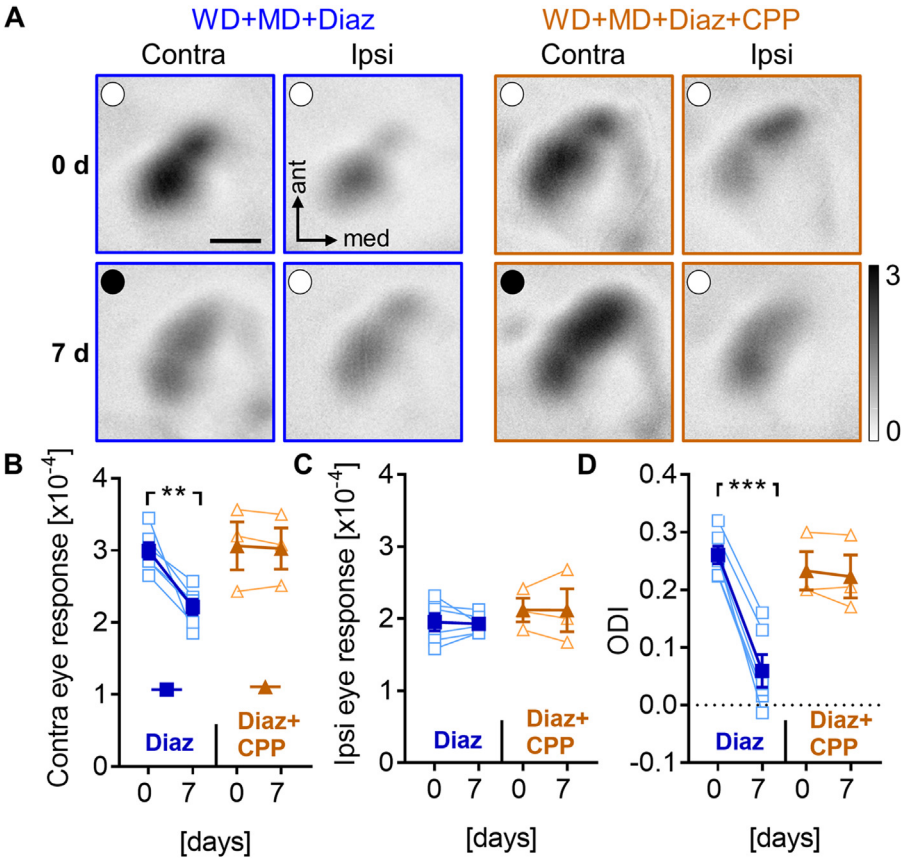

Fig. 5. Cross-modally restored "juvenile-like" OD plasticity after diazepam treatment depends on the NMDA receptor. (A) Representative V1 response maps evoked by contra (previously closed) or ipsilateral (open) eye stimulation, respectively, before and after MD in WD mice daily treated with diazepam (n = 6) or a cocktail containing diazepam and CPP, a competitive NMD receptor blocker (n = 3). (B) While diazepam treatment in monocularly deprived WD mice decreased V1 responsiveness to contralateral eye stimulation, additional CPP treatment abolished these changes. (C) There were no alterations in the V1 input strength through the ipsilateral eye in mice of both groups. (D) Additional CPP treatment prevented OD shifts in diazepam-treated mice after AD and MD. Open circles in A: open eye, closed circles in a: closed eye. In B-D: Open circles, squares and triangles represent measurements of individual animals and filled symbols represent mean ± s.e.m. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Scale bar: 1 mm.

with a decrease of V1 GABA levels (Fig. 2). Notably, Harauzov and colleagues (2010) could also demonstrate that reducing the inhibitory tone facilitated white matter stimulation induced long-term potentiation (LTP) in V1 slices, whereas the thresholds for LTD remained unchanged. They therefore proposed that this treatment would most likely induce "adult-like" OD plasticity after MD (Harauzov et al., 2010), since this type of V1 plasticity is indeed mediated by LTP-like mechanisms (Sawtell et al., 2003; Cooke and Bear, 2014). This idea is also in line with the present finding that both WD and AD, restored the "adult" form of OD plasticity (Figs. 3 and 4). At first glance, these results seem to indicate that the reduction of GABA levels in V1 is causal for the cross-modal restoration of OD plasticity.

Most previous studies demonstrating that a reduction of cortical GABA levels restores OD plasticity used *in vivo* brain microdialysis to obtain cortical probes (Sale et al., 2007; Maya-Vetencourt et al., 2008; Baroncelli et al., 2010). The GABA content of these probes was subsequently determined by HPLC analysis. This experimental approach measures extracellular GABA (Sale et al., 2007; Maya-Vetencourt et al., 2008; Baroncelli et al., 2010) which most likely represent the biologically active GABA in the synaptic clefts. In the present study, we determined GABA levels in micro punches of V1 tissue which contain both extracellular and intracellular GABA. Thus, we cannot make definite statements on the exact reduction of extracellular GABA after WD. However, as we found a general cross-modal reduction in V1 GABA levels (Fig. 2), we believe that this is accompanied with a general reduction of inhibition, as suggested previously (Harauzov et al., 2010; Spolidoro et al., 2011)

Enhancing GABAergic transmission with diazepam did not abolish cross-modally induced OD shifts but instead changed the signature of OD plasticity (Figs. 3 and 4). Hence, our data suggest that the reduction of GABA levels in V1 after WD cannot be the only reason for restoring OD plasticity. Instead, there must be additional mechanisms which mediate cross-modal plasticity. In other words, the WD induced decrease of GABA levels sets V1 of fully adult mice back into a plastic stage facilitating the induction of "adult-like" OD plasticity (Fig. 6). However, increasing GABAergic inhibition in V1 by diazepam activates other, so far unknown, mechanisms, which do not only require reduced GABA levels in V1, to mediate cross-modally induced "juvenile-like" plasticity. Such alternative mechanisms have been already described. For instance, magnesium treatment, which increased the expression of specific subunits of the NMDA receptor, has been shown to restore "juvenile-like" OD plasticity in fully adult mice without changing the inhibitory tone in V1 (Liu et al., 2015). Likewise, PSD-95 KO mice display a lifelong preservation of "juvenile-like" OD plasticity, which is not accompanied by a reduction of inhibition in V1, and, hence, cannot be prevented by diazepam administration (Huang et al., 2015). Again, these findings demonstrate that there are, indeed, mechanisms which can restore "juvenile-like" plasticity without reducing cortical inhibition in V1 (Fig. 6).

Generally, in accordance with our previous findings that both WD and AD reactivate "adult-like" OD plasticity in V1 of fully adult mice, we could here demonstrate that the same sensory deprivations (WD

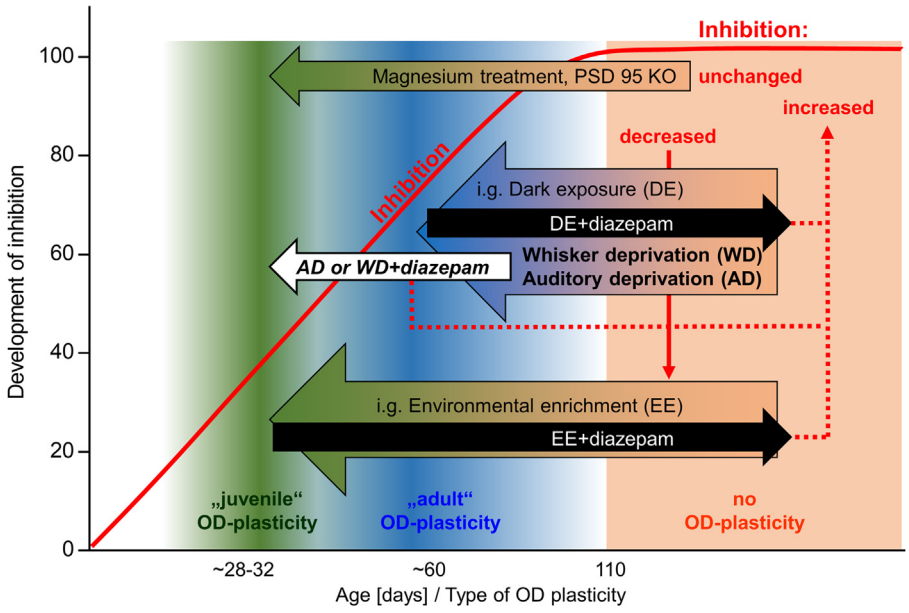

Fig. 6. Developmental increase of GABAergic inhibition in V1 is associated by a decline in V1 plasticity. Between 28 and 32 days of age relatively low inhibition levels cause high plasticity levels in mouse V1. MD during this critical period induces a depression of the contralateral (closed) eye input to V1, a signature of "juvenile" OD plasticity (Gordon and Stryker, 1996; Hensch and Fagiolini, 2005; Ranson et al., 2012). In young adult mice, at around 60 days of age, the quality of OD shifts changes, as MD causes potentiation of ipsilateral (open) eye input to V1, the characteristic feature of "adult" OD plasticity (Sato and Stryker, 2008; Ranson et al., 2012). In mice older than 110 days, when cortical inhibition is high, OD plasticity is completely absent (Lehmann and Lowel, 2008). However, interventions that decrease GABAergic inhibition in V1, such as dark exposure (DE) (He et al., 2006; Stodieck et al., 2014) or environmental enrichment (EE) (Sale et al., 2007; Greifzu et al., 2014), can restore "adult-like" and "juvenile-like" OD plasticity. Increasing GABAergic inhibition by diazepam, however, prevents OD plasticity after these interventions (Greifzu et al., 2014; Stodieck et al., 2014). We could previously demonstrate that whisker deprivation (WD) reinstalled the "adult-like" form of OD plasticity in fully adult mice older than 110 days (Teichert et al., 2018a). Here, we show that WD decreases GABA levels in V1, suggesting that this alteration is causal for the restoration of "adult-like" plasticity". However, as shown here, increasing GABAergic inhibition by diazepam, which should shift the impact of cortical GABA to the "no-OD-plasticity-stage" did not prevent OD shifts. Strikingly, the signature of OD plasticity changed from "adult" to "juvenile". Interestingly, this was also the case after diazepam treatment of AD mice. Thus, the cross-modal restoration of "juvenile-like" plasticity in V1 does not solely depend on alterations of the inhibitory tone, but also requires alternative, so far unknown cellular and molecular mechanisms.

and AD) can also reactivate "juvenile-like" plasticity in V1 after increasing inhibition. Collectively, these results suggest a high similarity of mechanisms taking place in V1 after depriving non-visual sensory modalities. However, the cross-modal interplay of primary sensory regions in normal mice appears to be asymmetric (Iurilli et al., 2012; Teichert and Bolz, 2017b). For instance, while sensory-evoked activity in the primary somatosensory (S1) and primary auditory cortex (A1) suppresses V1 responses (Iurilli et al., 2012; Teichert and Bolz, 2017b, a), V1 activity evokes depolarizations in S1 and has only little impact on A1 responses (Iurilli et al., 2012). Thus, it might be that deprivation of vision provokes different effects on the remaining sensory cortices such as S1 and A1.

There is increasing evidence that the "juvenile" form of OD plasticity is mediated by LTD-like mechanisms that decrease contralateral (closed) eye input (Kirkwood and Bear, 1994; Heynen et al., 2003; Espinosa and Stryker, 2012; Cooke and Bear, 2014), whereas the "adult" form occurs via LTP-like changes that lead to an increase of open eye input to V1 (Sawtell et al., 2003; Sato and Stryker, 2008; Ranson et al., 2012; Cooke and Bear, 2014). This idea is supported by the finding that both "juvenile" and "adult" plasticity require NMDA receptor activation (Sato and Stryker, 2008). In accordance with these findings we could recently show that cross-modally restored "adult" OD plasticity depends on NMDA receptor activation (Teichert et al., 2018a). As demonstrated in the present study, this is also the case when "juvenile" OD plasticity is induced by diazepam administration in WD mice (Fig. 5). Thus, these results suggest that diazepam administration in monocularly and whisker deprived mice, facilitates LTD-like mechanisms, whereas MD combined with WD alone facilitates LTP-like changes in V1. Generally, these results also demonstrate the central role of the NMDA receptor in cross-modal plasticity (Teichert et al., 2018a).

CONCLUSION

Here we provide evidence that cross-modally induced OD plasticity in fully adult mice older than 110 days (Teichert et al., 2018a) is accompanied by a reduction of GABA levels in the spared V1. This finding is in line with the current view that a decrease in the inhibitory tone is the central hub which mediates restoration of cortical plasticity. In contrast and surprisingly, our results also suggest that mechanisms other than reduced GABA levels mediate the cross-modal restoration of OD plasticity, as increasing the inhibitory tone did not abolish OD plasticity. However, the signature of OD plasticity changed from "adult-like" to "juvenile-like". While further research is needed to unravel the precise underlying molecular mechanisms, the present results emphasize the power of cross-modally induced plasticity to re-open a window of high plasticity in the fully adult cortex far beyond any sensory critical period. More general, while therapeutic interventions after sensory damage concentrate on the affected sensory modality, there might be a unique opportunity to sharpen and refine the spared senses and thereby partially compensate sensory deficits.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AUTHOR CONTRIBUTIONS

MT, MI and JB designed the study.
MT, MI, FW, CW performed the experiments.
MT, MI, and JB analyzed data.
MT and JB wrote the paper.

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RESEARCH ARTICLE

Visual deprivation independent shift of ocular dominance induced by cross-modal plasticity

Manuel Teichert[1,2☯], Marcel Isstas[1☯], Lutz Liebmann[3], Christian A. Hübner[3], Franziska Wieske[4], Christine Winter[4], Konrad Lehmann[1], Jürgen Bolz[1]*

1 Institute of General Zoology and Animal Physiology, University of Jena, Jena, Germany, **2** Synapses-Circuits-Plasticity, Max Planck Institute of Neurobiology, Martinsried, Germany, **3** Institute of Human Genetics, University Hospital Jena, University of Jena, Jena, Germany, **4** Department of Psychiatry, Technical University Dresden, Dresden, Germany

☯ These authors contributed equally to this work.
* Jurgen.Bolz@uni-jena.de

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1 Institute of General Zoology and Animal Physiology, University of Jena, Jena, Germany, **2** Synapses-Circuits-Plasticity, Max Planck Institute of Neurobiology, Martinsried, Germany, **3** Institute of Human Genetics, University Hospital Jena, University of Jena, Jena, Germany, **4** Department of Psychiatry, Technical University Dresden, Dresden, Germany

☯ These authors contributed equally to this work.
* Jurgen.Bolz@uni-jena.de

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Abstract

There is convincing evidence that the deprivation of one sense can lead to adaptive neuronal changes in spared primary sensory cortices. However, the repercussions of late-onset sensory deprivations on functionality of the remaining sensory cortices are poorly understood. Using repeated intrinsic signal imaging we investigated the effects of whisker or auditory deprivation (WD or AD, respectively) on responsiveness of the binocular primary visual cortex (V1) in fully adult mice. The binocular zone of mice is innervated by both eyes, with the contralateral eye always dominating V1 input over ipsilateral eye input, the normal ocular dominance (OD) ratio. Strikingly, we found that 3 days of WD or AD induced a transient shift of OD, which was mediated by a potentiation of V1 input through the ipsilateral eye. This cross-modal effect was accompanied by strengthening of layer 4 synapses in V1, required visual experience through the ipsilateral eye and was mediated by an increase of the excitation/inhibition ratio in V1. Finally, we demonstrate that both WD and AD induced a long-lasting improvement of visual performance. Our data provide evidence that the deprivation of a non-visual sensory modality cross-modally induces experience dependent V1 plasticity and improves visual behavior, even in adult mice.

Introduction

It has been demonstrated that the loss or deprivation of one sensory modality can have profound effects on the remaining senses. Such changes are broadly referred to as "cross-modal plasticity" and can improve the functionality of the intact senses [1–6]. Earlier studies suggested that these compensatory enhancements arise because the deprived cortex becomes driven by the spared sensory modalities, broadly referred to as "cross-modal recruitment" [1, 2, 6, 7]. However, there is increasing evidence that functional improvements of the remaining senses can be also attributed to rapid or long-term adaptive changes in the spared sensory cortices. For instance, we could recently show that auditory deprivation (AD) leads to a rapid

increase of visually evoked responses in the spared V1 which was accompanied by improvements of V1 spatial frequency and contrast tuning [4, 8]. While these changes appeared most likely due to a rapid disinhibitory effect [4], previous studies demonstrated that more prolonged sensory deprivations lead to plastic alterations in spared primary sensory cortices [6]. For example, a few days of visual deprivation in juvenile mice selectively strengthened layer 4-2/3 synapses in the somatosensory barrel cortex and sharpened the functional whisker-barrel map in layers 2/3 [9]. Similarly, one week of visual deprivation was shown to strengthen thalamo-cortical synapses in the spared primary auditory cortex (A1) of juvenile but also adult mice [10]. These plastic changes were accompanied by increased contrast sensitivity and frequency tuning of A1 neurons [10]. Moreover, in a recent study we could demonstrate that one to two weeks of whisker deprivation (WD) in fully adult mice massively enhanced spatial frequency and contrast tuning of the primary visual cortex (V1) and even markedly improved visually driven behavior [3]. These studies suggest that the ability of sensory cortices to undergo cross-modal plasticity is not restricted to sensory critical periods of early postnatal development, but can also take place in adults, although cortical plasticity levels decline with aging [11–13].

A valuable model of plasticity which typically displays an age dependent decline is the so called OD-plasticity. In young mice, for instance, a monocular deprivation (MD) for a few days shifts the OD away from the closed eye [12, 14]. However, in fully adult mice older than 110 days this type of plasticity is completely absent [11]. In terms of cross-modal plasticity we could recently show that both WD and AD cross-modally restore OD-plasticity in the spared V1 of such fully adult mice [15]. Specifically, combing WD or AD with monocular deprivation (MD) for 7 days induced a shift of the OD in the binocular zone of V1 which was mediated by an increased V1 responsiveness to open eye stimulation [15]. Collectively, these findings suggest that sensory deprivations lead to short-term and, in particular, long-term plastic neuronal alterations, which in turn improve the functionality of spared primary sensory cortices to compensate for the impairment of the deprived sense. However, the time course of events taking place in the spared cortices after the loss or deprivation of another sense is largely unknown. Moreover, the repercussions of late-onset sensory deprivations on functionality of the remaining sensory cortices are still poorly understood.

In order to address these issues we here investigated the cross-modal effects of WD or AD on V1 responsiveness and visually mediated behavior at 0, 3 and 7 days after the deprivation in fully adult mice far beyond their sensory critical periods. For this, we chronically measured V1 responses evoked by visual stimulation of the contralateral and ipsilateral eye using Fourier based periodic intrinsic signal imaging. Strikingly, we found that both WD and AD induced a marked shift of the OD in V1 after 3 days, which was mediated by a strong increase of V1 responses evoked by visual stimulation of the ipsilateral eye. Notably, this OD shift took place without preceding MD, the common paradigm to induce alterations of OD in the visual cortex [12, 14]. Intrinsic imaging after another 4 days of WD or AD revealed that V1 input through the ipsilateral eye and thus also OD completely recovered to baseline levels, suggesting that homeostatic mechanisms readjust activity levels in V1. Finally, we investigated the effects of WD or AD on behavioral visual performance. Strikingly, we found that both spatial frequency and contrast sensitivity thresholds of the optokinetic reflex (OKR) massively improved in a V1 dependent and V1 independent manner. Taken together, our results suggest that the deprivation of a non-visual sensory modality induces plastic changes in the binocular zone of V1 and leads to long-lasting improvements of visual performance, even in fully adult mice.

Material and methods

Animals

C57BL/6J (Jackson labs) mice were raised in a group of 2–3 in transparent standard cages (16.5x22.5 cm) on a 12 h light/dark cycle, with food and water available *ad libitum*. Between the chronic experiments each animal was housed alone in a standard cage. The environment in the cage was minimally enriched with cotton rolls and nest material. In our mouse facility the light intensity was about 150–170 lux. As demonstrated recently, these rearing conditions are not sufficient to extend OD plasticity into adulthood [15]. Animal housing in our institution is regularly supervised by veterinaries from the state of Thuringia, Germany. For the present study we used fully adult male and female mice (PD 120–240). All experimental procedures have been performed according to the German Law on the Protection of Animals and the corresponding European Communities Council Directive 2010 (2010/63/EU), and were approved by the Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz (Thuringia State Office for Food Safety and Consumer Protection) under the registration numbers 02-032/16 and 02-050/14. Every effort was made to minimize the number of animals used and their suffering. For or after the experiments stated below, almost all animals were sacrificed by cervical dislocation. Only for following Nissl-staining the mice were perfused transcardially with PBS followed by a 4% PFA (in PBS) solution.

Optical imaging of intrinsic signals

Mouse preparation for optical imaging. To investigate the effects of WD (n = 32) or AD (n = 6) on visually evoked activity of V1 we used Fourier based periodic intrinsic signal imaging [4, 16]. In addition, imaging experiments were performed in n = 9 untreated control mice. As described previously [3, 4], animals were initially anesthetized with 4% isoflurane in a mixture of 1:1 O_2/N_2O and placed on a heating blanket for maintaining body temperature (37.5˚C). Subsequently, mice received injections of chlorprothixene (20 μg/mouse i.m.) and carprofene (5 mg/kg, s.c.). The animal was fixed in a stereotaxic frame and we removed the skin of the left hemisphere to expose the visual cortex. The exposed area was covered with 2.5% agarose in saline and sealed with a standard microscope glass coverslip. Cortical responses were always recorded through the intact skull. During the experiment isoflurane inhalation anesthesia was applied through a plastic mask and maintained at 0.5–0.6%.

Mouse preparation for repeated imaging experiments. Repeated intrinsic imaging in the same mice was performed as described previously [15, 17]. Briefly, after the first imaging session the skin was re-sutured and animals were returned to their standard cages. During the subsequent days animals received a daily injection of carprofen (5 mg/kg, s.c.) for pain prevention. Before the next imaging session (day 3 and 7) the skin was re-opened and imaging was performed as described above.

Imaging of visual cortex. Responses of mouse primary visual cortex were recorded described previously [15, 18]. Briefly, the method uses a periodic stimulus that is presented to the animal for some time and cortical responses are extracted by Fourier analysis. In our case, the visual stimulus was a drifting horizontal light bar of 2˚ width, 100% (or 10%, respectively) contrast and with a temporal frequency of 0.125 Hz. The stimulus was presented on a high refresh rate monitor (Hitachi Accuvue HM 4921-D) placed 25 cm in front of the animal. Visual stimulation was adjusted so that it only appeared in the binocular visual field of the recorded hemisphere (-5˚ to +15˚ azimuth, -17˚ to +60˚ elevation). The stimulus was presented to the contra or ipsilateral eye or to both eyes for 5 min. Thus, the stimulus was repeated for about 35 times during one presentation period.

CCD camera recording procedure. Using a Dalsa 1M30 CCD camera (Dalsa, Waterloo, Canada) with a 135x50 mm tandem lens (Nikon, Inc., Melville, NY), we first recorded images of the surface vascular pattern via illumination with green light (550±2 nm) and, after focusing 600 μm below the pial surface, intrinsic signals were obtained via illumination with red light (610±2 nm). Frames were acquired at a rate of 30 Hz and temporally averaged to 7.5 Hz. The 1024x1024 pixel images were spatially averaged to a 512x512 resolution. We always imaged the left hemisphere of the animals.

Data analysis. From the recorded frames the signal was extracted by Fourier analysis at the stimulation frequency and converted into amplitude and phase maps using custom software [18]. In detail, from a pair of the upward and downward maps, a map with absolute retinotopy and an average magnitude map were computed. For data analysis we used the MATLAB standard as described previously [11, 19]. The magnitude component represents the activation intensity of the visual cortex. Since high levels of neuronal activity decrease oxygen levels supplied by hemoglobin and since deoxyhemoglobin absorbs more red light (610±2 nm), the reflected light intensity decreases in active cortical regions. Because the reflectance changes are very small (less than 0.1%) all amplitudes are multiplied with 10^4 so that they can be presented as small positive numbers. Thus, the obtained values are dimensionless. Amplitude maps were obtained by averaging the response amplitudes of individual pixels from maps to upward and downward moving bars. The ocular dominance index was computed as (C-I)/(C+I) with C and I representing the peak response amplitudes of V1 elicited by contralateral eye and ipsilateral eye stimulation, as described previously [17, 19]. To each condition we took at least three magnitudes of V1 responsiveness and averaged them for data presentation.

Whisker deprivation (WD) and auditory deprivation (AD)

WD and AD were always performed immediately before the first imaging session or optometry experiments (day 0). WD was performed as described previously [3, 15, 20]. Briefly, animals were deeply anesthetized with 2% isoflurane in a mixture of 1:1 O_2/N_2O applied through a plastic mask. The eyes of the animal were protected with silicon oil. Whiskers (macro vibrissae) were plucked bilaterally using fine forceps. Subsequently mice received an injection of carprofene (4 mg/kg, s.c.) for pain prevention and were returned to their standard cages. Over the following days whiskers were re-shaved every other day, and animals received a daily administration of carprofene (4 mg/kg, s.c.).

AD was always induced by bilateral malleus removal as described previously [3, 4, 8]. Briefly, animals were deeply anesthetized with 2% isoflurane in a mixture of 1:1 O_2/N_2O applied through a plastic mask. Additionally, mice received a subcutaneous injection of carprofene (4 mg/kg, s.c.) for pain prevention. The eyes of the animal were protected with silicon oil. The tympanic membrane was punctured and the malleus was removed under visual control through this opening using fine sterilized forceps. Great care was taken to avoid any destruction of the stapes and the oval window which is visible through the hearing canal (see [21]). Over the following days animals received a daily administration of carprofene (4 mg/kg, s.c.).

Monocular deprivation (MD)

We examined whether the cross-modally induced V1 activity changes depend on patterned visual input. For this, in one group of mice (n = 4) we sutured the contra and in another group we sutured the ipsilateral eye (n = 5). MD was always performed after the first imaging session, thus, during the same anesthesia like WD. For this, we increased the isoflurane concentration to 2% in a mixture of 1:1 N_2O and O_2. Lid margins of the contra or ipsilateral eye, respectively,

were trimmed and an antibiotic ointment was applied. Subsequently the eye was sutured. After MD animals received one injection of carprofene (4 mg/kg, s.c.) and were returned to their standard cages. All animals were checked daily to ensure that the sutured eye remained closed during the MD time. Over the following 3 days animals received a daily administration of carprofene (4 mg/kg, s.c.) for pain prevention.

Electrophysiology

Slice preparation for electrophysiological recordings. 350-μm-thick brain slices were prepared from 3-month-old mice (control: n = 4, 3 d WD: n = 3) in preparation aCSF (in mM): 2.5 KCl, 6 $MgSO_4$, 1.25 NaH_2PO_4, 0.25 $CaCl_2$, 260 D-glucose, 25.0 $NaHCO_3$, 2 sodium pyruvat, 3 myo inositol, 1 kynurenic acid. At room temperature slices equilibrated for at least 1 h in recording aCSF (in mM): 125 NaCl, 2.5 KCl, 1 $MgSO_4$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 10 D-glucose, 25.0 $NaHCO_3$, 2 sodium pyruvat, 3 myo inositol, 0.4 ascorbic acid, gassed with 95% O_2 / 5% CO_2, pH7.3.

Patch clamp recordings. Coronal brain slices were placed in a submerged recording chamber mounted on an upright microscope (BX51WI, Olympus). Slices were continuously superfused with aCSF (2–3 ml/min, 32 ˚C, pH 7.3). Patch clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) were performed as described previously [22]. mEPSCs were recorded in layer 4 pyramidal neurons V1. Layer 4 was identified based upon its relatively small cell size and high packing density compared to the surrounding layers. Pyramidal neurons were selected if they displayed a pyramidal-shaped cell body, in agreement with the morphology of principle neurons in the mouse cortex. Inhibitory neurons, which are usually smaller and exhibit very high input resistance values, were avoided [23]. mEPSCs were recorded at a holding potential of −70 mV for at least 5 min in aCSF. Data analysis was performed off-line with the detection threshold levels set to 3 pA for mEPSCs. mEPSCs were isolated by adding tetrodotoxin (0.5 μM, Tocris Bioscience) and bicuculline methiodide (20 μM, Biomol) to block action potential-induced glutamate release and $GABA_A$ receptor-mediated mIPSCs, respectively. 30 μM (2*R*)-amino-5-phosphonovaleric acid (dl-APV; Sigma-Aldrich) was added to suppress NMDA currents. The pipette solution contained the following (in mM): 120 $CsMeSO_4$, 17.5 CsCl, 10 HEPES, 5 BAPTA, 2 Mg-ATP, 0.5 Na-GTP, 10 QX-314 [*N*-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide], pH 7.3, adjusted with CsOH. The following parameters were determined: frequency and peak amplitude.

CPP, diazepam and saline injections

To investigate the role of the N-methyl-D-aspartate (NMDA)-receptor on V1 responsiveness and OKR thresholds we administrated the competitive NMDA-receptor blocker (WD+CPP: n = 8) (R,S)-3-(2-carbooxypiperazin-4-yl)propyl-1-phosphonic (CPP, Abcam). CPP was diluted in saline and injected intraperitoneally (i.p.) every 24 h at a dose of 12–15 mg/kg in a volume of 0.12 ml [15, 24]. To increase the level of cortical inhibition in WD mice for imaging experiments we intraperitoneally injected 0.12 ml diazepam solution (in saline, 1mg/kg; n = 4) daily. In control mice (n = 4), we daily injected the same volume of saline (i.p.).

High performance liquid chromatography (HPLC)

Brain micropunches were taken from 1 mm V1 slices at −3.28 from Bregma from control (n = 5) and WD mice (n = 6) and homogenized by ultrasonication in 20 vol of 0.1 N perchloric acid at 4 ˚C immediately after collection. A total of 100 ml of the homogenate was added to equal volumes of 1 N sodium hydroxide for measurement of protein content. The remaining homogenate was centrifuged at 17 000 g and 4 ˚C for 10 min. Glutamate and GABA levels

were determined using methods described previously [25]. Briefly, amino acids were precolumn-derivatized with o-phthalaldehyde-2-mercaptoethanol using a refrigerated autoinjector and then separated on a HPLC column (ProntoSil C18 ace-EPS) at a flow rate of 0.6 ml/min and a column temperature of 40 ˚C. The mobile phase was 50 mM sodium acetate (pH 5.7) in a linear gradient from 5% to 21% acetonitrile. Derivatized amino acids were detected by their fluorescence at 450 nm after excitation at 330 nm.

Optomotor system

To determine subcortically mediated vision thresholds for spatial frequency and contrast of the optomotor response after WD (n = 11), AD (n = 4) or in untreated control mice (n = 4), we used a virtual optomotor system [26]. Briefly, placed on a platform, freely moving animals were surrounded by moving vertical sine wave gratings of varying spatial frequencies and contrasts. Mice reflexively track grating by head movements (optokinetic reflex, OKR) as long as they can see it [26]. Thresholds for spatial frequencies were measured at 100% contrast and the contrast thresholds were determined at a spatial frequency of 0.2 cycles per degree (cyc/deg). From contrast thresholds contrast sensitivity was calculated (contrast sensitivity = (1/ contrast thresholds)x100)). We measured both spatial frequency and contrast sensitivity for each eye separately and, because they were almost identical, averaged these measurements for data presentation.

V1 aspiration

To investigate whether V1 is required for the observed effects of WD on the OKR, the monocular and binocular V1 was aspirated bilaterally in WD (n = 3) and control mice (n = 3). First, mice received an injection of carprofene (5 mg/kg) for pain prevention. The correct position of V1 was determined using intrinsic imaging as described previously [4]. Briefly, to localize V1, animals were stimulated with a moving 2˚ wide horizontal light bar presented on the monitor placed in the right or left visual field at a distance of 25 cm to stimulate right and left eye, respectively. The bar covered 79˚ azimuth. The obtained retinotopic color coded phase map was then merged with a picture of the skull vascular pattern. Through a burr hole V1 was aspirated bilaterally as described previously [27] and the skin was re-sutured. Animals received subcutaneous carprofene (5 mg/kg) daily for pain prevention.

Nissl staining

To demonstrate the efficiency of V1 aspiration experiments, we sacrificed mice tested in the Optomotry and performed a Nissl staining in the obtained brain slices. For this, brain sections were fixed in ethanol (95%) containing 5% acetic acid (99.5%) for 30 min. After washing with distilled water sections were incubated in a cresyl violet solution (0.1% in distilled water) for 4 min. After a further incubation in ethanol with ascending concentrations (50%, 70%, 95%, 99.9%) and xylol (98%), sections were embedded in depex (Serva). The sections were observed using a bright field microscope (Olympus) using a 10x objective.

Experimental design and statistical analysis

To investigate whether WD or AD affect the responsiveness of the visual cortex we performed before-after comparisons of optical imaging data by ANOVA with repeated measures followed by Bonferroni correction. Between-group comparisons were performed by one-way AVOVA, again, followed by Bonferroni correction. Electrophysiological measurements were compared either by a Kolmogorov-Smirnov or unpaired t-test. HPLC were also compared by an

unpaired *t-test*. To examine potential effects of the cross-sensory deprivation on the visually mediated OKR, behavioral data of control, WD and AD mice (spatial frequency and contrast thresholds) were first analyzed by a two-way ANOVA with repeated measurements. After this group data were compared by *post hoc* two-tailed unpaired student´s *t*-test. The resulting *p*-values were then Bonferroni corrected. In the graphs, the levels of significance were set as: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. Data were analyzed using GRAPHPAD PRISM and SPSS and are presented as means and standard error of the mean (s.e.m) or as measurements of individual animals.

Results

Cross-modally induced shifts of ocular dominance (OD)

We investigated the cross-modal effects of the deprivation of a non-visual sensory modality on visually evoked activity in the spared binocular V1 in fully adult mice (PD 120–240). For this, we induced either a somatosensory deprivation by bilaterally removing the macro-vibrissae (whisker deprivation, WD; n = 7) [3, 15] or an auditory deprivation (AD, n = 6) by bilateral malleus removal [4, 28] and performed repeated intrinsic signal imaging experiments directly after either WD or AD (day 0) and 3 and 7 days after WD or AD (Fig 1a). Intrinsic signal imaging allows repeated non-invasive measurements of V1 responses evoked by visual stimulation [17, 29] and its reliability has been profoundly validated by electrophysiological recordings [17, 30]. Since the binocular V1 of mice receives input of both eyes, we measured V1 activity evoked by visual stimulation of the contralateral and ipsilateral eye separately (Fig 1b). As a visual stimulus we used a drifting light bar of 100% contrast which was presented in the right binocular visual field (Fig 1b).

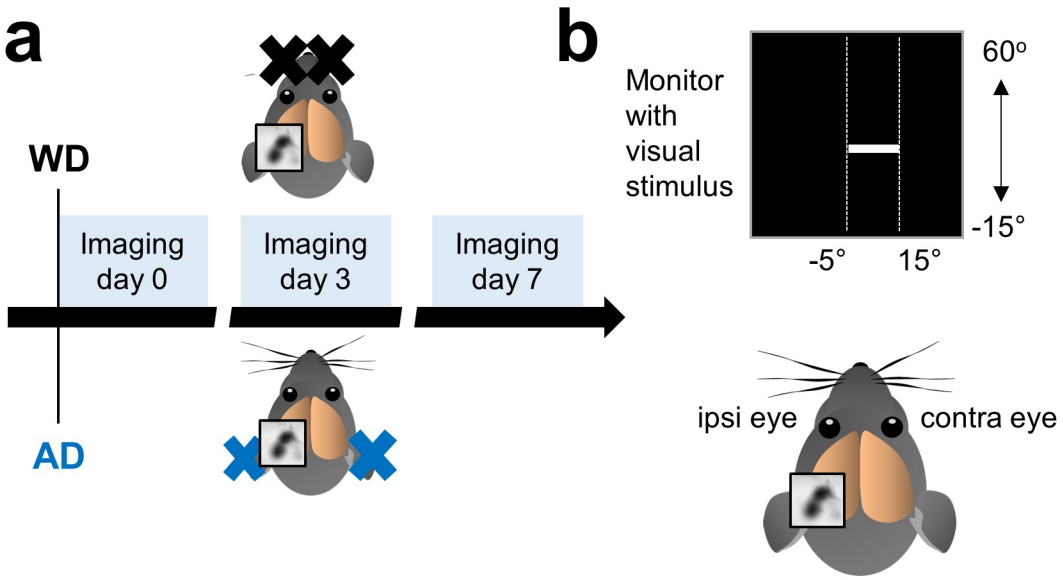

Fig 1. Experimental time course and schematic illustration of intrinsic signal imaging. (**a**) In one group of mice we performed WD by bilaterally plucking all macro-vibrissae and in another group we induced AD by bilateral malleus removal. The first imaging session (day 0) for mapping V1 started immediately after the deprivation followed by a second imaging session at day 3 and a third imaging session at day 7. (**b**) For visual stimulation we used an upward or downward moving white light bar with 100% contrast which was presented in the right binocular visual field. We always recorded V1 responses in the left hemisphere. Thus, according to the position of the recorded hemisphere the left eye represents the ipsilateral and the right eye represents the contralateral eye.

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First, we examined whether repeated optical intrinsic imaging *per se* affects visually evoked V1 activity in normal untreated mice (n = 5). Fig 2a depicts representative V1 activity maps evoked by the stimulation of either the contralateral (upper row) or the ipsilateral eye (lower row) obtained at 0, 3 and 7 days. Generally, darker activity maps indicate higher visually driven V1 responses. It is clearly visible that V1 input strength remained unchanged during the time tested, with the contralateral eye always dominating the input to V1, the normal OD ratio for the binocular region of V1 [31]. These results demonstrate that repeated intrinsic imaging provides reliable and stable measurements of sensory evoked V1 activity.

Next, we tested whether WD cross-modally affects responsiveness of V1 to contra- or ipsilateral eye stimulation. As shown in Fig 2b (upper row) V1 activity patches elicited by visual stimulation of the contralateral eye remained equally strong at 0, 3 and 7 days after WD. However, surprisingly, there was a marked increase of V1 responses evoked by ipsilateral eye stimulation 3 days after WD, which was followed by a decrease of V1 input strength back to the level of the V1 maps obtained at 0 days. These results suggest that WD provokes a transient shift of OD within the binocular zone of V1 after 3 days, which during the following 4 days then readjusted to the original level, probably due to homeostatic mechanisms.

We further investigated whether also the deprivation of another non-visual modality, the auditory sense, induces similar cross-modal effects in V1. Fig 2c shows representative V1 maps evoked by the stimulation of either the contralateral (upper row) or ipsilateral eye (lower row) obtained at 0, 3 and 7 days after AD. V1 maps elicited by visual stimulation of the contralateral eye remained stable over the whole time tested. However, like already found after WD, V1 response maps driven by the ipsilateral eye were markedly stronger 3 days after AD. This increase of elicited V1 activity was followed by a decrease back to starting levels 7 days after AD. Thus, our results indicate that the deprivation of non-visual sensory modalities leads to cross-modal alterations of OD. Notably, this took place without monocular deprivation (MD), the common traditional paradigm to induce OD shifts in mammals used up to now since its first description 55 years ago [12, 14, 32].

In control animals, neither the cortical response elicited by stimulation of either eye nor, accordingly, the ODI, showed a change over the days of measurement, which was confirmed by ANOVA with repeated measures (factor days across all three variables: $F_{6,12} = 1.087$, $p = 0.423$, $F<1.6$ and $p>0.25$ for each single variable, Fig 3a and 3b; Table 1). Thus, these data indicate that intrinsic signal imaging provides stable data of visually evoked V1 activity over the time course examined here.

After WD, however, the measured values changed across days ($F_{6,20} = 3.498$, $p = 0.016$), which was due to alterations of V1 activity evoked by ipsilateral eye stimulation ($F_{2,12} = 11.604$, $p = 0.002$) and ODI ($F_{2,12} = 11.632$, $p = 0.002$), but not contra eye responses ($F_{2,12} = 0.3$, $p = 0.746$, Fig 3d–3f; Table 1). Pairwise comparisons with Bonferroni correction revealed, that V1 input through the ipsilateral eye significantly increased on day 3 compared to day 0 ($p = 0.034$) and decreased again after 7 days of WD ($p = 0.021$, Fig 3e, Table 1). The corresponding decrease in ODI narrowly missed significance ($p = 0.056$) but was followed by a significant re-increase to starting levels ($p = 0.016$, Fig 3f; Table 1).

After AD, the values also massively changed over the course of the experiment ($F_{6,16} = 4.044$, $p = 0.012$). In detail, while V1 responsiveness to contralateral eye stimulation remained unaltered ($F_{2,10} = 0.402$, $p = 0.679$, Fig 3g), V1 activity evoked by visual stimulation of the ipsilateral eye ($F_{2,10} = 12.324$, $p = 0.002$, Fig 3h) and ODI ($F_{2,10} = 13.498$, $p = 0.001$, Fig 3i) were found to vary across readings. Pairwise comparisons with Bonferroni correction confirmed that V1 input through the ipsilateral eye increased significantly from day 0 to day 3 ($p = 0.039$) and decreased again after 7 days of AD ($p = 0.015$, Fig 3g, Table 1). As a direct consequence, the ODI followed suit, dropping on day 3 ($p = 0.016$) and rising again on day 7 ($p = 0.028$,

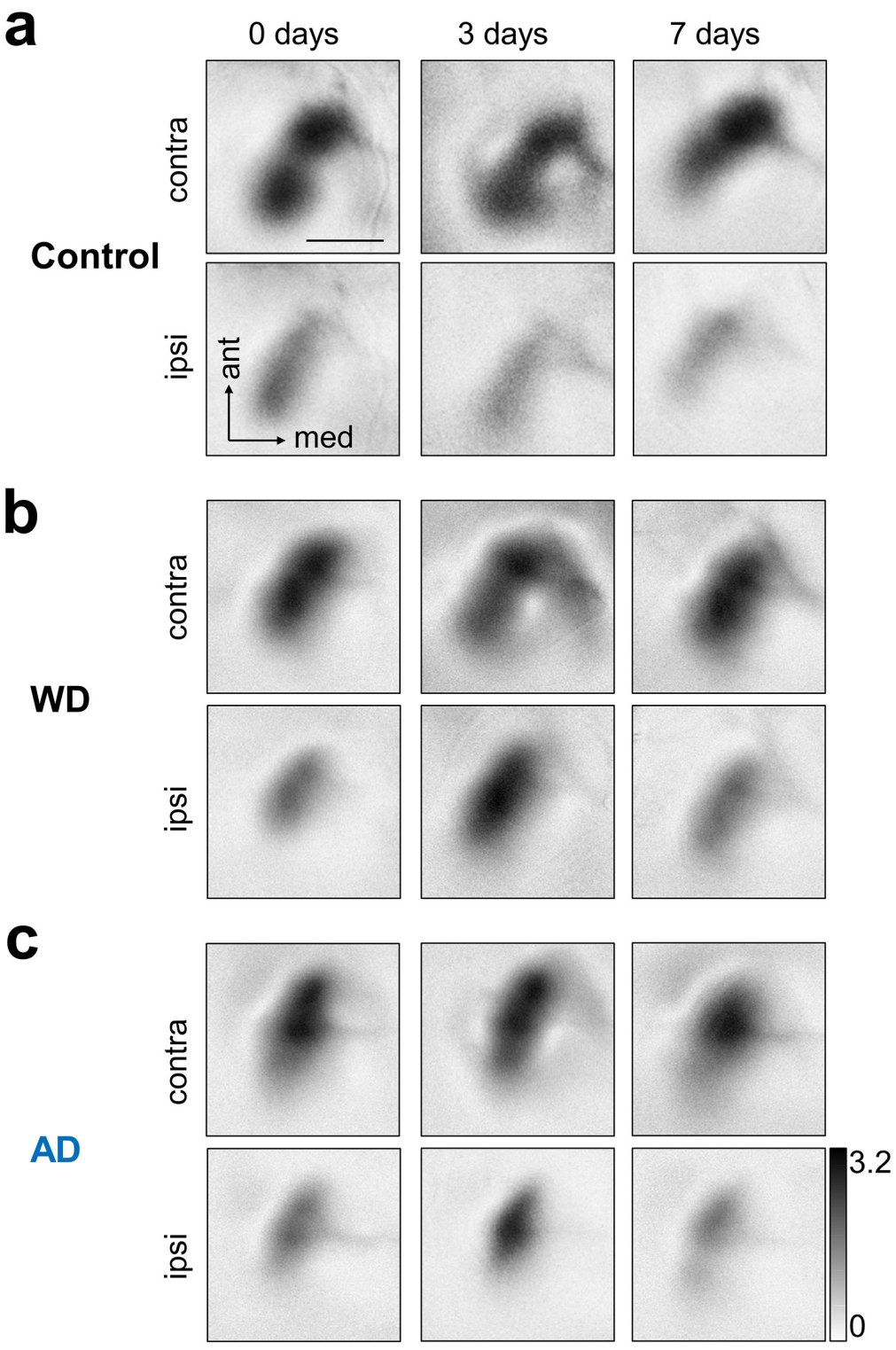

Fig 2. Representative V1 maps evoked by the stimulation of either the contralateral or ipsilateral eye at day 0, 3 and after 7 days. (**a**) Upper row: In normal control mice contralateral eye stimulation always evoked similarly strong V1 responses. Lower row: V1 responses evoked by ipsilateral eye stimulation were equally strong over the time tested but these responses were always weaker that V1 maps obtained after visual stimulation of the contralateral eye. (**b**) Upper row: Like in control mice, WD did not lead to alterations of V1 responses elicited by contralateral eye input. Lower row: However, V1 responses elicited by ipsilateral eye stimulation markedly increased 3 days after WD which

was followed by a decrease of V1 response strength 7 days after WD. (**c**) Upper row: Responsiveness of V1 to visual stimulation of contralateral eye remained stable after AD over the time tested. Lower row: 3 days after AD there was a massive increase of V1 responses evoked by ipsilateral eye stimulation which was followed by a recovery of V1 activity 7 days after AD. Thus, the deprivation in both senses (somatosensory or auditory) alters the OD within the binocular zone of V1. Scale bar: 1 mm.

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Fig 3i, Table 1). These data indicate that both WD and AD lead to an increased V1 activation to visual stimulation after 3 days which is, however, restricted to the ipsilateral eye input. Again, the restoration of V1 activity and ODI after 7 days might be due to homeostatic mechanism adjusting V1 inputs back to baseline levels.

We also compared our imaging data between the groups. Before the intervention (day 0), all variables were similar in all three groups (contra eye: $F_{2,15} = 0.076$, $p = 0.927$; ipsi eye: $F_{2,15} = 0.089$, $p = 0.916$; ODI: $F_{2,15} = 0.029$, $p = 0.972$, Fig 4a–4c). On the third day after sensory deprivation, however, ANOVA revealed a significant group effect for ipsilateral eye input to V1 ($F_{2,15} = 4.096$, $p = 0.038$) and ODI ($F_{2,15} = 8.726$, $p = 0.003$), but not contralateral eye input ($F_{2,15} = 0.719$, $p = 0.503$, Fig 4d–4f). Pairwise comparison with Bonferroni correction confirmed a significant increase V1 activity evoked by ipsilateral eye stimulation in WD animals ($p = 0.04$), and a significant decrease of the ODI in both AD ($p = 0.004$) and WD ($p = 0.014$) animals compared to control mice. On day 7 after WD or AD, all these differences disappeared again as V1 input through the ipsilateral eye was back to control levels (contra eye: $F_{2,15} = 0.518$, $p = 0.606$; ipsi eye: $F_{2,15} = 0.006$, $p = 0.004$; ODI: $F_{2,15} = 0.318$, $p = 0.732$, Fig 4g–4i).

Taken together, our data indicate that the deprivation of a non-visual sense leads to a selective increase of V1 activity evoked by stimulation of the typically "weaker", ipsilateral eye and thereby to changes of the OD within the binocular zone of V1. Thus, these results suggest that sensory deprivations of non-visual sensory modalities can cross-modally induce neuronal plasticity in the adult V1.

Cross modally induced OD changes cannot be explained by a saturation of V1 activity evoked by the contralateral eye

So far, we described that both WD and AD lead to a marked ODI shift which was mediated by a selective increase of V1 activity evoked by ipsilateral eye stimulation after 3 days, whereas the contralateral eye input to V1 remains unchanged. Since we were surprised by this unexpected cross-modal effect, we wondered whether the absence of V1 activity changes due to contralateral eye stimulation 3 days after deprivation might be caused by a saturation of the contralateral eye input to V1. To address this issue we first investigated whether V1 activity elicited by contralateral eye stimulation is already saturated in normal control mice (n = 4). For this, we measured V1 responses evoked by monocular ipsilateral and contralateral eye stimulation and also after binocular visual stimulation (Fig 5a). As expected, we found that V1 responses evoked by ipsilateral eye stimulation were always weaker than after stimulation of the contralateral eye (ipsi vs contra: 2.31±0.15 ($x10^{-4}$) vs 3.18±0.11 ($x10^{-4}$), $p = 0.008$; paired *t*-test; Fig 5b). Moreover, V1 activity evoked by contralateral eye stimulation was significantly weaker than after binocular stimulation (contra vs bino: 3.18±0.11 ($x10^{-4}$) vs 3.87±0.11 ($x10^{-4}$), $p = 0.003$; paired *t*-tests; Fig 5b). Thus, these data indicate that V1 responsiveness, as measured by intrinsic signal imaging, is not saturated when evoked by contralateral eye stimulation.

To further exclude a potential saturation effect, we again measured V1 responsiveness to contra and ipsilateral eye stimulation 0 and 3 days after WD (n = 4). However, this time we reduced the contrast of the visual stimulus from 100% to 10%, which generally decreases visually evoked V1 responses [3, 4, 33]. Hence, potential changes of V1 input from the contralateral

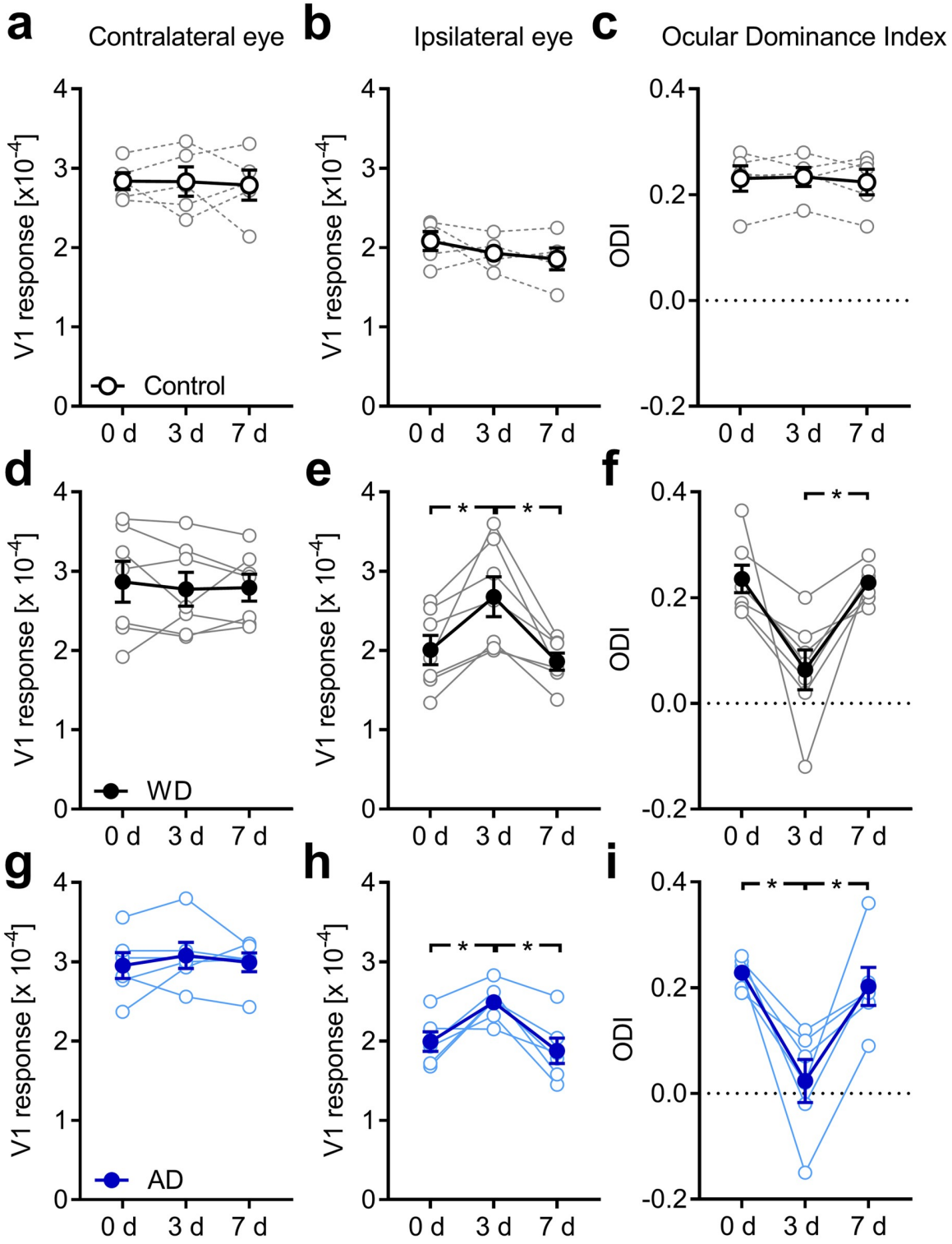

Fig 3. Both WD and AD shift OD in the binocular V1 in fully adult mice. (**a, b**) V1 activity evoked by visual stimulation of the contra or ipsilateral eye in untreated control mice (n = 5) remained unchanged at 0, 3 and 7 days. (**c**) Thus, over the same time course the ODI did not change underlining the reliability of repeated intrinsic signal imaging. (**d**) During one week after WD (n = 7), V1 activity elicited by contralateral eye stimulation was unaltered. (**e**) However, V1 responsiveness to ipsilateral eye stimulation markedly increased 3 days after WD followed by a recovery of V1 activity 7 days after WD. (**f**) These alterations of V1 responsiveness led to an ODI shift towards zero at day 3 which was followed by a readjustment of the ODI 7 days after WD. (**g**) After AD (n = 6) V1 activity elicited by contralateral

eye stimulation remained unchanged during the time tested. (**h**) However, V1 responses evoked by visual stimulation of the ipsilateral eye massively increased after 3 days of AD. After the 7 days V1 responses due to the ipsilateral eye input decreased back to the starting level measured at day 0. (**i**) Hence, the ODI displayed a dramatic shift towards zero 3 days after AD which was followed by a complete recovery after one week. Thus, the deprivation of a non-visual input altered OD in the spared V1. Open circles represent measurements of individual animals. Closed circles represent the means of each group ± s.e.m.; *p<0.05, repeated measures AVOVA.

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eye 3 days after WD can be detected. Quantification showed that V1 activity elicited by visual stimulation of the contralateral eye still remained unchanged between 0 and 3 days after WD (0 d vs 3 d: 1.77±0.18 ($x10^{-4}$) vs 1.68±0.13 ($x10^{-4}$), $p = 0.47$; paired *t*-test; Fig 5c) whereas ipsilateral eye input to V1 significantly increased again (0 d vs 3 d: 1.07±0.10 ($x10^{-4}$) vs 1.36±0.05 ($x10^{-4}$), $p = 0.01$; paired *t*-test; Fig 5d). Thus, the differential V1 activity changes caused a marked reduction of the ODI 3 days after WD (0 d vs 3 d: 0.18±0.009 vs 0.08±0.01, $p = 0.006$; paired *t*-test; Fig 5e). These data suggest that cross-modally induced OD changes in V1 are independent of the strength of the visual stimulus. Thus, the absence of V1 activity changes evoked by contralateral eye stimulation 3 days after WD, described above, is not caused by a saturation of the contralateral eye input to V1.

Cross-modally induced changes of OD require visual experience exclusively through the ipsilateral eye

It has been suggested that plastic changes in a spared primary sensory cortex require sensory experience through its main input [10]. Thus, we next asked whether patterned visual input through the contra or ipsilateral eye is necessary to provoke V1 activity changes 3 after WD. To address this question we first combined WD with MD of the contralateral eye (n = 4) and measured V1 responsiveness at 0 and 3 days using intrinsic signal imaging. We found that V1 responses evoked by visual stimulation of the contralateral (closed) eye remained unchanged whereas V1 activity driven by the ipsilateral (open) eye input was still increased 3 days after WD and MD (contra: 0 d vs 3 d: 2.79±0.13 ($x10^{-4}$) vs 2.81±0.14 ($x10^{-4}$), $p = 0.86$; ipsi: 0 d vs 3 d: 1.83±0.06 ($x10^{-4}$) vs 2.30±0.08 ($x10^{-4}$), $p = 0.002$; paired *t*-tests; Fig 6a and 6b). This again led to a significant reduction of the ODI (0 d vs 3 d: 0.24±0.03 vs 0.09±0.03, $p = 0.009$; paired *t*-test; Fig 6c), similar to WD mice with open contralateral eyes. Moreover, the percentage increase of V1 activity evoked by ipsilateral eye stimulation was statistically indistinguishable from WD mice without MD described in the first paragraph of the results (WD+MD (contra): 24.58%±2.38%; WD only: 35.71%±12.38%, $p = 0.46$; unpaired t-test). Thus, our data suggest

Table 1. The effects of WD and AD on V1 responsiveness and OD. Data are presented as means ± s.e.m.

	0 days	3 days	7 days
Contra (x10^{-4})			
Control (n = 5)	2.84 ± 0.11	2.83 ± 0.19	2.79 ± 0.19
WD (n = 7)	2.87 ± 0.26	2.77 ± 0.21	2.79 ± 0.17
AD (n = 6)	2.95 ± 0.16	3.08 ± 0.17	2.99 ± 0.12
Ipsi (x10^{-4})			
Control	2.08 ± 0.12	1.93 ± 0.09	1.86 ± 0.14
WD	2.01 ± 0.19	2.68 ± 0.25	1.86 ± 0.11
AD	1.99 ± 0.12	2.49 ± 0.10	1.88 ± 0.16
ODI			
Control	0.23 ± 0.02	0.23 ± 0.02	0.22 ± 0.02
WD	0.24 ± 0.03	0.06 ± 0.04	0.23 ± 0.01
AD	0.23 ± 0.01	0.02 ± 0.04	0.20 ± 0.04

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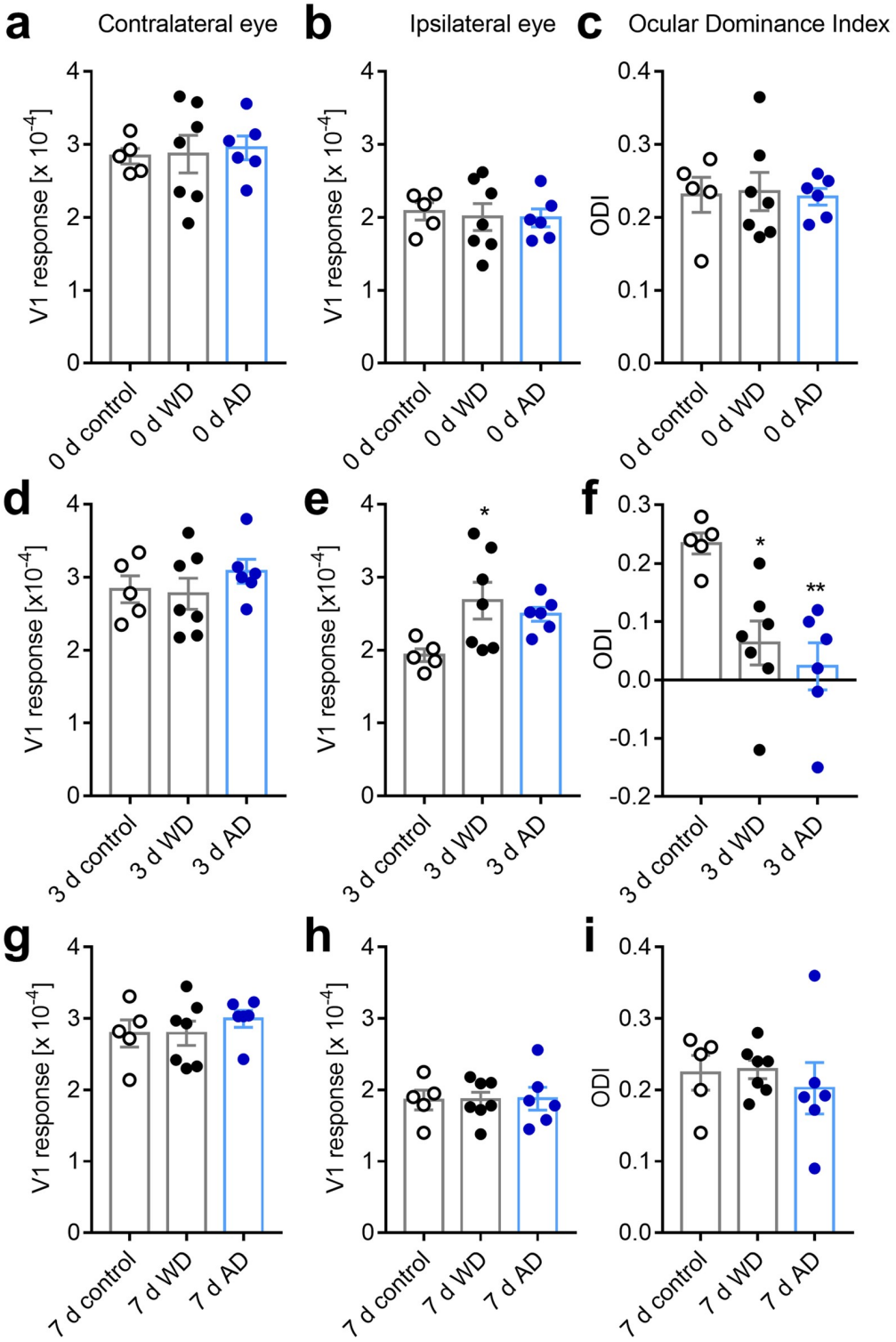

Fig 4. Both WD or AD induce an ODI shift compared to normal control mice. (**a, b**) V1 responses evoked by visual stimulation of either the contra or ipsilateral eye immediately after WD (n = 7) or AD (n = 6) were indistinguishable from values obtained in normal control mice (n = 5). (**c**) Hence, directly after WD or AD there was no alteration of the ODI. (**d**) After 3 days of either WD or AD V1 responses elicited by contralateral eye stimulation was not different from control values. (**d**) However, V1 activity evoked by stimulation of the eye ipsilateral to the recorded hemisphere was increased 3 days after WD or AD compared to control levels. (**f**) Thus, at this time point there was a significant shift of

the ODI towards zero. (**g**) After 7 days of WD or AD V1 responses elicited by the stimulation of contralateral eye were unchanged compared to the values of control mice. (**h**) Interestingly, V1 responsiveness to ipsilateral eye stimulation was re-adjusted to control levels after one week of either WD or AD. (**i**) Consequently, the ODI of WD or AD mice was completely restored back to control values after 7 days. Bars represent the means ± s.e.m. and open or filled circles represent measurements of individual animals; $^{*}p<0.05$, $^{**}p<0.01$, one way ANOVA.

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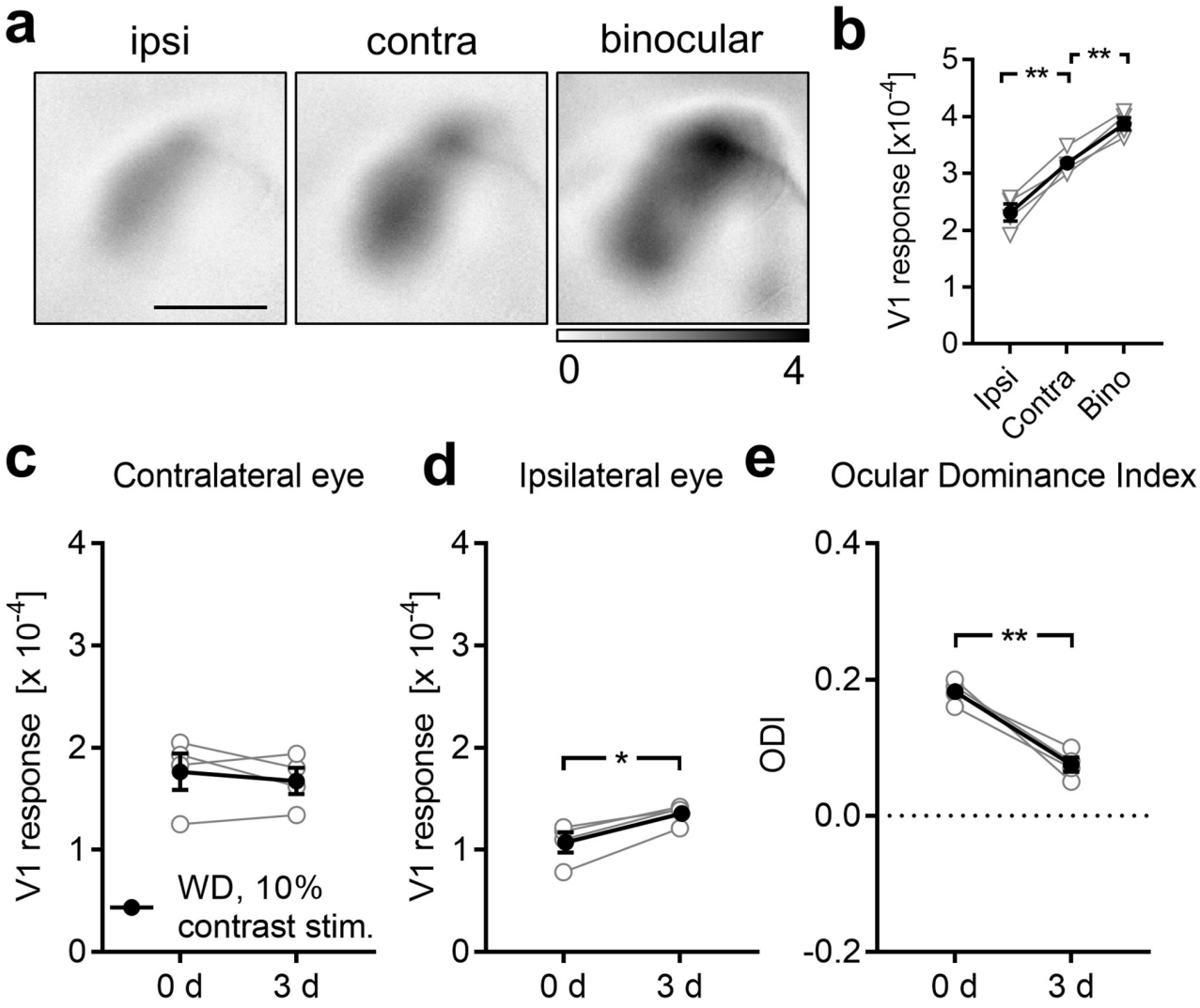

Fig 5. Exclusion of saturation of V1 responses evoked by contralateral eye stimulation. (**a**) Representative V1 amplitude maps evoked by visual stimulation of the ipsi and contralateral eye and elicited by binocular stimulation of normal untreated mice (n = 4). (**b**) V1 responsiveness to ipsilateral eye stimulation was always weaker compared to V1 responsiveness to contralateral eye stimulation. However, V1 activity elicited by visual stimulation of the contralateral eye was significantly weaker than V1 activity evoked by binocular stimulation. Hence, V1 responses, measured with intrinsic signal imaging, are not saturated by the input through the contralateral eye. (**c**) V1 responses evoked by contralateral eye stimulation with a visual stimulus of 10% contrast at 0 and 3 days after WD (n = 4) remained unchanged. (**d**) However, there was a potentiation of V1 responses to the input through the ipsilateral eye between 0 and 3 days after WD. (e) Thus, the ODI significantly shifted towards zero. Hence, visual stimulation with a weaker visual stimulus reveals the same effect of WD on V1 activity like visual stimulation with a strong visual stimulus. Open circles represent measurements of individual animals. Closed circles represent the means of each group ± s.e.m.; $^{*}p<0.05$, $^{**}p<0.01$; Scale bar: 1 mm.

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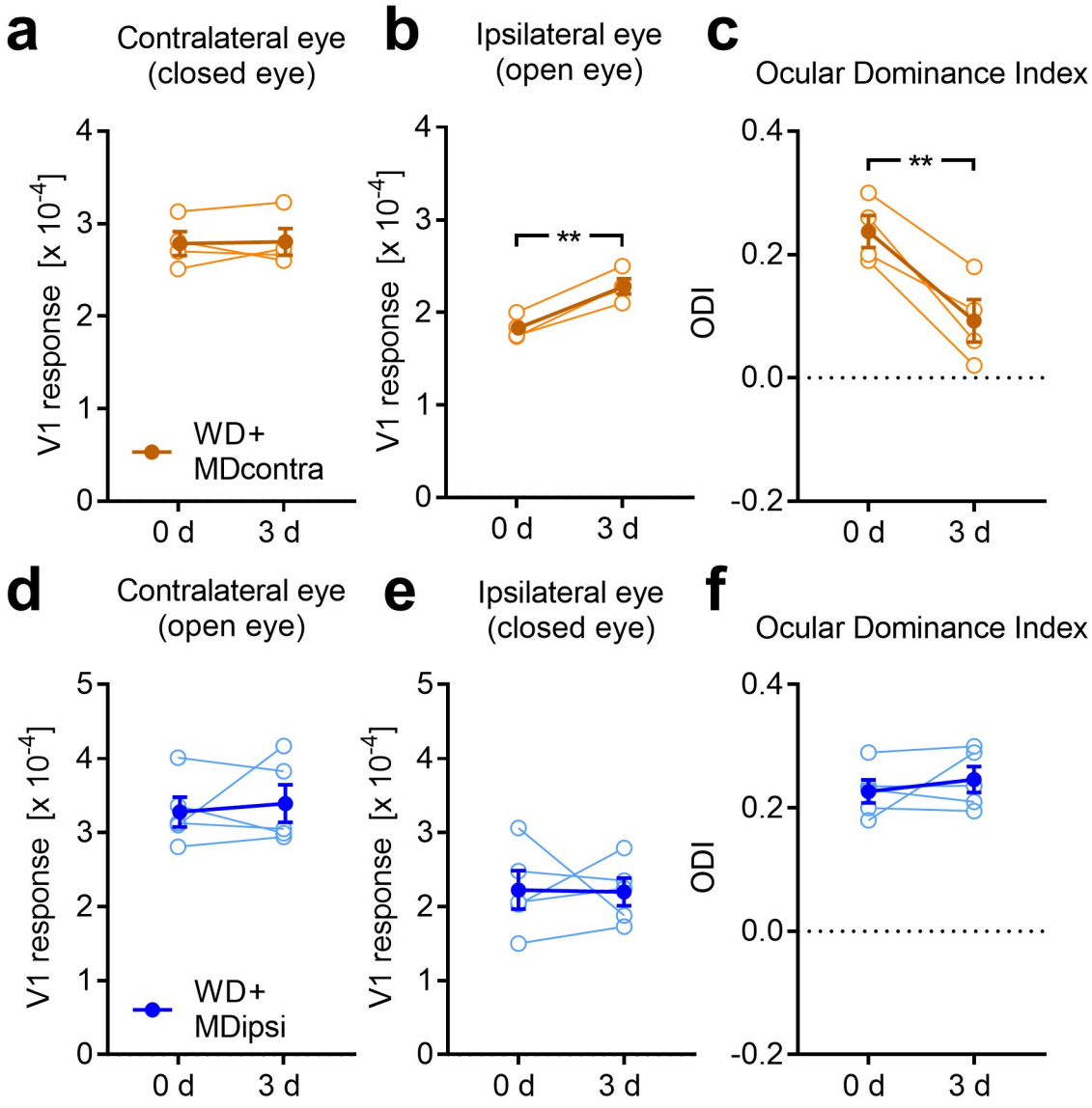

Fig 6. Cross-modally induced ODI shift requires patterned vision through the ipsilateral eye. (**a**) Combining WD with a MD of the contralateral eye (n = 4) did not lead to changes of V1 responses evoked by the contralateral eye between 0 and 3 days after WD. (**b**) However, there was a significant increase of V1 activity elicited by visual stimulation of the ipsilateral eye, like found after WD only. (**c**) Thus, the ODI markedly shifted towards zero. (**d, e**) In contrast, if we combined WD with a MD of the ipsilateral eye (n = 5), V1 activity evoked by both contra and ipsilateral eye stimulation remained statistically unchanged after 3 days. (**f**) Moreover, there was no ODI shift after this treatment suggesting that patterned vision through the ipsilateral eye is required for cross-modally induced OD shifts. Open circles represent measurements of individual animals. Closed circles represent the means of each group ± s.e.m.; $^{*}p<0.05$, $^{**}p<0.01$.

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that cross-modally induced changes of OD do not require patterned visual input through the contralateral eye.

Next, we investigated whether experience of patterned vision through the ipsilateral eye is required for WD induced changes of OD. Combining WD with a MD of the ipsilateral eye (n = 5) did not lead to changes of the contralateral eye input to V1 (0 d vs 3 d: 3.28±0.20 ($x10^{-4}$) vs 3.40±0.25 ($x10^{-4}$), $p = 0.67$; paired t-test; Fig 6d). However, this treatment abolished the increase of V1 responsiveness to ipsilateral eye stimulation after 3 days of WD (0 d vs 3 d:

2.23±0.26 (x10^{-4}) vs 2.20±0.19 (x10^{-4}), p = 0.94; paired t-test; Fig 6e). Hence, the ODI did not change after these interventions (0 d vs 3 d: 0.23±0.02 vs 0.25±0.02, p = 0.45; paired t-test; Fig 6f). These data suggest that cross-modal changes of V1 activity after WD exclusively depend on visual experience through the eye ipsilateral to the recorded hemisphere.

WD cross-modally increases mEPSC amplitudes in layer 4 of V1

It has been demonstrated that a sensory deprivation can re-induce synaptic plasticity of thalamo-cortical layer 4 synapses in a spared sensory cortex in adult mice [10]. Hence, to get insights into potential mechanisms that may underlie the cross-modal cortical changes described above, we next examined the effects of WD on the strength of layer 4 synapses of pyramidal cells in the spared V1. For this, we performed whole-cell recordings in acute V1 slices of normal control mice (11 cells, n = 4 mice) and animals 3 d after WD (6 cells, n = 3 mice). Fig 7a depicts a rpresentative mEPSC trace of a control cell and a WD cell. It is clearly visible that mEPSC amplitudes were increased after 3 days of WD. The cumulative distribution of mEPSC amplitudes of all cells examined was markedly shifted to the right, towards higher amplitudes (p = 0.0008; Kolmogorov-Smirnov test; Fig 7b). Hence, on average, there was a significant increase in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) mediated mEPSC amplitudes (control: 9.31±0.58 (pA), 3 d WD: 15.02±1.63 (pA), p = 0.0011; unpaired t-test; Fig 7b) suggesting a strengthening of excitatory synapses. However, we did not find changes in frequency of miniature excitatory postsynaptic currents (mEPSC) after WD (control: 2.42±0.85 (Hz), 3 d WD: 3.19±0.93 (Hz), p = 0.49; unpaired t-test; Fig 7c). Is has been described that changes in the distribution of mEPSC amplitudes can be either multiplicative, if the strength of all neurons excitatory synapses is changed by the same factor (synaptic scaling), or non-multiplicative, if the synaptic changes are not uniform across the sampled synapses [28, 34–36]. Hence, we used the standard method to examine in which manner WD cross-modally affected layer 4 synapses in V1: first, rank ordered mEPSC amplitudes of WD mice were plotted against rank-ordered mEPSC amplitudes of control animals. This plot was fitted by a linear regression revealing the scaling function, y = 1.818x (Fig 7d), as described previously [34, 37]. Then, we transformed individual mEPSC amplitudes of WD mice with this equation and constructed a cumulative plot (WD scaled, Fig 7e). The resulting distribution of the scaled WD data was significantly different from the distribution of control data (p = 0.007, Kolmogorov-Smirnov test; Fig 7e) suggesting that only a subset of synapses in layer 4 pyramidal cells was strengthened after WD.

In conclusion, our data indicate that WD cross-modally increases the strength of excitatory V1 layer 4 synapses. These may include thalamo-cortical synapses driven by the ipsilateral eye. Typically, strengthening of thalamo-cortical synapses leads to an increased sensory driven responsiveness of primary sensory cortices [10, 38]. Consistent with these observations is our finding that V1 responses evoked by ipsilateral eye stimulation were increased 3 d after WD, as revealed by intrinsic imaging. Taken together, these data suggest that WD cross-modally re-induces synaptic plasticity in the spared V1.

WD cross-modally increases the E/I ratio in V1

Experience dependent V1 plasticity after MD that leads to changes in OD, typically declines with aging and is completely absent in fully adult mice [11], as used in the present study. However, previous studies could demonstrate that increasing the cortical excitation/inhibition (E/I) ratio is a central hub for the restoration of visual plasticity in the adult V1 [39–42]. Hence, as a next step, we examined whether WD for 3 days leads to cross-modal changes in V1 glutamate and GABA levels. For this, we quantified levels of glutamate and GABA by post-

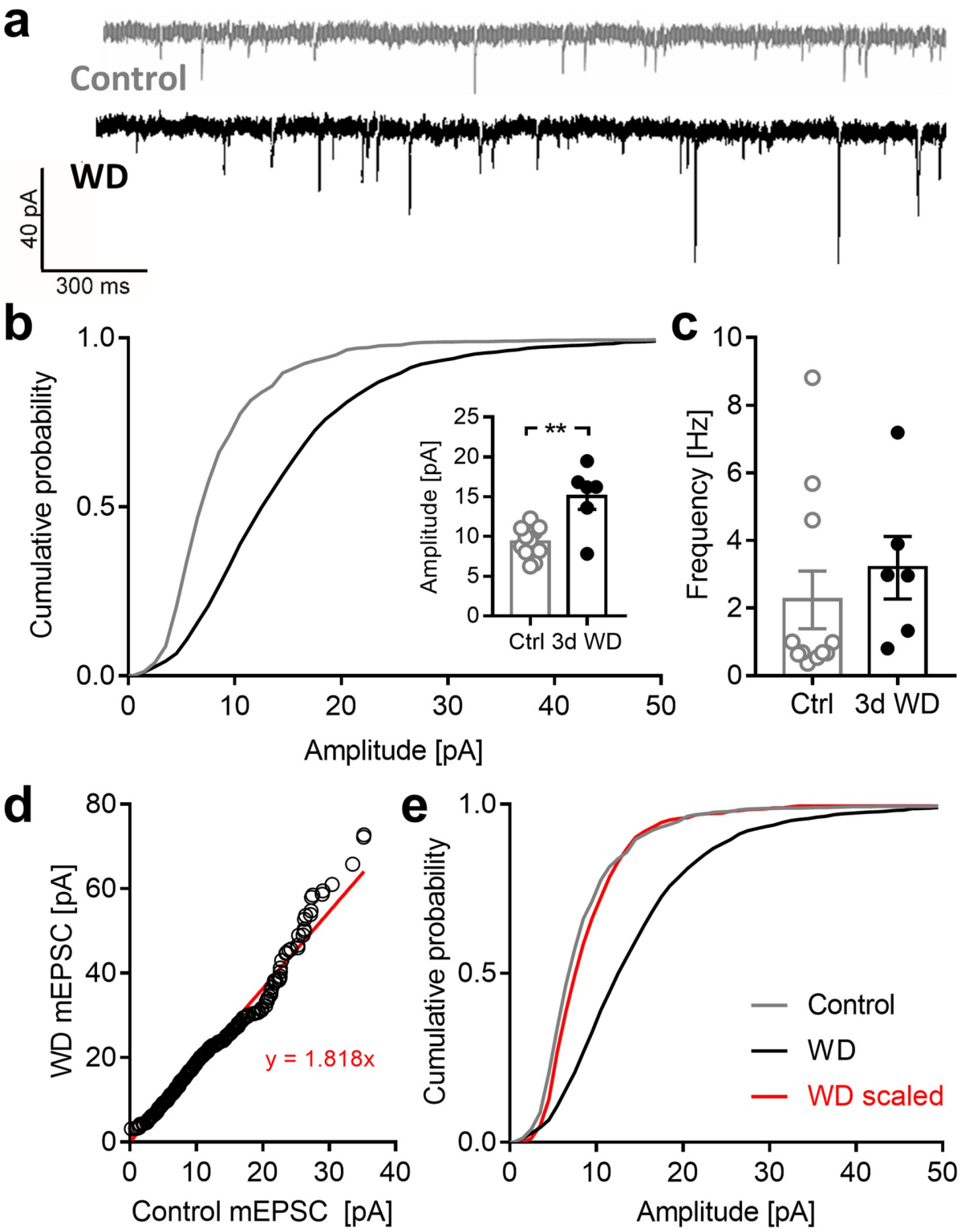

Fig 7. WD cross-modally increases mEPSC amplitudes in V1 layer 4. (**a**) Representative traces of mEPSCs recorded in control mice (n = 4) and 3 d after WD (n = 3) (**b**) Cumulative distribution of all mEPSC amplitudes was right shifted in WD mice compared to control animals. Hence, the mean amplitude of mEPSCs was significantly increased in WD mice. These results suggest that WD induces synaptic plasticity in V1 layer 4. (**c**) Mean frequency of mEPSCs was unaltered after WD. (**d**) Plot of rank ordered mEPSC amplitudes from control and WD mice. The red line represents a linear regression of the data points. (**e**) Cumulative histograms of mEPSC amplitudes. Individual mEPSC amplitudes of WD mice were transformed with the equation y = 1.818x. The distribution of the transformed values is significantly different with the

distribution of control values (Kolmogorov-Smirnov test). Bars represent means together with s.e.m., Open and filled circles represent measurements of individual animals; **p<0.01.

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mortem HPLC analyzes of V1 tissue from control mice (n = 5) and WD mice 3 days after WD (n = 6). Quantification showed that there was a significant increase in V1 glutamate levels 3 days after WD (control vs 3 d WD: 71.07±1.5 (nMol/mg protein) vs 77.70±1.79 (nMol/mg protein), $p = 0.02$; unpaired *t*-test; Fig 8a). Moreover, V1 GABA content slightly decreased by about 6% after WD, which was, however, not statistically significant (control vs 3 d WD: 9.80±0.19 (nMol/mg protein) vs 9.26±0.39 (nMol/mg protein), $p = 0.27$; unpaired *t*-test; Fig 8b). Due to the differential regulations of glutamate and GABA levels in V1 after WD, the glutamate/GABA ratio significantly increased 3 days after WD (control vs 3 d WD: 7.25±0.12 vs 8.11±0.30, $p = 0.02$; Fig 8c). These results suggest that WD cross-modally alters the E/I balance in V1 in favor of excitation, which might in turn set the adult V1 back into a plastic stage.

Cross-modal changes of V1 activity depend on V1 GABA levels and NMDA receptor activation

We next investigated whether the increase of the V1 glutamate/GABA ratio after WD was related to cross-modally induced V1 activity changes. Hence, to compensate for the increase of glutamatergic excitation, we artificially raised cortical GABAergic inhibition by daily systemic administration of diazepam in WD mice (n = 4) and measured V1 responsiveness at 0 and 3 days using intrinsic imaging. Diazepam, administrated systemically or locally, is a

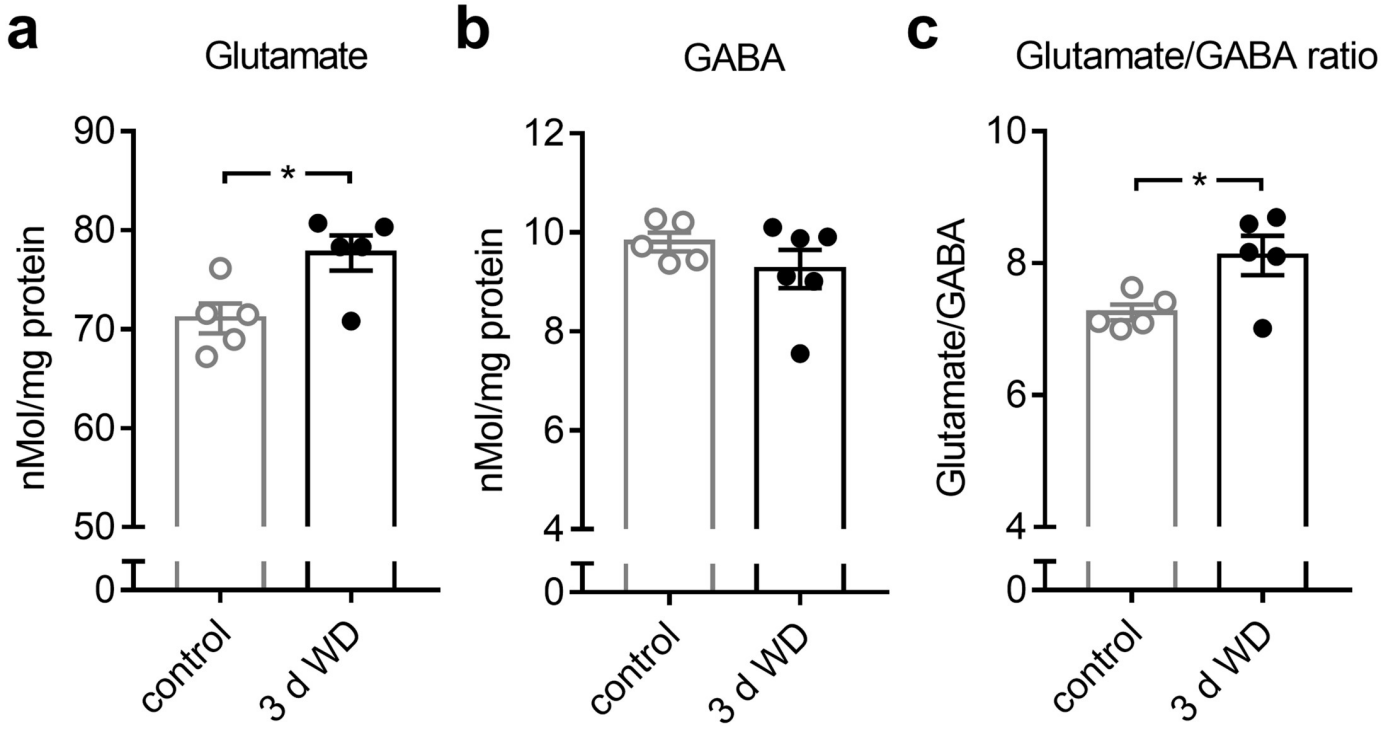

Fig 8. Concentration changes of neurotransmitters in V1 after WD revealed by post-mortem HPLC analyzes. (**a**) Compared to the V1 glutamate level of control mice (n = 5), there was a significant increase of V1 glutamate content 3 days after WD (n = 5). (**b**) There was slight but not significant reduction of the V1 GABA concentration at 3 days after WD (n = 6) compared to controls. (**c**) The glutamate/GABA ratio was markedly increased at 3 after WD. Bars represent means together with s.e.m., Open and filled circles represent measurements of individual animals; *p<0.05.

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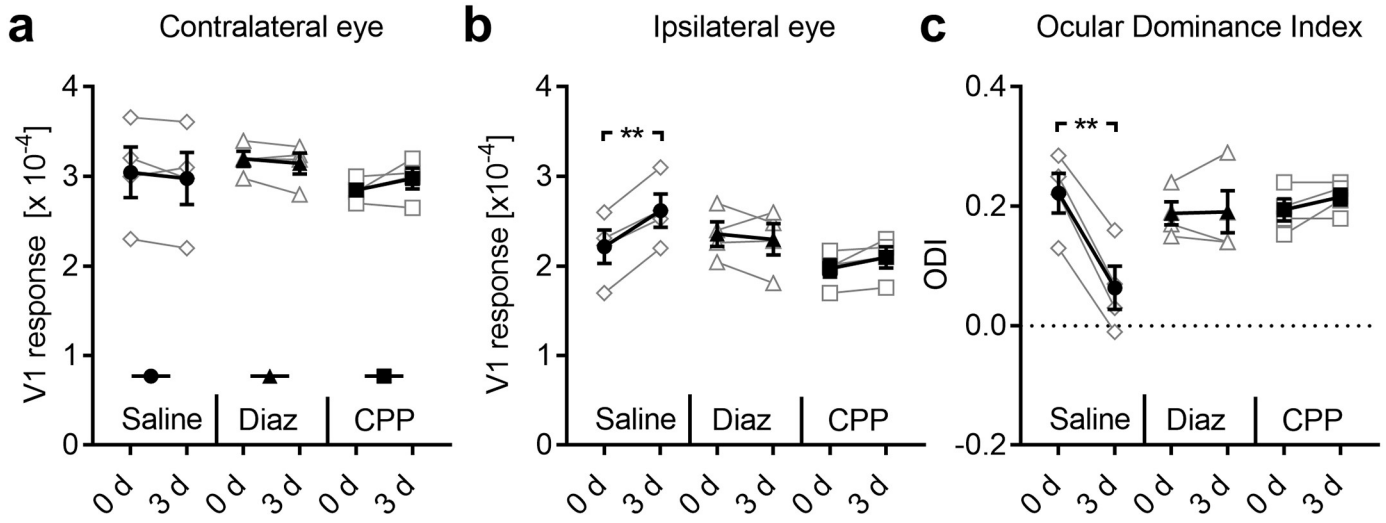

Fig 9. Both increasing inhibition and blocking NMDA receptor activation block cross-modally induced V1 plasticity. (**a**) In WD mice treated with saline (n = 4) or diazepam (n = 4) or CPP (n = 4) V1 responses evoked by visual stimulation of the contralateral eye remained unchanged. (**b**) There was a significant increase of V1 responses elicited by ipsilateral eye stimulation in WD+Saline mice. However, these changes were completely abolished by diazepam or CPP injections. (**c**) We found a significant reduction of ODI in saline treated WD mice, whereas ODI did not change after diazepam of CPP administration. Taken together, our data suggest that cross-modally induced alterations of V1 OD depend on increased glutamateric excitation and NMDA receptor activation. Open circles represent measurements of individual animals. Closed circles represent the means of each group ± s.e.m.; $^{**}p<0.01$.

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common tool for enhancing cortical inhibition, since it increases GABA receptor mediated currents [43–45]. In control animals (n = 4) we also performed WD but administrated saline systemically. As expected, saline treatment did not influence the cross-modal effects of WD on V1, as V1 activity evoked by visual stimulation of the contralateral eye was unchanged during the time tested, whereas the ipsilateral eye input significantly increased at day 3 after WD (contra: 0 d vs 3 d: $p = 0.38$; ipsi: 0 d vs 3 d: $p = 0.006$; paired *t*-tests; Fig 9a and 9b; Table 2). Consequently, ODI significantly decreased after WD (0 d vs 3 d: $p = 0.002$; paired *t*-tests; Fig 9c; Table 2). However, in WD animals treated with diazepam, both the contralateral and ipsilateral eye input strength in V1 and thereby the ODI also remained unchanged at 3 days (contra: 0 d vs 3 d: $p = 0.36$; ipsi: 0 d vs 3 d: $p = 0.61$; ODI: 0 d vs 3 d: $p = 0.89$; paired *t*-

Table 2. The effects of diazepam and CPP administration on cross-modally induced V1 activity changes after WD. Data are presented as means ± s.e.m.

	0 days	3 days
Contra (x10^{-4})		
WD+Saline (n = 4)	3.04 ± 0.28	2.97 ± 0.29
WD+Diaz (n = 4)	3.19 ± 0.09	3.14 ± 0.12
WD+CPP (n = 4)	2.85 ± 0.06	2.97 ± 0.12
Ipsi (x10^{-4})		
WD+Saline	2.21 ± 0.19	2.61 ± 0.19
WD+Diaz	2.35 ± 0.14	2.30 ± 0.17
WD+CPP	2.00 ± 0.10	2.09 ± 0.12
ODI		
WD+Saline	0.22 ± 0.03	0.06 ± 0.04
WD+Diaz	0.19 ± 0.02	0.19 ± 0.04
WD+CPP	0.19 ± 0.02	0.22 ± 0.01

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tests; Fig 9a, 9b and 9c; Table 2). Thus, increasing cortical inhibition abolished the WD induced activity changes in V1. These results suggest that the WD induced increase of the V1 E/I ratio is causal for the OD shift observed 3 days after WD.

Next, we tested the hypothesis that WD induced V1 activity changes might rely on NMDA receptor (NMDAR) activation. Previous investigations could demonstrate an involvement of NMDARs in experience dependent V1 plasticity, as systemic administration of the competitive NMDA receptor antagonist CPP or genetic deletion of cortical NMDARs abolished plastic alterations in V1 [15, 24, 46]. Moreover, we could recently show that blocking NMDAR activation by systemic administration of CPP abolished cross-modally induced restoration of ocular dominance plasticity after 7 days of monocular deprivation [15]. Hence, here, WD mice received daily injections of CPP (n = 4) and we measured V1 responsiveness again at 0 and 3 days. V1 responsiveness to contralateral eye stimulation as well as V1 activity elicited by visual stimulation of the ipsilateral eye did not change during the time tested (contra: 0 d vs 3 d: $p = 0.25$; ipsi: 0 d vs 3 d: $p = 0.14$; paired *t*-tests; Fig 9a and 9b; Table 2). Thus, the ODI remained unchanged in these mice (0 d vs 3 d: $p = 0.21$; paired *t*-tests; Fig 9c, Table 2). These data show that systemic administration of CPP blocks WD induced cross-modal plasticity in V1. Taken together, our results suggest that NMDA receptor activation is necessary to provoke cross-modal strengthening of sensory driven activity in a spared sensory cortex.

WD cross-modally improves visual performance

As a next step we investigated whether the V1 response alterations, observed after WD or AD are also reflected at the level of visually mediated behavior. In a recent study we could already demonstrate that WD markedly refined V1 mediated visual performance as revealed by visual water task experiments [3]. Another example of visual behaviors is the so called optokinetic reflex (OKR), a head and eye movement, mediated by subcortical structures, which stabilizes images on the retina [47]. Interestingly, previous studies could show that V1 activity can modulate the OKR [27, 47]. We therefore hypothesized that the observed cross-modally induced changes of visually driven V1 activity (after WD or AD) might also lead to changes of the OKR. We therefore investigated the repercussions of WD and AD on spatial frequency and contrasts sensitivity of OKR using a virtual optomotor system [26].

First, we investigated the effects of WD on visual acuity. For this OKR thresholds obtained after visual stimulation of either the right or left eye were measured daily for a period of 10 days. Baseline values of WD mice (n = 4, Fig 10a and 10b) were always measured before WD, whereas values measured on day 0 represent measurements obtained 4–5 hours after the surgery for WD. Control mice (n = 4) remained untreated. Quantitative analysis using two-way ANOVA with repeated measurements revealed significant influences of group ($F_{1,6} = 1165.94$, $p<0.0001$) and time ($F_{11,66} = 46.45$, $p<0.0001$) and a significant interaction between the two ($F_{11,66} = 46.45$, $p<0.0001$). Post hoc analysis showed that reflex sensitivity for spatial frequency was unchanged in control animals (n = 4) over the whole time period tested. However, in the WD group (n = 4), there was a significant gradual enhancement of spatial frequency sensitivity of the OKR reaching a peak 3 days after WD, about 12% above control level (3 days: control vs WD: 0.40±0.001 (cpd(cycles per degree)) vs 0.45±0.0023 (cpd), $p<0.0001$; unpaired *t*-test followed by Bonferroni correction; Fig 10a). Spatial frequency thresholds levels then dropped down over the next two days, but persisted at a level about 5% above control values up to 10 days after WD (10 days: control vs WD: 0.40±0 (cpd) vs 0.42±0.00086 (cpd), $p<0.0001$; unpaired *t*-test followed by Bonferroni correction, Fig 10a). Contrast sensitivity of OKR was measured in the same control and WD mice at 0.2 cpd. Group ($F_{1,6} = 656.48$, $p<0.0001$) and time ($F_{11,66} = 69.23$, $p<0.0001$) had a significant influence on contrast thresholds and there

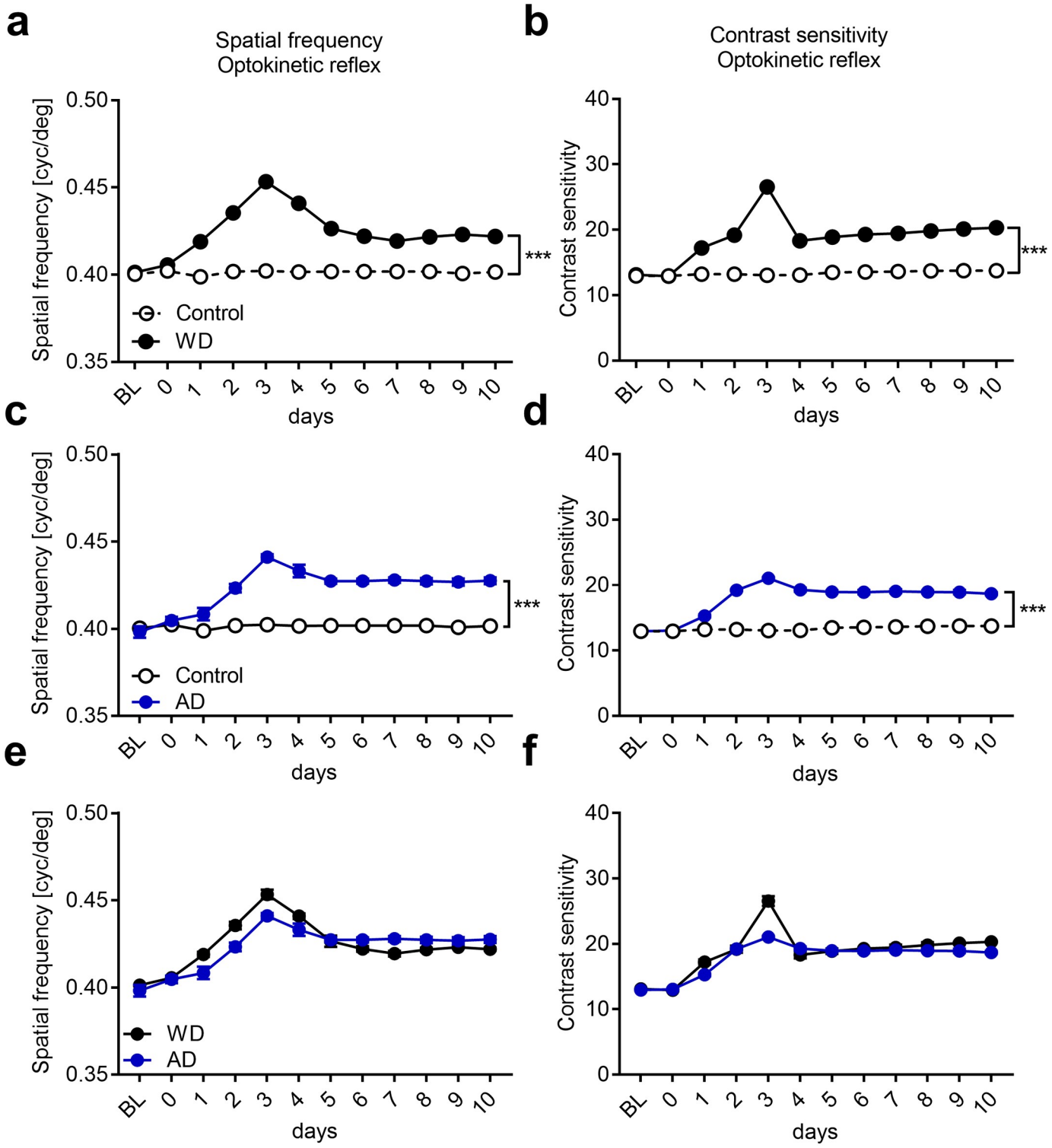

Fig 10. Both WD and AD cross-modally provoke a potentiation of the visual OKR. (**a**) In control mice (n = 4), spatial frequency thresholds did not change over the whole time period tested. However, after WD (n = 4) there was a marked improvement of the spatial frequency sensitivity which reached a peak on day 3. Subsequently, spatial frequency thresholds levels slightly decreased and remained at a stable level above control values until 10 days after WD. (**b**) Contrast sensitivity of the OKR in control mice remained unchanged over 10 days. After WD, contrast thresholds massively improved until day 3. After a slight decrease at 4 days after WD, values then remained at a stable level above control values until 10 days after WD. (**c**) We used values of the same control mice like in Fig 4a. AD (n = 4) led to a marked increase of spatial frequency thresholds peaking at 3 days. Subsequently, spatial frequency sensitivity slightly decreased until day 5 after AD

and remained at a stable level above control values until 10 days after AD. (**d**) Contrast thresholds markedly increased until day 3 after AD which was followed by a slight decrease to a stable level above contrast values of control mice. (**e, f**) WD or AD led to similar improvements of the OKR. Open and filled circles represent mean values together with the s.e.m. However, vertical lines of s.e.m. are often occluded by data symbols. ***p<0.001.

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was a significant interaction between both ($F_{11,66}$ = 69.23, p<0.0001, tow-way ANOVA with repeated measurements). While contrast thresholds of control mice did not change over the whole time period tested, they gradually increased by almost 100% until day 3 after WD (3 days: control vs WD: 13.03±0.16 vs 26.55±0.74, p<0.0001, unpaired t-test followed by Bonferroni correction, Fig 10b) suggesting a substantial enhancement of contrast sensitivity due to WD. Subsequently, OKR contrast thresholds decreased again but then remained about 50% above control values between 4 and 10 days after WD (10 days: control vs WD: 13.73±0.26 vs 20.31±0.10; p<0.0001, unpaired t-test followed by Bonferroni correction; Fig 10b). Taken together, our results suggest that WD cross-modally improves behavioral OKR spatial frequency and contrast sensitivity.

As a next step, we examined whether also AD (n = 4) affects the visual OKR. Quantitative analysis using a two-way ANOVA with repeated measurements showed that group ($F_{1,6}$ = 654.78, p<0.0001) and time ($F_{11,66}$ = 24.3, p<0.0001) had a significant influence on spatial frequency thresholds. In addition, we found a significance interaction between group and time ($F_{11,66}$ = 20.65, p<0.0001, two-way ANOVA with repeated measurements). As shown in Fig 10c there was a gradual increase of spatial frequency sensitivity until 3 days after AD (3 days: control vs AD: 0.40 ±0.001 (cpd) vs 0.44±0.002; p<0.0001; unpaired t-test followed by Bonferroni correction; Fig 10c) which was followed by a slight decrease over the next two days to a stable level above control values until day 10 after AD (10 days: control vs AD: 0.40±0 vs 0.43±0.002, p = 0.0002; unpaired t-test followed by Bonferroni correction; Fig 10c). Group ($F_{1,6}$ = 317.79, p<0.0001) and time ($F_{11,66}$ = 143.39, p<0.0001) also had a significant influence on contrast thresholds and there was a significant interaction between both ($F_{11,66}$ = 112.92, p<0.0001, two-way ANOVA with repeated measurements). Contrast thresholds also significantly increased until 3 days after AD (3 days: control vs AD: 13.03±0.16 vs 12.06±0.16, p<0.0001, unpaired t-test followed by Bonferroni correction; Fig 10d) and then remained at a higher level above control measurements until day 10 (10 days: control vs AD: 13.73±0.26 vs 18.7±0.21, p<0.0001, unpaired t-test followed by Bonferroni correction; Fig 10d). These data indicate that AD can cross-modally improve OKR sensitivity, too. Notably, both spatial frequency and contrast sensitivity changes found after AD were similar to changes observed after WD (Fig 10e and 10f). Taken together, our results strongly suggest that the deprivation of a non-visual modality leads to marked improvements of subcortically mediated visual behavior.

Cross-modally induced enhancements of the OKR are partially V1 dependent

So far, we described that both WD and AD lead to a potentiation of the OKR. Interestingly, the highest values of OKR thresholds of spatial frequency and contrast as well were obtained 3 days after WD or AD, and thus, exactly at the same time point when visually driven V1 reached its peak. These results suggest that V1 might be involved in mediating OKR potentiation. In order to address this issue we combined WD and bilateral V1 aspiration (WD+V1aspi, n = 3) and measured spatial frequency and contrast thresholds of the OKR over the following 10 days. In mice of the control group we only aspirated V1 bilaterally (V1aspi only, n = 3). Baseline values were always measured before V1 aspiration, measurements at day 0 were obtained 4–5 h after WD and V1 aspiration.

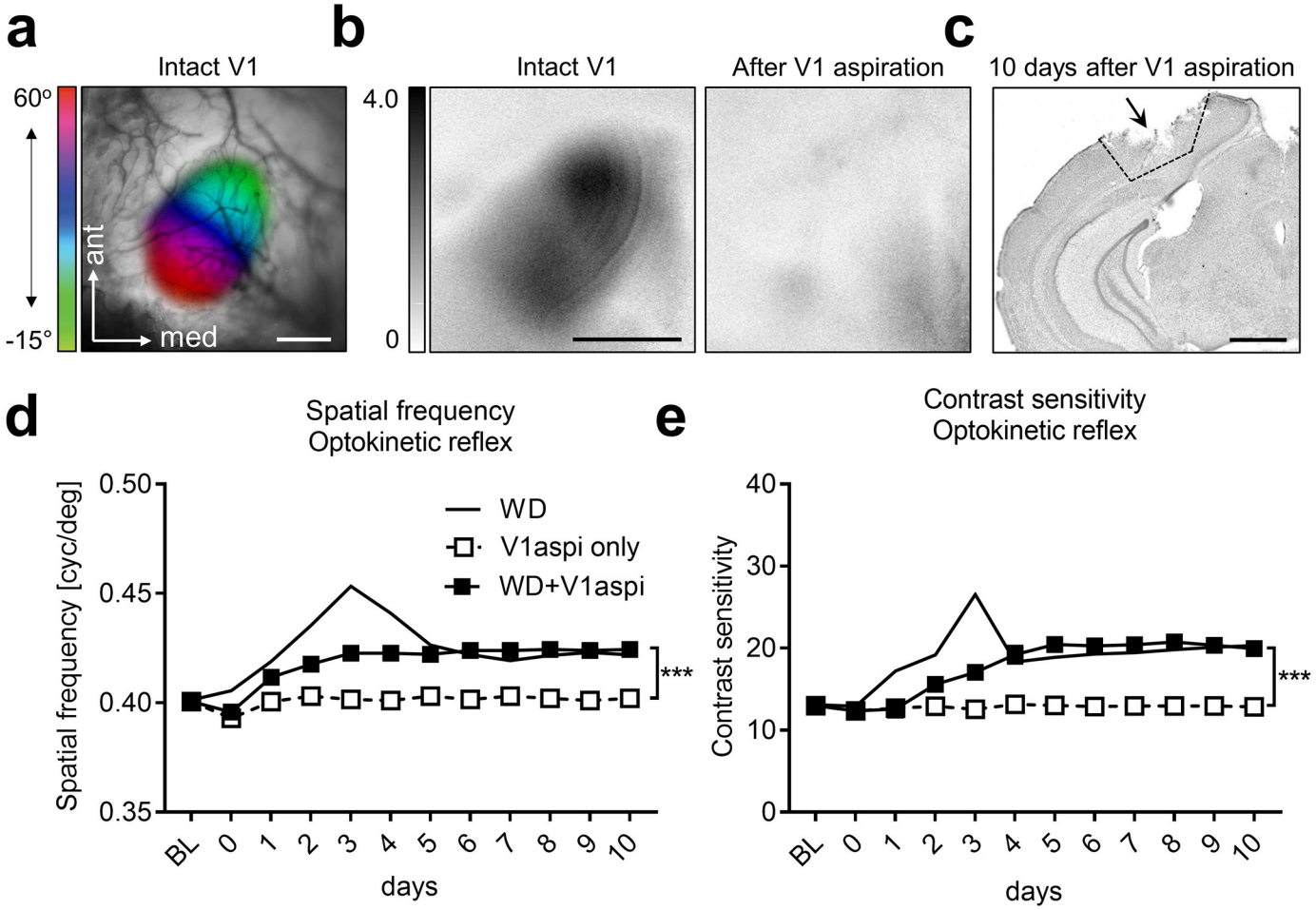

Fig 11. Aspiration of V1 reveals V1 contribution to cross-modally induced enhancements of the OKR. (**a**) V1 was located using intrinsic signal imaging. (**b**) Representative V1 amplitude maps elicited by visual stimulation before and after V1 aspiration. It is clearly visible that after V1 aspiration visually evoked cortical responses were absent demonstrating the efficiency of the aspiration surgery. (**c**) Nissl stained brain slice obtained 10 days after V1 aspiration. (**d**) Spatial frequency thresholds remained unchanged in mice after V1 aspiration only (n = 3). After combined WD and V1 aspiration (n = 3) spatial frequency sensitivity slightly increased until day 3 and remained at this level for the remaining 7 days. Interestingly, the strong peak of spatial frequency thresholds obtained 3 days after WD only (n = 4) was absent in mice after WD and V1 aspiration whereas the long-lasting improvement was almost identical in mice of both groups. (**e**) In WD only, V1aspi only and WD+V1aspi mice, the course of contrast sensitivity thresholds closely followed the course of spatial frequency thresholds in mice of the same groups. Taken together our data suggest that WD leads to V1 dependent and V1 independent improvements of the OKR. Open and filled squares represent mean values together with the s.e.m. However, vertical lines of s.e.m. are often occluded by data symbols.

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For aspiration surgery we located the correct position of V1 using intrinsic signal imaging. Fig 11a depicts a representative visually evoked retinotopic polar map of V1, which was merged with a picture of the cortical blood vessel pattern of a normal mouse. Fig 11b (left) shows the corresponding amplitude map. We then aspirated V1, guided by blood vessel landmarks, through a small trepanation and performed a second optical imaging session to validate the efficiency of the surgery. As expected, after V1 aspiration visually evoked responses in the V1 area were completely abolished (Fig 11b, right, not quantified). These experiments confirm that the surgery for V1 aspiration was efficient and reliable since it completely abolished visually elicited V1 activity. Fig 11c shows a representative example of a brain slice 10 days after the aspiration of V1.

Quantitative analysis using a two-way ANOVA with repeated measurements showed that group ($F_{1,4}$ = 77.27, p = 0.001) and time ($F_{11,44}$ = 32.83, p<0.0001) had a significant influence on spatial frequency thresholds. In addition, there was a statistically significant interaction between group and time ($F_{11,44}$ = 18.00, p<0.0001, two-way ANOVA with repeated measurements). As shown in Fig 11d the spatial frequency sensitivity of mice in that we only aspirated V1 (n = 3) remained almost unchanged for 10 days. However, if we combined WD and V1 aspiration, spatial frequency thresholds of the OKR slightly increased until day 3 and remained at this level for the whole time period tested (3 days: V1aspi only vs WD+V1aspi: 0.40±0.001 vs 0.42±0.001, 0.004; 10 days: 0.40±0.001 vs 0.42±0.001, p = 0.002; paired t-test followed by Bonferroni correction; Fig 11d). Interestingly, the peak of spatial frequency sensitivity at day 3, which we obtained in mice that only received a WD, was abolished in animals after concurrent WD and V1 aspiration. In contrast, the stable level of increased spatial frequency thresholds (between day 5 and 10) which was present in WD mice with combined V1 aspiration was practically identical to the increased stable level reached after WD only. Thus, these results suggest that the marked initial increase and decrease of spatial frequency sensitivity during the first 5 days after WD is mediated by V1. However, the long lasting improvement of spatial frequency thresholds after WD appeared to be V1 independent.

A similar result was obtained for contrast sensitivity of the OKR. Quantitative analysis using a two-way ANOVA with repeated measurements revealed significant influences of group ($F_{1,4}$ = 44.05, p<0.003) and time ($F_{11,44}$ = 37.08, p<0.0001) and a significant interaction between both ($F_{11,44}$ = 31.83, p<0.0001). In mice in which we only aspirated V1 contrasts thresholds remained unchanged for 10 days (Fig 11e). When we combined WD and V1 aspiration, contrast thresholds gradually increased until day 5 and remained at this level for the following 5 days (5 days: control vs WD: 13.03±0.79 vs 20.43±0.54, p = 0.02; 10 days: 12.84±0.67 vs 19.94±0.29, p = 0.008; paired t-test followed by Bonferroni correction; Fig 11e). However, the peak of contrast sensitivity found in animals which only received a WD at day 3, was not present in mice after combined WD and V1 aspiration whereas the stable level of enhanced contrast thresholds (4–10 days) was almost identical in animals of both groups (Fig 11e). These data suggest that the transient strong improvement of contrast thresholds of the OKR found in mice after 3 days of WD alone is mediated by activity changes observed in V1. In contrast, the long lasting enhancement of contrast sensitivity does not require V1. Taken together, these results indicate that WD improves subcortically mediated visual spatial frequency and contrast sensitivity in both a V1 dependent and V1 independent manner.

Cross-modally induced potentiation of OKR requires NMDA receptor activation

We next examined whether NDMA receptors contribute to improvements of the OKR. For this, WD mice received daily CPP injections (WD+CPP; n = 4) and we measured spatial frequency and contrast thresholds again for 10 days. In these mice both spatial frequency and contrast sensitivity remained unchanged over the whole time period tested and were not different from values of untreated control animals (n = 4) (spatial frequency: group: $F_{1,6}$ = 4.84, p = 0.07; time: $F_{11,66}$ = 1.02, p = 0.44; interaction: $F_{11,66}$ = 1.961, p = 0.149; contrast sensitivity: group: $F_{1,6}$ = 1.63, p = 0.35; time: $F_{11,66}$ = 6.8, p = 0.008; interaction: $F_{11,66}$ = 2.097, p = 0.16; two-way ANOVA with repeated measurements; Fig 12a and 12b). Hence, CPP administrations abolished both the visual cortex dependent and independent improvements of the OKR thresholds observed in WD mice. These results suggest that potentiation of the OKR induced by the deprivation of a non-visual sense also depends on NMDAR activation, as shown above for the changes in V1 activity after WD.

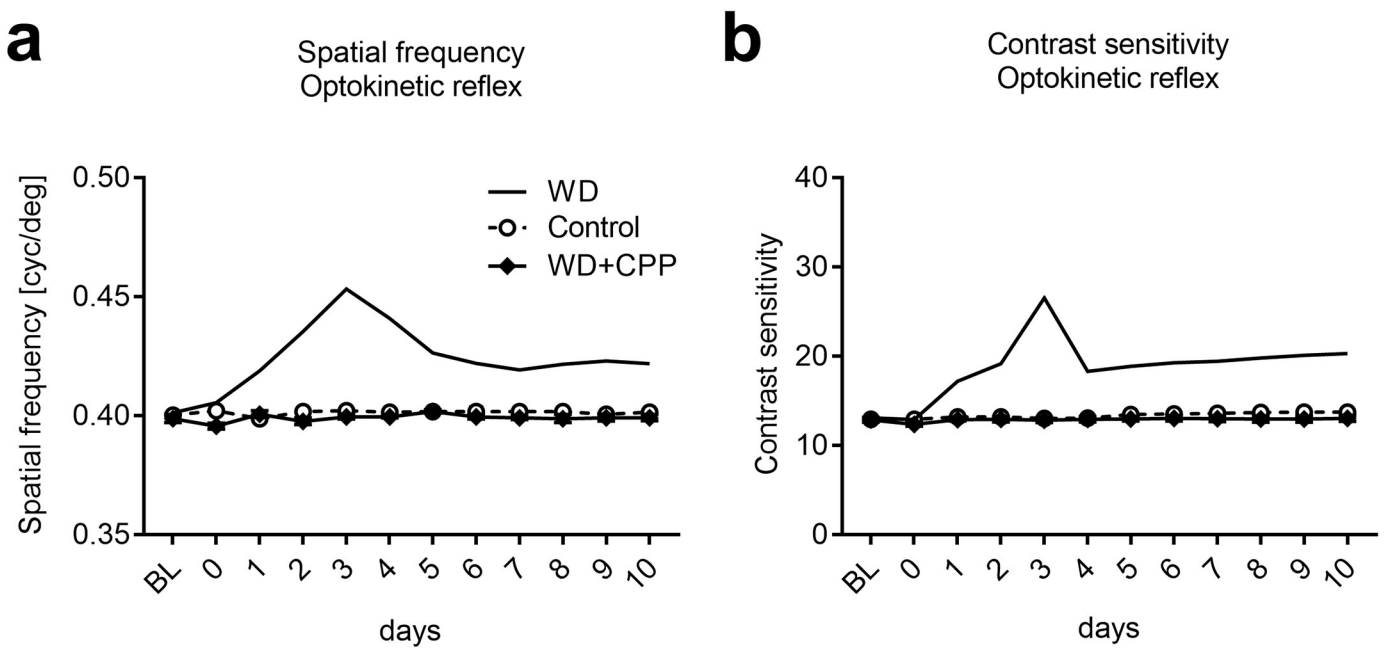

Fig 12. Cross-modally induced OKR potentiation requires NMDAR activation. (**a,b**) In both control mice (n = 4) and WD mice which received daily injections of CPP (n = 4) spatial frequency and contrast thresholds remained completely unchanged for 10 days. Hence, blocking NMDARs abolished both V1 dependent and V1 independent potentiation of OKR thresholds found after WD only.

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Discussion

In the present study we investigated the cross-modal effects of WD and AD on visually evoked V1 responses and visually mediated behavior in fully adult mice. Strikingly, we found that both WD and AD transiently shifted the OD in V1 towards the input through the ipsilateral eye. These changes required patterned vision through the ipsilateral eye and were accompanied by an increase of the E/I ratio in V1, suggesting a cross-modal restoration of V1 plasticity. Moreover, the observed changes in V1 activity partially mediated potentiation of the OKR, a visual behavior predominantly mediated by subcortical structures. These results indicate that the late-onset loss of a non-visual sensory modality dramatically alters neuronal processing at different stages of the visual pathway of adult mice, which in turn improves visually dependent behavior.

It has been demonstrated that prolonged monocular visual deprivation (MD, 5–7 days) in "young adult" mice around 60 days of age leads to a shift of the OD which is mediated by an increase of V1 activity elicited by open (ipsilateral) eye stimulation [24, 46, 48]. However, as the capacity of the brain to undergo experience dependent plastic changes massively declines with aging [12, 13], this type of cortical plasticity is completely absent in mice older than 110 days [11]. Strikingly, we show here that 3 days of WD or AD in mice of this age lead to V1 activity changes which resemble the type of OD plasticity in younger mice, since OD changes were also mediated by an increased V1 responsiveness to ipsilateral eye stimulation (Figs 3 **and** 4). Hence, our data provide evidence that WD or AD can rapidly restore V1 plasticity in fully adult mice. However, in contrast to the classical, now more than 50 years old paradigm showing that MD can induce alterations of OD [12, 14, 32], the cross-modally induced OD shifts described here took place without any visual deprivation. Thus, to the best of our knowledge, we demonstrate here for the first time that, at least in mice, OD in V1 can be altered by deprivations of non-visual sensory modalities, too.

Previous studies suggested that changes in the cortical E/I ratio in favor of excitation are the central hub for the restoration of cortical plasticity [49, 50]. For instance, dark exposure [39, 43], environmental enrichment [41, 44] and fluoxetine administrations [42], all cause lower inhibition and thus higher excitation levels in V1 and restore OD plasticity in V1 of fully adult mice. Moreover, these OD shifts can be prevented by artificially increasing cortical GABAergic inhibition by diazepam, a positive allosteric modulator of GABAA receptors [42–44], suggesting that a reduction of cortical inhibition is the common threat that re-induces cortical plasticity. We found that 3 days of WD or AD also raise the cortical E/I ratio in V1, in this case however, by increasing glutamate concentration in V1 (Fig 8). This was accompanied by a marked OD shift (Figs 3 **and** 4). These results are in accordance with previous studies demonstrating that treatments causing higher glutamate release in cortical synapses can reestablish cortical synaptic plasticity in adult mice [51, 52], suggesting that levels of the excitatory neurotransmitter glutamate also play an important role in regulating cortical plasticity. In addition, we found that increasing cortical inhibition by diazepam, and thus, re-decreasing the E/I ratio in V1, could abolish these OD shifts (Fig 9), indicating that the artificially increased GABAergic inhibition could compensate for increased glutamatergic excitation. Thus, our results suggest that increased cortical glutamate levels and thus, the increase of the E/I ratio in V1 was necessary for cross-modally induced OD changes. Hence, it can be assumed that deprivation of a non-visual sensory modality reinstates higher plasticity levels in V1, which then allow the visual input to re-shape V1 circuits. However, determining cortical GABA and glutamate levels by post mortem HPLC analysis of brain micro punches, as performed in the present study, is a relativity crude method to measure the cortical E/I ratio. This method cannot distinguish between intracellular GABA and the biologically active extracellular GABA in the synaptic cleft. Thus, to get a deeper mechanistically view into the cross-modally cortical alterations of the E/I balance, future studies could use the more precise *in vivo* micro dialysis to measure GABA and glutamate levels [41, 42, 45] or apply electrophysiological approaches [44]. However, as we found a general cross-modal increase of glutamate levels, it is likely that is, indeed, accompanied by increased cortical excitation.

There is increasing evidence that MD induced OD plasticity in young adult mice is mediated by long term potentiation (LTP)-like synaptic changes that lead to an increase of ipsilateral eye input to V1, as they require the NMDA receptor [24, 46, 48, 53]. In contrast, NMDAR dependent plasticity is also completely absent in fully adult mice older than 110 days [7, 46, 53–55]. However, here we show that the cross-modally induced OD shift after 3 days of WD or AD in mice of this age, also depends on NMDA receptors (Fig 9), suggesting that NMDAR function is reestablished in V1 after non-visual sensory deprivations. This conclusion is further supported by important recent studies: First, both WD and AD together with MD for 7 days can cross-modally restore NMDA dependent OD plasticity in fully adult mice [15, 56]. And second, AD has been shown to reactivate thalamocortical plasticity in V1, such as LTP, which was accompanied by a potentiated function of NMDAs in the adult V1 [57]. All these studies indicate a pivotal role of this particular receptor in mediating cross-modal plasticity in spared primary sensory cortices. However, as we administrated the NMDAR blocker CPP systemically, we cannot make statements on the precise location where this receptor is required to mediate cross-modal adaptations. It might be the visual cortex [24, 46, 57] but, as recent studies demonstrated that already neurons in the thalamus play a role in OD plasticity [58–60], it is possible that NMDARs, are already required in earlier structures of the visual pathway.

Previous studies have demonstrated that the deprivation of one sense for only a few days strengthens thalamo-cortical and layer 4 to 2/3 synapses in a spared primary sensory cortex [9, 10, 36]. In accordance with this finding, we here demonstrate that WD cross-modally increased the AMPAR mediated mEPSC amplitudes in layer 4 of V1, suggesting a

strengthening of layer 4 synapses (Fig 7). At least a part of these strengthened synapses most likely represent thalamo-cortical synapses for several reasons: first, strengthening of thalamo-cortical synapses leads to increased sensory driven responsiveness of primary sensory cortices [10, 38]. This is in line with our imaging results, as evoked V1 activity was increased 3 d after WD. And second, visual input through the ipsilateral eye is required to mediate V1 activity alterations (Fig 6) suggesting an involvement of synapses and NMDARs on the visual pathway from the ipsilateral eye to V1. In summary, these results suggest that cross-modal plasticity, even in the adult spared sensory cortex is a form of experience dependent synaptic plasticity similar to long-term potentiation (LTP), as already demonstrated recently [6, 57]. Furthermore, our results demonstrate a high importance of V1 input through the ipsilateral eye for cross-modal plasticity in V1. However, further studies are required to examine the precise mechanisms underlying the surprising finding that exclusively the pathway of the ipsilateral eye to the binocular V1 seems to be affected by the deprivation of non-visual senses.

Previous studies reported that a prolonged sensory deprivation (for 7 days) leads to a decrease of AMPAR mediated mEPSC amplitudes in layers 2/3 of the spared primary sensory cortex [20, 61]. Hence, it was speculated that an initial strengthening of synapses in the remaining sensory cortices (after 2–3 days) is followed by a decrease in synaptic transmission after 7 days of sensory deprivation [9]. Our functional data of V1 responsiveness support this hypothesis as 7 days after WD or AD V1 responses were completely restored back to baseline levels (Figs 3 **and** 4). Thus, our data suggest that the restoration of normal OD levels is mediated by homeostatic mechanisms like synaptic down-scaling [20, 61] and/or cross-modally induced reduction of lateral input strength in layers 2/3 as shown previously [36].

Here we show that 3 days of either WD or AD alone induced a strengthening of V1 input through the ipsilateral eye, which was, however, followed by a recovery of normal V1 activity levels after 7 days (Figs 3 **and** 4). Recently, we also showed that 7 days of WD or AD alone did not lead to alterations of V1 activity [15]. However, when we combined WD or AD with MD of the contralateral eye for 7 days, responsiveness in V1 evoked by ipsilateral (open) eye stimulation was enhanced [15, 56]. Remarkably, in the present study, the same enhancement was observed 3 days after WD or AD alone (Fig 3), or 3 days of WD or AD combined with MD of the contralateral eye (Fig 6). These results strongly suggest that a prolonged MD for 7 days in WD or AD mice keeps V1 input through the ipsilateral eye at a higher level. In other words, under these MD conditions there is no recovery of V1 activity elicited by ipsilateral eye stimulation.

What might be a potential explanation for these differential changes in V1 responsiveness? As mentioned above, the downregulation of the overshooting V1 activity in WD or AD mice without MD is most likely mediated by homeostatic mechanisms, which readjust neuronal activity levels after perturbations [20, 36, 61, 62]. However, if WD or AD is combined with MD, activity levels in V1 are generally lower. Therefore, homeostatic mechanisms are not induced.

We recently showed that 7–12 days of WD dramatically improved V1 mediated visual acuity and contrast sensitivity, as measured by behavioral visual water task experiments [3]. Hence, the transient increase of V1 responsiveness 3 days after WD (or AD) described in the present study might be a necessary cortical alteration for later V1 dependent visual improvements that might compensate for the loss of somatosensation or audition, which in normal rodents provides essential information about their environment [7]. Here, we further demonstrate that both WD and AD also provoked a fast and a long-lasting improvement of the optokinetic reflex (OKR), another type of visual behavior mainly mediated by subcortical structures including the cerebellum and vestibular nuclei [47]. Previous studies demonstrated enhanced OKR sensitivity in rodents after MD [27], vestibular impairments [47, 63] or daily

threshold testing from eye opening into adulthood [64]. Here, however, we provide the first evidence that OKR improvements can also be induced by depriving somatosensation or audition (Figs 10 **and** 11). We found that both, WD and AD resulted in three distinct phases of altered OKR sensitivity: During the first phase, OKR sensitivity markedly increased and peaked at 3 days after WD or. The second phase was characterized by a drop of OKR thresholds, lasting for 2–3 days. In phase three OKR thresholds stabilized and remained at a level above control values. Phase one and two appeared to be V1 dependent. These result are in line with previous studies demonstrating that V1 is involved in enhancements of OKR sensitivity [27, 47, 64]. Interestingly, the transient activity peak in V1 3 days after WD or AD temporally matched the peak of OKR improvements. These results suggest that cortico-fugal projections might transmit cross-modally induced V1 activity changes to subcortical structures which then act to mediate the compensatory potentiation of OKR. This hypothesis is supported by the findings that cortico-fugal projections can indeed modulate sensory induced behaviors [65, 66]. However, the third phase, where OKR sensitivity remained at a stable level above baseline for at least 10 days, was V1 independent as mice with removed whiskers (WD) and aspirated V1 also reached this enhanced level (Fig 11). However, we have to mention that V1 aspiration, as performed in the present study, is a relatively crude way to investigate the necessity of V1 in OKR changes, as also fibers of passage might be affected. Future studies should, hence, use more refined alternatives such as silencing V1 using muscimol or by optogenetical activation of inhibitory neurons in this region. However, as it has been shown that V1 aspiration and silencing V1 using muscimol affect the OKR in a similar manner [27], we believe that our approach, indeed, revealed a crucial role of V1 in mediating cross-modally induced OKR changes. Together with our previous finding that WD leads to an enhancement of visual performance in the visual water task [3], our OKR data indicate that the deprivation of non-visual senses provokes a general long-lasting compensatory improvement of visually mediated behaviors. Interestingly, like cross-modally provoked V1 activity changes, potentiation of the OKR could be completely abolished by antagonizing NMDARs (Fig 12). These results then suggest that NMDAR in different structures of the visual pathway are instrumental in the mediation of cross-modal effects.

In summary, we could demonstrate that the deprivation of non-visual sensory modalities transiently changes OD in V1. We postulate that reducing either somatosensory or auditory input cross-modally re-installs V1 plasticity in fully adult mice, allowing visual inputs to compensatorily re-shape V1 circuits. While further studies are needed to clarify the precise mechanisms underlying this novel and surprising finding, the present results already emphasize the power of cross-modal plasticity to re-open a window of high plasticity in the fully adult cortex far beyond any sensory critical period.

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Author Contributions

Conceptualization: Manuel Teichert, Marcel Isstas, Jürgen Bolz.

Data curation: Manuel Teichert, Marcel Isstas.

Formal analysis: Manuel Teichert, Marcel Isstas, Konrad Lehmann.

Investigation: Manuel Teichert, Marcel Isstas, Lutz Liebmann, Franziska Wieske.

Project administration: Jürgen Bolz.

Resources: Christian A. Hübner, Christine Winter, Jürgen Bolz.

Supervision: Jürgen Bolz.

Validation: Manuel Teichert, Marcel Isstas.

Writing – original draft: Manuel Teichert.

Writing – review & editing: Manuel Teichert, Marcel Isstas, Jürgen Bolz.

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5. Major discussion

Here, I investigated the effects on primary visual cortex, caused by the manipulation of another sense. I wanted to know, what happens in V1 and on the behavioral level, if audition or somatosensory sensation is deprived for a longer period. First it was of interest, if somatosensory deprivation, in form of whisker plucking improves visual performance in fully adult and free behaving mice. For this, visual thresholds were determined using the visual water task. After detecting thresholds, mice were divided into two groups. In one group, whiskers were plucked, and the other animals received a sham-operation as a control group. After seeing that visual abilities were up to 40 percent better in whisker-deprived animals, optical imaging experiments were performed to detect changes in V1 to visual stimuli with different spatial resolution and contrast. After 7-12 days after whisker deprivation V1 responded stronger to visual stimuli with a higher spatial frequency and lower contrast, after whisker plucking, suggesting better visual abilities in these mice, like seen by the behavioral tests. Hence, I supposed plastic changes in V1 of fully adult mice, leading to the question, if the deprivation of a non-visual sense is also able to restore ocular dominance plasticity in fully adult, which do not display any changes of the ODI in response to monocular deprivation any more. For this, I measured V1 response to the stimulation of each eye and calculated the ODI by using the method of period optical imaging of intrinsic signals, immediately after plucking the whiskers. Afterwards the dominant eye was closed for seven days and then ODI was determined again (after reopening the eye, of course). Results show, that the ODI decreases after one week of monocular deprivation, because of the potentiation of ipsilateral eye inputs, in animals without whiskers, but not in the control group. This effect needs the NMDA receptor, as blocking this receptor by the systemic administration of 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), a competitive NMDA receptor antagonist, prevented the potentiation of ipsilateral eye input. The receptor is involved in the strengthening of synapses between neurons, leading to the conclusion, that the open eye potentiation depends on LTP, maybe at Layer 4 and 2/3 synapses, receiving input from the thalamus, as the method of optical imaging of intrinsic signals mainly measures here. Next to the involvement of the NMDA receptor, the overall GABA content in V1 was reduced after WD, which could suggest a decrease in GABAergic inhibition, which is often accompanied with the restoration of ocular dominance plasticity. In order to substitute the loss of GABA, I systemically administrated Diazepam, an allosteric GABA-A receptor agonist, which should have prevented the ODI shift. But this was not the case. To my surprise, the Diazepam administration did not prevent the ODI shift. I saw a shift

caused by a decrease of contralateral eye response in V1, which is normally restricted to the juvenile form of ocular dominance plasticity. Ipsilateral eye input to V1 was unchanged. This shift could be prevented by the additional administration of CPP, what suggests, that the decrease of V1 response to the stimulation of the contralateral eye also depends on NMDA receptor functioning, perhaps a LTD-like mechanism. The results lead to the assumption that there are unknown mechanisms involved in cross-modal plasticity. Having checked what happened in V1 after seven days of auditory or somatosensory deprivation, I was interested if there is a time course during this week. For this I plucked the whiskers and imaged the animals immediately. Again, after three days and seven days. Here, I measured the activity in V1 evoked by the stimulation of the contra- or ipsilateral eye. At day three after whisker plucking there was a decrease in the ODI due to a stronger responsiveness to the stimulation of the eye ipsilateral to the recorded hemisphere, which was absent seven days after somatosensory or auditory deprivation. This was a surprise, because this phenomenon is normally only seen after the manipulating vision, in form of suturing the contralateral eye, for example. For further characterization I combined monocular deprivation of the ipsi- or the contralateral eye, to figure out, if the potentiation of the ipsilateral eye needs visual input. I found, that suturing the contralateral for three days had no effect on the potentiation but suturing the ipsilateral prevented the ODI shift and amplitudes did not change. Moreover, whole-cell recordings in V1 slices of animals three days after whisker plucking showed an increase in AMPA receptor mediated mEPSC amplitudes, suggesting a strengthening of excitatory synapses. Additionally, the excitatory/inhibitory balance was shifted after three days of whisker deprivation in favor of excitation, revealed by HPLC analysis of V1 tissue, which is in line with the imaging and electrophysiological data. Next, I tried to compensate the increase of excitation by the systemic administration of diazepam and could not detect an ipsilateral eye potentiation in V1 after three days of whisker plucking with the method of optical imaging any more, indicating that the increase of E/I-balance is involved in the shift of the ODI. After seeing that mEPSC are increased, I administrated CCP to block NMDA receptor function and found no shift after three days of whisker plucking, suggesting an involved of these receptors in the so far described effect. As we found that seven to twelve days of whisker deprivation is sufficient to improve vision in fully adult and behaving animals and that three days of whisker plucking increased V1 response, I asked if there is also a cross modal evoked effect on the optokinetic reflex, a head and eye movement, which stabilizes images on the retina, mediated by subcortical structures, involving the colliculi superiors, for example. To answer this question, I used the optomotor system to detect thresholds for spatial frequency and contrast in untreated fully adult

animals. Then I plucked whiskers, or a conductive hearing loss was induced by the removal of the malleus and animals were tested again daily. After sensory deprivation optokinetic response increased and peaked three days after deprivation. Then levels dropped down over the next two days but persisted above baseline level till the end of experiment. The peak after three days of somatosensory deprivation is in line with the optical imaging results, where visually driven V1 response reached its peak. This suggests an involvement of V1 in the enhancement of the optokinetic reflex. Hence, I bilaterally removed V1 in another group of animals and found that the three-days-peak stayed out, but thresholds slightly increased and persisted at the same level above baseline as seen five days after whisker removal in the group with visual cortex and plucked whiskers. This indicates that the initial peak is mediated by V1, but there also must be alterations in the subcortical pathway mediating the optokinetic reflex. I also could show, that for the V1 dependent peak and for the V1 independent increase of the optokinetic reflex NMDA receptors are involved, as the administration of CPP prevented both.

5.1 Methodological considerations

Different techniques were used to investigate the effects on V1 by manipulating the auditory or somatosensory system. In the following section I will discuss the advantages and limitations of the techniques used for my work.

Here, I mainly used the technique of optical imaging of intrinsic signals, which allows to determine cortical response to sensory stimulation by measuring changes of reflectance due to hemodynamic response (Grinvald et al., 1986). For behavioral studies the visual water task was used, a two-choice discrimination task, which enables determining threshold for visual acuity and contrast sensitivity (Prusky et al., 2000, Prusky and Douglas, 2004). But there is also a visual reflex, the optokinetic reflex, triggered by subcortical structures. For the detection of changes in thresholds for spatial resolution and contrast tuning I used the optomotry system (Prusky et al., 2004). .

5.1.1 Periodic optical imaging intrinsic imaging of mouse visual cortex

The technique of periodic optical imaging of intrinsic signals is a widely used method to visualize and quantify cortical activity caused by sensory stimulation (Grinvald et al., 1986, Kalatsky and Stryker, 2003, Greifzu et al., 2014, Fu et al., 2015, Lehmann and Lowel, 2008,

Isstas et al., 2017, Teichert and Bolz, 2017, Rodriguez et al., 2018, Kalatsky et al., 2005). Here, a periodic stimulus is combined with continuous data acquisition. Using Fourier analysis, the stimuli evoked activity can be separated from noise, resulting from breathing, heart beat and diffuse cortical activity. It allows a very fast data acquisition (within minutes) with a high spatial resolution over large cortical areas (Bonhoeffer and Hubener, 2016) and findings acquired with this technique has been confirmed by electrophysiological results (Kaneko et al., 2008b, Kaneko and Stryker, 2014). Moreover, as the technique is minimal invasive, it is possible to obtain data through the intact skull of the mice. Hence, it enables multiple imaging sessions in the same animal.

Here, I used the method of optical imaging of intrinsic to measure stimuli evoked activity in V1. For this it is possible to measure activity evoked by the stimulation of both eyes, as binocular input (**manuscript 1 and 4**), or resulting from each single eye (monocular vision). In the second case it is possible to calculate the ocular dominance index (ODI) from the visual evoked response in V1 triggered by each single eye. Hence, the ODI reflects the ocular dominance (see 1.3). This makes is possible to detect plastic changes after an intervention (**manuscript 1, 2, 3 and 4**). Moreover, it is not only possible to obtain data from visual cortex. Some studies also used optical imaging to characterize the functional architecture of the barrel field (Grinvald et al., 1986, Frostig et al., 1990, Masino et al., 1993, Narayan et al., 1994, Iordanova et al., 2015, Knutsen et al., 2016) and map plasticity (Feldman and Brecht, 2005).

5.1.2 Visual water task

The visual water task is a psychophysical, two choice discrimination task, for determining V1 dependent visual acuity thresholds and contrast sensitivity in rodents. In detail, mice learn to associate a sine-wave grating with the escape from the water. Now, the animals have to decide for one of two screens. One shows the sine-wave grating and the other an isoluminant homogenous grey, in a randomized manner. Under the screen, showing the grating, an escape-platform is hidden under the water surface. The spatial frequency (in cycles per degree) or/and contrast (in percent) can be modulated by computer software. Mice will perform with less mistakes, if they can see the grating. At some point they fail to find the screen, which shows the sine-wave grating. The last frequency seen, is then defined as the threshold for acuity or contrast sensitivity. For this study (**manuscript 1**), we determinated both, plucked all whiskers in one group and continued with the measurements. An advantage of this kind of behavioral

setup is, that it is possible to assess what the animal really sees. Next to invasive techniques, like electrophysiological measurements, visually-evoked potentials, for example (Ridder and Nusinowitz, 2006) or analyzing the retina itself (Martin, 1986, Collin and Pettigrew, 1989) the visual water task provides a non-invasive method, which is an advantage for longitudinal measurements. But on the other hand, it took quite a long time. For example, training-phase needs about one to two weeks, while the test-phase needs much longer. All in all, the animals used for this study had to swim for about three months.

5.1.3 The Optomotry-System

With the Optomotry-system it is possible to detect thresholds for the visually driven optokinetic reflex (OKR). This reflex is triggered by subcortical structures, including colicullus superior, cerebellum and vestibular nuclei (Liu et al., 2016) and is characterized by a head and eye movement which stabilizes images on the retina, when the visual field is rotated around the animal. Here, we used this technique to determine the thresholds for visual acuity and contrast sensitivity after whisker or auditory deprivation. It is a quite fast method and does not need any animal training. As the experimenter tracks the head movements by himself, it is necessary that a second person confirmes data. Literature shows, that efforts are done to automate animal tracking (Segura et al., 2018)

5.2 A prolonged time of whisker deprivation enhances visual abilities in fully adult mice

Here we could show, that a prolonged period of whisker deprivation enhances visual abilities in fully adult mice. For this, we determined visual acuity and contrast sensitivity using the visual water task animals, then plucked all main whiskers in one group of mice and investigated the effects on visual function.

It is shown for humans, that congenitally deaf individuals display superior visual abilities (Neville and Lawson, 1987, Bottari et al., 2010) and also for laboratory animals (Lomber et al., 2011). But it remains elusive, if such improvements also take place after late onset sensory loss in fully adult animals and if the spared senses are better on the behavioral level. In **manuscript 1** we show that somatosensory deprivation, in form of whisker plucking, improves visual acuity

and contrast sensitivity in fully adult animals by 40% after 7 to 12 days of whisker deprivation. One could argue, that this improvement is due to a phenomenon called perceptual learning. Here, visual abilities improve in animals caused by a prolonged training near their visual thresholds (Hager and Dringenberg, 2010, Wang et al., 2016). Wang et colleagues could show, that in C57BL/6 mice, the same strain used here, visual acuity is about 0.47 cpd in naive animals, for example, which fits to our data. After a prolonged period of training along their visual threshold, they showed an improvement of about 55% (Wang et al., 2016). But our results show, that the improvement of visual abilities depend on plucking the whiskers, as the control group did not display such a strong improvement. Moreover, in another group of mice, which never swam before, visual abilities were also measured by the method of periodic optical imaging of intrinsic signals (OI). These animals also showed better vision after a brief period of somatosensory deprivation, indistinguishable from the results obtained by using the Visual Water task.

The potential mechanisms underlying cross-modal refinements of vision

It has been shown that prolonged periods of sensory deprivation strengthens the thalamo-cortical input to the spared cortices in young and adult animals (Petrus et al., 2014, Rodriguez et al., 2018), which could lead to a refinement of spared sense. In detail, one week of visual deprivation increased sensitivity and frequency tuning of A1 neurons and refined intracortical circuits (Petrus et al., 2014, Meng et al., 2017). Visual deprivation also sharpened the functional whisker barrel map and increased serotonin levels in the spared somatosensory cortex (Jitsuki et al., 2011). Hence, the observed refinement of visual abilities might also depend on these mechanisms. Moreover, the strengthening of thalamo-cortical synapses and the refinement of intracortical circuits was also found as a result of perceptual learning (Wang et al., 2016), which makes these mechanisms to a common feature of the improvements.

Here, we provided data showing, that behavior relevant vision improves after somatosensory deprivation in fully adult mice. Future studies will have to investigate the underlying cellular and molecular mechanisms. We also do not know, if the effect is long-lasting. Hence, it would be interesting to see, if after the regrowth of whiskers, the improvement will persist or not. The answer to these questions would provide a better understanding of brain dynamics in response to a temporary loss of one sense and might also be of clinical relevance.

5.3 Sensory loss restores plasticity in the spared cortex

As we could show that a prolonged period of sensory deprivation provoked plastic changes in V1, we asked the question, if somatosensory or auditory deprivation could also restore ocular dominance plasticity in V1. For this, we combined one week of WD or AD with monocular deprivation and found that this the case (**manuscript 2**). The observed OD shifts were mediated by an increase of V1 response elicited by open eye stimulation. This is also seen in young adult animals and is the "normal" form of adult ocular dominance plasticity (Ranson et al., 2012, Sato and Stryker, 2008, Sawtell et al., 2003). This potentiation needs visual experience (Sawtell et al., 2003) and depends on NMDA receptor function, involved in LTP-mechanisms, as it could be shown, that the administration of a NMDA receptor antagonist, like CPP, prevents the open eye potentiation (Sawtell et al., 2003, Sato and Stryker, 2008). As antagonizing NMDA receptor also blocks cross-modal induced OD shifts, similar mechanisms might take place in animals in which ocular dominance plasticity is normally absent. Furthermore, studies show, that changes in inhibitory transmission are involved in the restoration of adult ocular dominance plasticity. For example, antagonizing GABA A receptors reduced the inhibitory tone in V1, which facilitated the OD shift in fully adult animals (Harauzov et al., 2010) and also food restriction decreased the inhibitory tone, leading to plasticity in adult visual cortex (Spolidoro et al., 2011). To figure out if WD or AD also reduces the inhibitory tone in V1 HPLC experiments were performed (**manuscript 3**). Results show, that seven days after sensory deprivation, the overall GABA concentration is reduced in V1, suggesting a reduction in the inhibitory tone. As several studies could show that OD shifts can be prevented by increasing the inhibition (Sale et al., 2007, Greifzu et al., 2014, Stodieck et al., 2014) I administrated Diazepam, an allosteric GABA-A receptor agonist, to substitute for the reduced GABA level in V1 and surprisingly this did not prevent the OD shift, but led to the juvenile form of ocular dominance plasticity characterized by the reduction of visually evoked activity of the closed eye (**manuscript 3**). Hence, the data suggest that the reduction in GABA-levels cannot be the only reason. So, it might be possible that cross-modally induced shifts in ocular dominance also involve so far unknown mechanisms. For instance, another study could show, that magnesium treatment alters the expression of NMDA receptor subunits, leading to restore juvenile plasticity, not affecting the inhibitory tone in visual cortex (Liu et al., 2015a). Maybe alterations in PSD 95 are also responsible for the observed effect, as PSD 95 KO mice display lifelong juvenile like plasticity (Huang et al., 2015). The described decrease of contralateral (closed) eye input could also be mediated by LTD-like mechanisms, suggested by a line of studies which show that this is

typical for juvenile like ocular dominance plasticity (Kirkwood and Bear, 1994, Heynen et al., 2003, Espinosa and Stryker, 2012, Cooke and Bear, 2014).

In summary we could show, that whisker or auditory deprivation combined with monocular deprivation can restore ocular dominance plasticity in fully adult mice, far beyond their critical period. This depends on NMDA receptor functioning, revealed by CPP injections, suggesting LTP-like mechanisms. This was accompanied with a reduction in V1 GABA-levels. Sensory deprivation combined with MD and the administration of Diazepam did not prevent the OD shift, but instead lead to juvenile plasticity. Thus, these results suggest that diazepam administration in monocularly and whisker deprived mice, facilitates LTD-like mechanisms, whereas MD combined with WD alone facilitates LTP-like changes in V1.

5.3.1 A prolonged period of sensory loss displays a time-course of changes taking place in the spared visual cortex

As we found, that WD or AD combined with MD leads to an OD shift after seven days in fully adult animals, we next asked about the time-course during the first week of sensory loss. For this, we carried out repeated optical imaging sessions in every animal tested. We determined the ODI directly after sensory loos (0d), three (3d) and seven days (7d) later without manipulating vision in form of MD, for example. As 0d and 7d do not differ from each other, we found a decreased ODI after three days of sensory deprivation (WD or AD), due to the potentiation of ipsilateral eye input to binocular V1. This was accompanied by an increase of the E/I ratio. As we decreased the E/I ratio with Diazepam, the ODI shift was abolished, also after blocking NMDA receptors with CCP. Others could show before that in young animals OD plasticity needs NMDA receptor function (Sato and Stryker, 2008, Sawtell et al., 2003). In manuscript 2 and manuscript 4 we show that NMDA receptor functioning is also a feature of cross-modally induced changes in the ODI of fully adult animals. As we administrated the NMDA receptor blocker CCP systemically, we know, that V1 NMDA receptors are blocked, but also the ones of earlier structures of the visual pathway, which can be involved in OD plasticity, like the lateral geniculate nucleus (Jaepel et al., 2017). Moreover, we could show, that the OD shift needs visual input through the ipsilateral eye, while closing the contralateral eye had no effect. This suggests, that the altered OD is experience dependent.

This is the first study which could show, that it is possible to alter the OD by deprivations of non-visual sensory modalities without the additional manipulation of the visual system, like monocular deprivation, for example.

5.3.2 The deprivation of a non-visual sense enhances the optokinetic reflex

After seeing that visual acuity and contrast sensitivity are affected by WD (**manuscript 1**), I asked, if sensory loss also alters the optokinetic reflex as several studies demonstrate changes in OKR sensitivity after manipulating vision, like monocular deprivation (Prusky et al., 2006), or non-visual systems, like vestibular impairments (Liu et al., 2016, McCall and Yates, 2011). Moreover, it was shown that an increased V1 activity also alters the OKR, while removing V1 has no effect on OKR baseline values (Prusky et al., 2006). In manuscript 4, we show that WD or AD also lead to changes of the OKR. After determining thresholds, I plucked all whiskers or a conductive hearing loss was induced, by Malleus removal. During the first two days after sensory deprivation OKR thresholds increased, with a peak at day three. After two more days, threshold decreased, but persisted above baseline level, till the end of experiments. In another group CCP was administrated, and the enhancement of the OKR was prevented, suggesting an involvement of NMDA receptors somewhere on the accessory pathway. Moreover, I could show, that removing V1 combined with WD or AD also enhanced the OKR, but that the initial peak failed to appear. Levels reached by these animals were indistinguishable from those found five days after sensory deprivation with intact V1. Hence, V1 removal just abolished the initial peak, seen on day three after WD or AD, but not the compensatory improvement, suggesting that changes in V1 activity enhanced OKR during the first days but that the compensatory improvement is cortex independent. Future studies will show, on which are the underlying mechanisms and where this improvement takes place.

5.4 General remarks

In the present study I could show that a late onset of sensory loss in fully adult animals has effects on the spared sensory system, also on the level of primary sensory cortices. In detail, the loss of audition or somatosensation, caused by whisker removal has huge impact on V1 functioning, as it improves vision, visual triggered reflexes and restores experience-dependent ocular dominance plasticity, far beyond the animal's critical period. We could detect changes from the behavioral to the cellular level, which gives a good picture of what happens, when a sense is lost later in life. But the picture is not finished as some mechanisms are unknown. So, it might be interesting to know, if the improvement of vision after whisker deprivation really depends on thalamo-cortical synapse strengthening. In vivo electrophysiological experiments would have answered that question. Moreover, it would be interesting to follow the question why the administration of GABA restored the juvenile form of ocular dominance plasticity. It would be nice to replace speculations with data. Then it would be very interesting to replace the systemic CCP and GABA injections by a locally to V1 restricted method, like mini-pumps or something.

6. Summery

In this thesis I investigated the effects of somatosensory loss in form of whisker removal and auditory loss in form of malleus removal on the spared primary visual cortex in fully adult mice. For this I used a behavior test to determine visual abilities. With the so called visual-water-task, a two-choice discrimination task, it is possible to examine what a rodent really sees, meaning which spatial resolution and how much contrast is necessary that mouse modulates its behavior. For untreated animals, we found values, which fit to literature. Next, we devided these animals in two groups. In one group we plucked all whiskers, the other just got a sham operation and served as control. After 12 days of whisker deprivation the thresholds for spatial acuity and contrast sensitivity changed in the animals without whisker, but not in the control group, indicating, that the deprivation of somatosensation leads to a compensatory mechanism, that refines vision. It was amazing to see that this cross-modal improvement was about 40%. We confirmed data by using an imaging technique for primary visual cortex, known as optical imaging of intrinsic signals, which is a minimal invasive method to quantify and visualize stimulus evoked cortical activity. Here, data show that 12 days after whisker removal V1 responded stronger to lower contrast and spatial resolution. We could not clarify the underlying mechanisms, but literature suggest a strengthening of thalamo-cortical inputs to V1 responsible for the strong improvement of vision after somatosensory deprivation. After showing for the first time, that somatosensory deprivation, in form of whisker plucking leads to better behavior relevant vision in fully adult animals and the involvement of V1, I asked, if the deprivation of somatosensation or audition, in form of bilateral malleus removal could also restore adult ocular dominance plasticity in the spared primary visual cortex in fully adult animals. To test this the input strength of each eye in the binocular part of V1 was measured by using the method of optical imaging of intrinsic signals immediately after WD and again after one week of WD. Here, there was no change in ocular dominance. But combining WD or AD with the monocular deprivation of the contralateral eye for one week shifted ocular dominance in favor of the open eye, a phenomenon typical for the adult form of ocular dominance plasticity. This shows, that sensory deprivation has huge impact on the spared senses even in animals far beyond their critical period. Moreover, I could show, that the shift towards the open eye depends on NMDA receptor functioning, as antagonizing this receptor by the systemic administration of CCP prevented the observed effect. This suggests, that the strengthening of the ipsilateral eye depends on LTP. Another mechanism involved in the restoration of experience-dependent plasticity in V1 is the reduction of GABA levels, implicating a lower level of inhibition. So, we

tested GABA levels in V1 punches using HPLC and found them really reduced. In line with this finding I tried to compensate this loss of inhibition by the systemic administration of diazepam an allosteric GABA-A receptor agonist in order to prevent the shift. To our surprise it did not prevent ocular dominance plasticity per se. In the animals treated with diazepam it switched to the juvenile form of ocular dominance plasticity which is characterized by weakening of deprived eye input to V1. This suggests so far unknown mechanisms involved in cross-modal plasticity phenomenons. As we always deprived one eye for seven days, we were also interested in the question what happens during this week. So, we performed an additional optical imaging session in another group of mice after three days of WD or AD to figure out what happens. We started without depriving animals' vision by monocular deprivation and were surprised to observe changes in ocular dominance after three days, mediated by a potentiation of ipsilateral eye input. These results could be confirmed by electophysiological findings, which show, that AMPAR mediated mEPSC amplitudes were increased in layer IV of V1 on day three after WD accompagined with increased Glutamate levels, indicating that the observed switch in ocular dominance is due to LTP like mechanisms. On day seven after WD or AD amplitudes and the ocular dominance index was the same as measured on day zero, meaning immediately after WD or AD.

After showing before, that WD has the power to improve vision and after seeing, that three days of WD or AD provokes an increase of V1 amplitudes, we asked, if the optokinetic reflex (OKR), a visual reflex triggered by subcortical structures, is also affected. Literature shows, that OKR thresholds change when there are changes in V1 response strength. To figure out we used the optomotry system. We found a fast and long-lasting improvement of the OKR after WD or AD. This improvement showed three distinct phases. From day one to day three after WD or AD we observed an enhancement of OKR sensitivity with its peak on day three (phase one). Then OKR thresholds dropped during the next two to three days (phase two) but remained above control levels till the end of the experiment (phase three). Aspirating V1 showed, that phase one is cortex dependent, but not the long-lasting improvement as seen in phase three. This suggests the long-lasting improvement depends on plastic changes in the subcortical structures triggerning the OKR. As the chronic administration of CCP abolished OKR improvement, we could show that even in subcortical structures NMDAR play an important role.

All together this work shows, that the late onset of sensory loss has an enormous impact on the spared primary sensory cortex of fully adult animals with behavioral relevant consequences.

7. Zusammenfassung

Lange wurde davon ausgegangen, dass sich kreuzmodale Interaktionen zwischen den Sinnen erst in höheren kortikalen Arealen zeigen. Ich weise in dieser Arbeit nach, dass es aber schon auf Ebene der primären sensorischen Kortices zu kreuzmodalen Interaktionen kommt, die sich zell- und molekularbiologisch nachvollziehen lassen, und verhaltensrelevante Auswirkungen haben.

Zugrunde liegen meine Untersuchungen zur Feststellung der jeweiligen Auswirkung des Verlustes des Hörens, sowie des Verlustes der Tasthaare auf das visuelle System und das Sehen von adulten Mäusen.

Die dazu erforderliche Bestimmung der Sehleistung frei beweglicher Mäuse erfolgte mit dem „Visual-Water-Task“, einem geeigneten Verhaltenstest. Meine Versuche ergaben, dass sich die Sehleistung (räumliches Auflösungsvermögen und Kontrastsensitivität) der Mäuse innerhalb von ca. 12 Tagen nach Entfernung der Tasthaare gegenüber der unbehandelten Kontrollgruppe um bis zu 40% verbessert hat. Diese Ergebnisse konnten durch die bildgebende Methode des “Optical imaging of intrinsic signals“ bestätigt werden. Hierbei handelt es sich um ein minimal invasives, bildgebendes Verfahren, mit dem kortikale Aktivitäten in Folge periodischer Reizungen eines Sinnesorgans visualisiert und quantifiziert werden können. Es wurde bewiesen, dass der primäre visuelle Kortex kreuzmodal provozierten Veränderungen unterliegt. Die behandelten Tiere zeigten stärkere visuelle Aktivitäten im primären visuellen Kortex bei schwächeren visuellen Reizen, als die unbehandelte Kontrollgruppe. Nach Feststellung dieser Veränderungen, die wahrscheinlich auf einer Verstärkung des thalamo-kortikalen Inputs beruhen, war für mich die Frage naheliegend, ob die Deprivation des Hörens oder der Tasthaare auch die okulare Dominanzplastizität wiederherstellen kann, die bei unter Standardbedingungen gehalten adulten Tieren fehlt. Dazu habe ich das kontralateral zur untersuchten Hemisphäre liegende Auge der Tiere verschlossen (monokulare Deprivation) und zusätzlich die Tasthaare oder den Malleus entfernt. Nach sieben Tagen zeigten die Tiere eine Verschiebung in der okularen Dominanz hin zum offenen (ipsilateralen) Auge. Dieser Effekt ist abhängig vom NMDA-Rezeptor. Ein gezieltes Hemmen dieses Rezeptortyps durch die systemische Gabe von CPP, einem potenten NMDA-Rezeptor-Hemmer, verhinderte die beobachtete Verschiebung in der okularen Dominanz. Dies legt nahe, dass die Potenzierung der Amplituden des offenen Auges auf einem LTP-ähnlichen Effekt beruhen. Durch HPLC-Untersuchungen von V1-Gewebe wurde aufgezeigt, das der Gesamt-GABA-Gehalt gesenkt

war, was darauf schließen ließ, dass auch die Inhibition gesenkt ist. Ich versuchte, den niedrigen GABA-Gehalt durch die chronische Gabe von Diazepam, einem GABAA-Rezeptor-Agonisten, zu kompensieren, um die Verschiebung in der okularen Dominanz zu verhindern. Die Diazepam-Gabe verhinderte die Potenzierung der Amplituden des offenen Auges. Überraschenderweise zeigte sich aber weiterhin eine verschobene okulare Dominanz, bedingt durch eine Abschwächung der Amplituden des zuvor geschlossenen Auges. Dies ist untypisch für adulte Tiere, aber bekannt bei sehr jungen Tieren, denen man während der kritischen Phase ein Auge verschließt. Dieses Ergebnis lässt auf bisher unbekannte Plastizitätsmechanismen schließen.

Weiterhin stellte sich die Frage, was in den genannten sieben Tagen passiert. Ich begann mit Tieren, denen lediglich Tasthaare oder Malleus entfernt wurden, ohne das visuelle System durch eine monokulare Deprivation zu manipulieren. Es wurden Optical imaging experimente direkt nach der Deprivation durchgeführt, und dann nochmals drei und sieben Tage danach. An Tag drei zeigte sich eine Verschiebung in der okularen Dominanz, während sich die Werte an Tag sieben nicht von denen an Tag null unterschieden. Diese erstaunlichen Ergebnisse konnten durch elektrophysiologische Untersuchungen bestätigt werden. Es ließ sich aufzeigen, dass die EMPSC-Amplituden in Schicht IV des primären visuellen Kortexes an Tag drei nach Deprivation erhöht waren. Dieses lässt den Rückschluss zu, dass auch hier NMDA-Rezeptoren in den Effekt involviert sind. Um deren Beteiligung nachzuweisen, gab ich einer Gruppe von Tieren CPP, um den Rezeptor zu blockieren, was die zuvor beobachtete Verschiebung in der okularen Dominanz verhinderte.

Weiterhin konnte ich durch Verhaltensversuche nachweisen, dass sich der optokinetische Reflex, ein subkortikal gesteuerter Folgereflex, der erfolgt, wenn sich die Umwelt relativ zur Retina bewegt, der deprivierten Tiere verbesserte. Diese Verbesserung zeigte ihren Höhepunkt nach drei Tagen. Die Werte fielen dann bis Tag fünf wieder, persistierten jedoch bis zum Ende des Experiments oberhalb der Grenze unbehandelter Tiere. Der starke Anstieg, mit seinem Höhepunkt an Tag drei nach Deprivation blieb aus, wenn den Tieren der visuelle Kortex entfernt wurde. Dies legt nahe, dass der rasche Anstieg mit der verstärkten Amplitude in V1 an Tag drei nach Deprivation einhergeht, und somit k ortexabhängig ist. Ich konnte bei den entkortifizierten Tieren jedoch auch eine kortexunabhängige Verbesserung nachweisen, die in ihrer Stärke dem persistierenden Anstieg nach Tag fünf entsprach. Nach der Gabe von CPP blieben sämtliche Anstiege aus. Die derart behandelten Tiere unterschieden sich nicht von der unbehandelten Kontrollgruppe, was auch hier einen LTP-ähnlichen Mechanismus nachweist, der für die Verbesserungen nach Deprivation verantwortlich ist.

Zukünftige Studien werden dazu beitragen, die zugrunde liegenden Mechanismen der kreuzmodalen Interaktionen besser zu verstehen.

8. References

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9. Declaration of authorship

With this declaration I confirm:

- that I am familiar with the relevant course of examination for doctoral candidates (Promotionsordnung, Fassung vom 23.09.2019)
- that I have composed and written this dissertation myself
- that I have not used any sections of text from a third party or from dissertations of my own without identifying them as such, and I have acknowledged all sources within the work
- that I have not enlisted the assistance of a doctoral consultant and that no third parties have received either direct or indirect monetary benefits from me for work connected to the present thesis
- that I have not already submitted the dissertation as an examination paper for a state or other scientific examination
- that I have not submitted the same, a substantially similar, or a different dissertation to another postsecondary school.

Jena,

10. Danksagung

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11. Curriculum vitae

Persönliche Daten:

Vor- und Zuname: Marcel Isstas

Adresse: Camsdorfer Strasse 6, 07749 Jena

Geburtsdatum /-ort: 29.07.1978 in Bendorf a.R.

Familienstand: ledig

Kinder: Ole Isstas, 1.12.2008

Fiete-Onno Isstas, 06.03.2011

Thore Krüger. 20.04.2016

Schulbildung:

1985-1989:	Grundschule Staakenweg, Oldenburg
1989-1991:	Orientierungsstufe Eversten, Oldenburg
1991-1996:	Realschule Brüderstrasse, Oldenburg
1997-2000:	BBSII, Oldenburg, Erwerb der allg. Hochschulreife

Ausbildung:

15.09.00-1.10.03:	Studium (Biologie und Sozialwissenschaften) an der Universität Oldenburg ohne Abschluss
15.10.06-30.9.14	Studium Biologie an der FSU Jena

Hauptfach: Zoologie mit Schwerpunkt Neurobiologie

1. **Nebenfach:** Humananatomie
2. **Nebenfach:** evolutionäre Anthropologie

Weitere Berufstätigkeit:

01.02.02-01.08.02:	Angestellt bei Agravis Mischfutter GmbH, Oldenburg
01.07.02-01.01.03:	Bauhelfer/Verkäufer und Schausteller auf Mittelaltermärkten
01.01.03-01.04.03:	Auslandsaufenhalt

01.04.03-01.01.04: Bauhelfer/Verkäufer und Schausteller auf Mittelaltermärkten

01.01.04-26.06.05: Selbstständigkeit im Messebau

26.06.05-01.06.06: Angestellt bei Personaldienstleister W.I.R.

01.06.06-01.10.06: Übernahme durch die Glasverarbeitende Gesellschaft Bremen

01.10.14-01.02.15: Interner Mitarbeiter bei Personal Office

15.10.06-05.03.15: Studentische Hilfskraft am MPI für chem. Ökologie und Biogeochemie sowie am Institut für allg. Zoologie und Tierphysiologie

15.02.16-28.02.19 Wissenschaftlicher Mitarbeiter am Institut für allg. Zoologie und Tierphysiologie (Doktorand)

Seit 01.08.2019: Lehrkraft im Thüringer Schulwesen

Memberships:

- Neurowissenschaftliche Gesellschaft seit 2013
- Verband Biologie, Biowissenschaften und Biomedizin Deutschland (VBio) seit 2019

12. List of publications

12.1 Peer-reviewed papers:

Balog J., Hintz F., **Isstas M**., Teichert M., Winter C., Lehmann K. ***Social hierarchy regulates ocular dominance plasticity in adult male mice.*** Brain structure & function. 2019 Sept; https://doi.org/10.1007/s00429-019-01959-w

Teichert M[1], **Isstas M[1]**, Liebmann L, Huebner CA, Wieske F, Winter C, Bolz J ***Visual deprivation independent shift of ocular dominance induced by cross-modal plasticity.*** PlosOne. 2019 Mar; 14(3): e0213616

Teichert M[1], **Isstas M[1]**, Wieske F, Winter C, Bolz J. ***Cross-modal Restoration of Juvenile-like Ocular Dominance Plasticity after Increasing GABAergic Inhibition***. Neuroscience. 2018 Oct 6;393:1-11. PubMed PMID: 30300702.

Teichert M[1], **Isstas M[1]**, Zhang Y, Bolz J. ***Cross-modal restoration of ocular dominance plasticity in adult mice.*** The European journal of neuroscience. 2018 Jun;47(11):1375-84. PubMed PMID: 29761580.

Teichert M, **Isstas M**, Wenig S, Setz C, Lehmann K, Bolz J. ***Cross-modal refinement of visual performance after brief somatosensory deprivation in adult mice.*** The European journal of neuroscience. 2018 Jan;47(2):184-91. PubMed PMID: 29247462

Isstas M, Teichert M, Bolz J, Lehmann K (2017) ***Embryonic interneurons from the medial, but not the caudal ganglionic eminence trigger ocular dominance plasticity in adult mice***. Brain structure & function 222:539-547.

12.2 List of posters:

Lehmann K, **Isstas M**, Teichert M, Knöllker V, Bolz J (2014) Cells from the medial, but not the caudal ganglionic eminence induce ocular dominance plasticity in adult mice. FENS-meeting, Mailand

Isstas M, Teichert M, Bolz J, Lehmann K (2013) Transplantation of neurons from the embryonic ganglionic eminences into the matured cortex. NWG-meeting; Göttingen

Teichert M, **Isstas M**, Döding A, Lehmann K, Bolz J (2012) Fibroblasts display neuron-like features after transplantation into the adult mouse cortex. SFN-meeting, New Orleans

12.3 Papers in preparation:

Isstas M, Teichert M, Zhang Y, Apte S, Bolz J ***Whisker manipulation cross-modally affects visually evoked neuronal activity of primary visual cortex in adult mice.***

Marcel Isstas
Camsdorfer Straße 6
07749 Jena
marcel.isstas@gmx.de
015757520934

Dekanat der Fakultät für Biowissenschaften
Der Friedrich-Schiller-Universität Jena
Bachstraße 18k
07743 Jena

Jena, den 13.5.1021

Betreff: Korrektur zur Dissertationsschrift *Cross-modal changes in primary visual cortex induced by somatosensory manipulation* eingereicht von Marcel Isstas (Promotionsverfahren seit dem 19.4.2021 eröffnet).

Sehr geehrte Damen und Herren,

im Folgenden bitte um die Korrektur und Richtigstellung folgender Punkte in meiner Dissertationsschrift (Titel: *Cross-modal changes in primary visual cortex induced by somatosensory manipulation*).

In meiner oben genannten Schrift wurde für folgende Veröffentlichung (Manuskript 1: „*Cross-modal refinement of visual performance after brief somatosensory deprivation in adult mice*“) ein Eigenanteil von 30% am „Study Design“ angegeben. Von diesem Punkt möchte ich zurücktreten, da ich in den „Contributions“ der genannten Veröffentlichung namentlich nicht genannt bin.

Ich bitte dies zu entschuldigen.

Weiterhin findet sich in meiner Schrift bei den Angaben zu den Eigenanteilen für folgende Veröffentlichungen („Manuskript 2: *Cross-modal restoration of ocular dominance plasticity in adult mice*“ und Manuskript 3 „*Cross-modal Restoration of Juvenile-like Ocular Dominance Plasticity after Increasing GABAergic Inhibition*“) unter dem Punkt „Preparation of the manuscript“ ein Eigenanteil von 20% bzw. 30%. In den „Contributions“ der jeweiligen Veröffentlichungen bin ich jedoch unter Punkt „Wrote the paper“ nicht namentlich genannt.

Hier oblag das Schreiben der Veröffentlichungen Herrn Prof. Dr. Jürgen Bolz und Dr. Manuel Teichert. Ich hatte lediglich einen Anteil am Editing-, Proofreading und Review-Prozess. Ich bitte, um Kenntnisnahme und bitte die unpräzise Aussage und Angabe in meiner Schrift zu entschuldigen.

Ich hoffe, dass die Korrektur und die Stellungnahme für die Kommission akzeptabel sind, und ich bitte nochmals um Entschuldigung für diese Diskrepanzen.

Mit freundlichen Grüßen