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Developmental function of PirB restricts adult ocular dominance plasticity

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1 Summary

Early visual input induces changes in functional connectivity which can either lead to the stabilisation of appropriate synaptic connections or the elimination of inappropriate ones in the visual system. Monocular deprivation (MD) is a widely used paradigm to study changes in ocular dominance (OD) in the binocular visual cortex of higher mammals. Closure of one eye for several days leads to a shift in OD which reflects changes in the response kinetics of the deprived and the non-deprived eye. The molecular machinery which underlies this type of experience-dependent plasticity is still elusive. A recent genetic screen in the lab of Carla Shatz has identified that the family of MHCI receptors are expressed in the developing visual cortex and regulated upon neuronal activity. They hypothesized that MHC receptors might be required for consolidation of longlasting changes in synaptic strength. To investigate the role of MHCI in OD plasticity, I used a transgenic mouse lacking the MHCI receptor paired-immunoglobulin-like receptor B (PirB). To determine OD in the mouse visual cortex, I used optical imaging of intrinsic signals which measures the activity of neuronal populations elicited from either eye stimulation.

Beforehand I investigated OD plasticity in adult mice (C57Bl6) which is still questioned to be present after MD. I confirmed earlier findings which have shown robust MD induced changes of either eye in the visual cortex of adult mice.

In the next chapter I explored eye specific kinetics during the critical period (postnatal days (P)19-32) in PirB KO mice. Closed eye depression occurred more rapidly and was stronger in KO mice in comparison to WT mice. I was also interested whether the mechanisms of OD plasticity in adult PirB KO (P90) mice differed from that juvenile PirB KO mice. Interestingly I observed a tendency for similar eye specific kinetics in adult PirB KO mice and in juvenile WT mice, which lead to the speculation that removal of PirB might reinduce juvenile like plasticity in adult mice. A recent study in the lab investigated the effect of

prior experience and could show that OD plasticity in adult mice was enhanced due to an inital MD in juvenile mice and a subsequent MD of the same eye in adulthood. Would PirB play a role in this type of enhanced plasticity? Surprisingly I explored that OD plasticity in PirB KO mice is the same after a single or repeated exposure to MD, suggesting that the capacity for plasticity in these mice is near saturation. In the last chapter I addressed the question whether the representation of both eyes in the binocular visual cortex is different in PirB KO mice in comparison to WT mice. Therefore I showed stimuli in the central and peripheral visual field of adult non-deprived and deprived PirB KO mice. I found enhanced response strength in the open eye after peripheral visual field stimulation in deprived PirB KO mice in contrast to WT mice.

Overall I assessed stronger and more rapid functional plasticity in PirB KO mice during development and adulthood. Hence I postulate that PirB might act as a molecular brake limiting OD plasticity.

2 Introduction

2.1 The mouse visual system

Although mice are nocturnal animals with a low visual acuity of 0.5 cycles/degree (Prusky et al. 2004, Gianfranceschi et al. 1999) in comparison to higher mammals such as cats (about six cycles/degree, (Ikeda 1979)), studying the mouse visual system, and in particular its development and plasticity, has become attractive as mice are easily amenable to genetic modifications. Transgenic mice lacking or overexpressing distinct proteins can give insights into the role of these factors during the development and plasticity of the visual system. The organization of the mouse visual system is basically similar to that of higher mammals (Fig. 2.1).

The first processing steps are carried out by the various cell types in the retina. Retinal ganglion cells project mainly to two major nuclei, the lateral geniculate nucleus (LGN) and the superior colliculus (SC). While the main function of the SC is the control of eye movements, the LGN is the relay station to the visual cortex. The vast majority (about 95%) of retinogeniculate fibers cross at the optic chiasm from the retina to the LGN, such that the LGN receives mostly contralateral eye input, with only a small region of the LGN receiving input from the so-called temporal crescent in the nasal retina of the ipsilateral eye (Dräger 1975, Wagor et al. 1980). These separate, eye specific regions of the LGN convey visual input directly to the monocular and binocular part of the primary visual cortex. The primary visual cortex is located in the posterior pole of the occipital lobe; its main function is the processing of form and movement information. About one third of primary visual cortex comprises the binocular region, localized laterally in the primary visual cortex, and receiving inputs from both eyes. This is the first station of the visual pathway where single neurons can be excited by both eyes (Dräger 1975, Wagor et al. 1980, Gordon and Stryker 1996). In

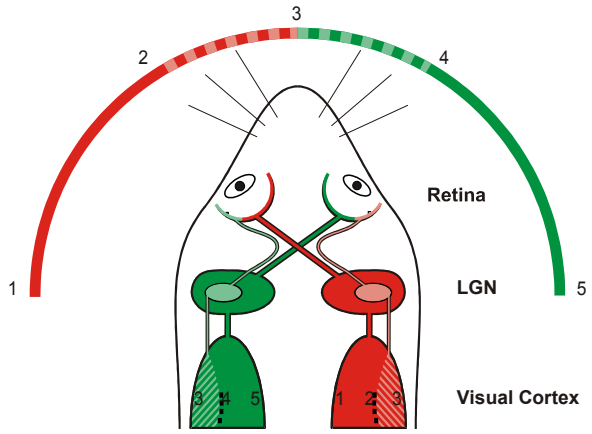

Figure 2.1: Scheme of the mouse visual system. Each side of the visual field and the corresponding visual pathways are coloured in red and green. In the binocular visual field, light hits the temporal retinas of each eye (in light green and red). Visual information is processed from the temporal retina to the ipsilateral region of each LGN. Further visual input from both LGN converge onto the binocular visual cortex (striped region). The retinotopic organisation in the visual field and the visual cortex is outlined (see digits). The center of the visual field (digit '3') is represented in both hemispheres of the binocular visual cortex. From Hübener et al., 2003.

higher mammals, cortical cells primarily driven by one eye or the other are clustered into so called ocular dominance (OD) columns (Hubel and Wiesel 1977, LeVay et al. 1978).
Since traditionally many studies on visual system development and plasticity have been carried out in higher mammals, I will briefly point out the main differences in the organization of the visual cortex between mice and higher mammals. In contrast to mice, the visual cortex of most carnivores and primates is organized into columns, spanning the six cortical layers. Cells within a column share similar preferences for certain visual stimulus parameters (Hubel and Wiesel 1977). For instance, OD columns are independently innervated by fibers either from the ipsi- or contralateral eye. In contrast, the binocular region of rodent visual cortex lacks a columnar organization for OD (Métin et al. 1988, Schuett et al. 2002), though there is a very weak clustering of cells dominated by the same eye (Mrsic-Flogel et al. 2007). Binocular cells respond differentially to stimulation of both eyes: Some cells are equally responsive to the two eyes, but others respond more strongly to one eye or the other (Dräger

1975, Gordon and Stryker 1996, Antonini et al. 1999). The afferents from the temporal retina do not cross the optic chiasma and project only into the binocular zone. Most of the cells in mouse binocular visual cortex are stronger responsive to the contralateral eye.
Similarly, rodents lack orientation columns (Ohki et al. 2005), though the majority of cells in the primary visual cortex of mice respond selectively to the orientation of visual stimuli such as edges or bars (Dräger 1975, Mangini and Pearlman 1980, Hübener 2003, Niell and Stryker 2008). The average radius of the receptive field of single neurons is larger in the primary visual cortex of mice (in the range of five to seven degrees in layer 2/3, (Niell and Stryker 2008)) in comparison to cats (two degrees, (Hubel and Wiesel 1963)) and monkeys (in the range of one degree, (Dräger 1975, Mangini and Pearlman 1980, Métin et al. 1988)). As many other sensory systems, the mouse visual system is organized in a topographic manner, such that neighboring stimuli in the visual field excite neighboring neurons at different levels of the visual pathway (Fig. 2.1).

2.1.1 Visual system wiring and plasticity

As in many other parts of the brain, the development of the visual system can be divided into two principle phases. Initially, specific molecular cues guide outgrowing fibers to their target structures. In a second step, activity dependent remodeling then leads to the final circuitry. The role of molecular guidance cues has probably been demonstrated best for the formation of the retinotopic map in the SC. In 1963, Sperry proposed his chemoaffinity hypothesis, stating that map formation occurs through a concentration gradient of molecular signals, which attract or repel outgrowing axons, which themselves express certain receptors in a graded fashion (Sperry 1963). In the case of the retino-collicular projection , the naso-temporal axis of the retina is mapped onto the anterior-posterior axis of the SC, and the dorso-ventral axis of the retina is mapped onto the medio-lateral axis of the SC. Mapping along these axes occurs through the expression of two families of receptor tyrosine kinases, EphA and EphB, and their ephrin ligands. Members of the EphB family are expressed along the dorso-ventral axis in the retina and their ephrin-B binding partners in the SC form a gradient from medial to lateral. It was shown that transgenic mice, lacking EphB2 and EphB3, display an impaired mapping of the mediolateral axis of the SC (Hindges et al. 2002).

EphAs are expressed along the nasal-temporal axis of the retina and a ephrin-A gradient exists along the posterior-anterior axis of the SC. Double KO mice, lacking ephrin-A2 and ephrin-A5 display mapping abnormalities along the antero-posterior axis (Feldheim et al. 2000). Thus, EphA and EphB molecules repel retinocollicular afferents to control the initial SC mapping.

In the second phase of visual system development, after coarse connections have been formed, the level and pattern of electrical activity determines the further refinement of connections. A well studied example is the segregation of retinal afferents into eye specific layers in the LGN (Sretavan and Shatz 1986). Initially, projections from each eye are distributed over the entire LGN. Later during development, eye-specific layers are then formed through the retraction of one eye's retinogeniculate fibers from the territory of the other. The segregation into eye specific layers is activity-dependent (Shatz and Stryker 1988). It was shown that prenatal, intracranial tetrodotoxin (TTX) infusion which blocks retinal activity, prevented the segregation of retinogeniculate afferents. Initially it was assumed that visually evoked activity drives these processes. An elaborate study by Meister et al. (1991) showed that it is in fact spontaneous activity which lead to the refinement of connections in the retina. Using multielectrode arrays, they found that spontaneous activity is highly correlated in neighboring retinal ganglion cells (RGCs) and that this spontaneous activity spreads across the retina in a wave-like manner. This retinal activity is relayed via the optical nerve into the LGN. According to Hebb's postulate that synapses strengthen when the pre and postsynaptic cell are synchronously active (Hebb 1949), retinogeniculate fibers which receive input from either eye, innervate neighboring cells in the LGN. The induction of eye specific regions in the LGN is thought to depend on the fact that retinal waves are generated independently in each eye, thus leading to correlated activity within each retina, and decorrelated activity between the eyes (Sretavan and Shatz 1986, Hebb 1949). Synchronization of ganglion cell firing occurs through cholinergic amacrine cells (Feller et al. 1996). Blockage of nicotinergic acetylcholine receptors (nACHR) abolished correlated activity between neighboring RGCs, and as a consequence, retinogeniculate afferents fail to segregate into eye specific regions (Rossi et al. 2001).

Taken together, the wiring of the visual system depends on an interaction between molecular cues and activity-dependent mechanisms, which lead to the maturation of neuronal circuits.

During and after the second, activity dependent phase of development, neuronal circuits become susceptible to alterations of the sensory input. The next chapter will focus on specific forms of experience-dependent plasticity and the resulting changes that occur in the mouse visual cortex during development.

2.2 Ocular dominance plasticity during visual development

In 1963, Wiesel and Hubel were the first to describe that alteration of vision during development leads to changes in ocular dominance of single cells in the visual cortex (Wiesel and Hubel 1963). Later, Shatz and Stryker showed that plasticity was also present at the level of OD columns (Shatz and Stryker 1978). Using the paradigm of monocular deprivation (MD), already used by Wiesel and Hubel, their study demonstrated that the patches of geniculocortical afferents serving the deprived eye were smaller in comparison to naïve animals. In contrast, patches of the fibers serving the non-deprived became larger. This physiological change induced by MD is referred to as OD shift and since the early studies of Hubel and Wiesel, assessing changes in OD has been widely investigated in different mammals (Mower 1991, Gordon and Stryker 1996, Galuske et al. 1996, Hofer et al. 2006).

In mice, OD columns are not present, but OD shifts at the level of individual neurons can nonetheless be induced by MD: To this end, one eye is sutured shut, typically for several days, resulting in strongly decreased visual input in the deprived eye. Immediately following reopening of the closed eye, OD shifts can then be assessed with different techniques. One of the first studies in juvenile mice used single cell recordings (Gordon and Stryker 1996) and observed a decrease in the number of cells responsive to deprived eye stimualtion. OD shifts were also detected by optical imaging (Hofer et al. 2006) and visual evoked potential (Sawtell et al. 2003) which measure changes in the response amplitude after MD. Basically, all these techniques show that responses to deprived eye stimulation decrease, while open eye responses increase after MD of juvenile mice.

Such shifts in OD are most readily induced during a specific developmental period, the so-called "critical period". In general, critical periods are known as phases during which specific parts of the brain are highly susceptible to alterations in sensory input. In higher mammals, OD shifts were in fact almost only observed during their respective critical pe-

riods. In mice, the critical period for OD plasticity lasts from postnatal day (P)19 until P32. Which factors control the timing of the critical period in this system? It has been shown that neuronal activity as well as molecular cues are important (Gordon and Stryker 1996, Hensch et al. 1998). The onset of the critical period is directly linked to the level of cortical inhibition, and depends crucially on the maturation of fast spiking basket cells (Hensch et al. 1998). It was demonstrated that both, the onset of the critical period and the development of inhibitory circuits could be delayed by rearing animals in the dark (Mower 1991). A key finding by Huang et al. (1999) was that the start of the critical period can be preponed in mice overexpressing brain-derived neurotrophic factor (BDNF). The authors could show that this effect was caused by an advanced maturation of cortical inhibition in BDNF overexpressing mice. Thus, the level of inhibition might be important for initiating the critical period. To proof this directly, Hensch and colleagues (1998) created a transgenic mouse lacking glutamic acid decarboxylase 65 (GAD65), the enzyme synthesizing the inhibitory transmitter gamma-aminobutyric acid (GABA). These GAD65 KO mice displayed a reduction in basal inhibition due to reduced GABA release. Investigations of OD plasticity revealed that the OD shift was absent after four days of MD between P28 and P32, while age matched WT mice showed a shift. This indicates that the critical period is impaired in GAD65 KO mice as a consequence of disrupted cortical inhibition.

Furthermore, the Hensch group showed that OD shifts can be reinduced in GAD65 KO mice through intracranial injection of the GABA agonist Diazepam. In line with these experiments it was also shown that Diazepam induced a premature critical period in WT mice, which were younger than P19, and normally display no shift in OD after MD. Thus, enhanced levels of inhibition accelerated the induction of the critical period (Hensch et al. 1998, Fagiolini and Hensch 2000). In summary it seems that a specific level of intracortical inhibition must be reached to trigger the start of the critical period. Closure of the critical period is initiated through maturation of intracortical inhibition which adjusts the balance between excitation and inhibition (Fagiolini et al. 2004, Morishita and Hensch 2008). In the subsequent chapter I will describe several mechanisms serving OD plasticity during the critical period, but also beyond this phase in adulthood.

2.2.1 Concepts of ocular dominance plasticity

In the field of OD plasticity, there is an ongoing discussion about the underlying mechanisms. It is postulated that OD plasticity during development is susceptible to changes in visual input leading either to strengthening or weakening of existing synapses which is often followed by structural rearrangements. In the following sections, I will introduce two principal mechanisms which play a role in OD plasticity.

2.2.1.1 Competition based theory

One class of mechanisms thought to be involved in OD plasticity is the competition based theory. In essence, this theory states that inputs from the two eyes compete for synaptic space on postsynaptic neurons in the visual cortex. The competition is driven by the level and pattern of neuronal activity in the inputs and their postsynaptic partners. Hebb was the first who postulated that a change in synapse strength occurs when the presynaptic neuron contributes to activate the postsynaptic neuron (Hebb 1949). Hebb's hypothesis was strengthened by the discovery of long-term potentiation (LTP) in the hippocampus (Bliss and Lomo 1973). During LTP induction, pre- and postsynaptic neurons are concurrently active above a certain threshold, leading to an enhancement of synaptic transmission. Long-term depression (LTD) is the opposite effect and was initially induced in the hippocampus through persistent low frequency stimulation (Lynch et al. 1977). This leads to a decrease and decorrelation of presynaptic input to the postsynaptic neuron which in turn induces a weakening in synapse strength. In the visual cortex it was shown that after MD the response of the deprived eye was weakened through an LTD-like mechanism (Heynen et al. 2003). It is known that induction of LTD leads to the dephosphorylation of a certain aminoacid (serin 845) and the internalization of AMPA receptors. Indeed Heynen et al. (2003) observed this molecular fingerprint after MD in the visual cortex of rats.

The visual input through the non-deprived eye is higher than in the deprived eye which, according to Bear and colleagues, leads to the induction of LTP, i.e. a strengthening of synapses from the non-deprived eye. As one component of LTP is located on the presynaptic side, it was suggested that a retrograde messenger transfers information from the post- to the presynapse. Potential candidates, which could serve as signaling factors, are members

of the family of neurotrophins (NT), such as BDNF. It is important to note that the role of NTs in OD plasticity is confusing, as the effects of various NTs seems to differ for very similar experimental approaches. And it is known that NTs are released from the postsynaptic cell in an activity-dependent way. The amount of NT is restricted in postsynaptic neurons. This might lead to presynaptic competition for NT supply in the visual cortex (Maffei et al. 1992, Thoenen 1995). It was observed that MD, by causing decreased activity in the closed eye, led to the downregulation of NTs in the rat binocular visual cortex. The OD shift normally following MD could be prevented by applying the NT nerve growth factor (NGF). These results were taken to indicate that a suficient supply of NGF in the visual cortex saves deprived eye inputs from being weakened after MD. But it is questionable whether NGF is a suitable NT candidate, as the density of NGF tyrosine kinase receptors (TrkA) is very low in the visual cortex (Bonhoeffer 1996, Maffei et al. 1992). It has been speculated that the high concentrations of NGF in these experiments might have led to cross activation of other NT receptors, such as the BDNF receptor TrkB.

Several labs have focused on the finding, that the decreased levels of NTs following MD are associated with structural changes like shrinkage of axonal arbors of LGN afferents as well as reduced cell body sizes of thalamocortical cells in the LGN (Riddle et al. 1995, Bonhoeffer 1996, Thoenen 1995). Riddle and Katz (1995) showed that the shrinkage of LGN cell bodies after MD could be prevented by injection of neurotrophin 4 (NT4) into the visual cortex.

Surprisingly, intraventricular injection of BDNF had a paradoxical effect in that it led to a *strengthening* of the deprived eye after short-term MD in kittens (Galuske et al. 1996). Galuske et al. interpreted this finding through an increase in cortical inhibition. It is known that GABAergic neurons express high levels of the TrkB receptor and modification of inhibitory circuits via BDNF might lead to the paradoxical OD shifts (Widmer and Hefti 1994). Another recent study focused on a new role of the BDNF receptor TrkB (Kaneko et al. 2008). The study revealed that the receptor is mainly involved in the recovery of deprived eye function after eye reopening. TrkB was inhibited, which prevented the recovery of the initially deprived eye. This is in fact an argument against the competition based theory and favors the view that TrkB signaling is involved in the increase in functional response or even in the induction of new connections. Despite these confusing findings, the idea that geniculocortical afferents from the deprived and non-deprived eye compete for a limited

supply of NTs, which are released from postsynaptic neurons and lead to strengthening of synapses with correlated input, is still attractive.

The first studies which illustrated that correlated activity leads to synaptic strengthening have used single unit recordings. It was shown that following MD more cortical cells respond to stimulation of the non-deprived than the deprived eye (Wiesel and Hubel 1963, Blakemore et al. 1978). An important hint pointing to competition between each eye's inputs comes from studies with binocular deprivation (BD). Wiesel and Hubel (1965) demonstrated that after BD most cells remained responsive to visual stimulation of both eyes. This result supports the competition based theory, since the activity levels of both eyes' thalamocortical afferents are equally low, and thus the same amount of retrograde factor is provided to each set of fibers.
It was reported that complete recovery from MD can be induced after eight days in mice (Hofer et al. 2006). The effect of visual recovery is difficult to explain by the competition based theory. However, results from reverse lid suture experiments showed that visual recovery is enhanced in comparison to animals with normal binocular experience after MD. In the reverse suture experiment, the non-deprived eye is sutured shut and the initially deprived eye remains open. Here the competition model predicts that the initial deprived eye is strengthened as the activity level of the newly deprived eye is lower. LTD like changes were also observed in the monocular cortex following brief MD (Heynen et al. 2003). However, a study by Reiter and Stryker (1988) demonstrated a shift in OD despite activity blockade in postsynaptic cortical cells. Pharmacological silencing of the visual cortex in combination with MD led to an OD shift towards the *deprived eye*, akin to the above described results of Galuske et al. (2000). Thus, hebbian mechanisms alone might not be able to explain all aspects of OD plasticity.

2.2.1.2 Homeostatic plasticity

An alternative (or additional) mechanism which does not rely on competition between synapses for a hypothetical trophic factor is *homeostatic plasticity*. Here, overall synaptic strength depends on a neuron's activity level. The more a cell spikes, the weaker will its

inputs be, and vice versa, a phenomenon called synaptic scaling (Turrigiano 1999, Turrigiano and Nelson 2004). In addition, the excitability of a neuron might also change depending on its past activity levels . In general, homeostatic plasticity has been invoked as a mechanism preventing excessive, and potentially harmful neuronal activity under conditions of too many strengthened synapses. This phenomenon was first investigated in culture, where the activity was globally manipulated (Turrigiano et al. 1998). In this study, the firing rate of interconnected excitatory pyramidal neurons and inhibitory interneurons was raised by adding the GABA antagonist bicuculline. After two days of bicuculline treatment, the overall firing rate was readjusted to the basal firing rate. This indicates that before adding the drug, homeostatic mechanisms alter synaptic properties to compensate for changes in overall activity levels. With regard to the visual system it was demonstrated that excitatory synapses onto pyramidal cells in the visual cortex were scaled up after mice were reared in the dark (Desai et al. 2002).

A specific variant of homeostatic plasticity hypotheses is the Bienenstock-Cooper-Munro model (BCM, (Bienenstock et al. 1982)) which postulates that synapses can be bi-directionally modified (via LTP or LTD) depending on ongoing neuronal activity (or stimulation frequency). The synaptic change depends on the input intensity which can either induce potentiation or depression. A critical value termed "the modification threshold" determines the sign of the postsynaptic change. Above threshold, high calcium influx through the N-methyl-D-aspartate receptor (NMDAR) leads to LTP. In case of a low threshold moderate calcium influx induces LTD. Potentiation of synapses occurs when the presynaptic activity leads to a postsynaptic response which exceeds the modification threshold. On the other hand synapses are depressed when the response of the postsynaptic neuron is below this critical value.

Importantly, the modification threshold is not a fixed value but it is sliding depending on the history of neuronal activity: the higher the past activity, the higher the threshold. Initially strengthened synapses can be weakened more easily and further strengthening is more difficult. Thus, this sliding plasticity threshold leads to facilitation of LTP and LTD depending on recent neural activity. According to BCM theory, the shift in OD after MD takes place because the activity of the deprived eye inputs is below the modification threshold , which leads to LTD between these inputs and the postsynaptic cells in the cortex. A study sup-

ported this hypothesis by comparing the physiological changes after MD which lowers retinal activity with those of intraocular TTX injections which abolishes retinal activity (Rittenhouse et al. 1999). The shift in OD was significantly greater after lid suture than after TTX injections, showing that a low level of activity is crucial to induce LTD and shifts in OD.
The latter finding corresponds to an anatomical study in mouse visual cortex, which showed that after short periods of MD thalamocortical arbors from the deprived eye were significantly decreased in size in comparison to normal animals (Antonini and Stryker 1993). No expansion in non-deprived eye arbors was observed after short-term MD, indicating that in accordance with the BCM model the OD shift is initially induced by LTD of deprived eye inputs.
Cortical responses are not impaired after binocular deprivation which can be also explained with the BCM model. The induction threshold for LTP is reduced due to lowered postsynaptic activity levels, which in turn leads to a lowered probability for LTD induction. The BCM model can also explain the effects resulting from recovery after MD. A behavioral study focused on the recovery of visual function after MD. Full recovery of the deprived eye was accomplished after a brief period of binocular vision (Mitchell and Gingras 1998). An explanation could be that the activity of the initially deprived eye is now correlated again with the activity pattern of the non-deprived eye, leading to an increase in absolute activity and in modification threshold . Additionally it was shown that the responsiveness of monocular neurons driven by the deprived eye was enhanced (Mrsic-Flogel et al. 2007). This finding cannot be explained with the BMC model which would predict that the response decrease during the duration of MD (Clothiaux et al. 1991, Blais et al. 1999).
In recent years a couple of studies have postulated that not a single mechanism is involved in the induction of OD plasticity after MD but that the concerted action of homosynaptic and hebbian rules shapes the visual system (Desai et al. 2002, Turrigiano and Nelson 2004, Mrsic-Flogel et al. 2007).

I have discussed the various physiological mechanisms leading to OD plasticity. But I have not yet addressed, that the timing of certain events is relevant for inducing synaptic modifications. There is evidence that the repeated pairing of pre and postsynaptic activity can lead to long-term changes in synaptic strength (Markram et al. 1997, Bi and Poo 1998,

Zhang et al. 1998). Spike-timing dependent plasticity (STDP) is based on the timing of action potentials from the pre- and the postsynaptic cell which leads to synaptic modifications. The synapse becomes potentiated when the presynaptic input repeatedly precedes the postsynaptic input on a millisecond timescale. In contrast LTD of synapses occurs if presynaptic spikes follow postsynaptic spikes within a time period which is longer than the LTP window. STDP can also induces changes in neuronal response in vivo in the visual cortex (Schuett et al. 2001, Meliza and Dan 2006).

Until recently it was believed that MD is only affected by molecular cues which are derived from the cortical level. Lately it was shown in rats that MD decreases the amounts of retinal BDNF in the deprived eye retina (Mandolesi et al. 2005) and that OD shifts can be prevented by either injecting BDNF into the deprived eye or by lowering endogenous BDNF expression in the open eye through injection of antisense oligonucleotides. Thus it was shown for the first time that retinal BDNF modulates OD plasticity. The embryonic homeodomain protein Otx2 was also identified to play a role in OD plasticity although it is expressed along the visual patchway, icluding retina and LGN (Sugiyama et al. 2008). In the visual cortex Otx2 is transported and taken up via parvalbumin positive cells. Otx2 loss of function via antibody infusion or inhibiting the protein synthesis via siRNA injection into the eye prevented critical period plasticity after MD. Overexpression of Otx2, via cortical infusion into the visual cortex, pushed the development of parvalbumin cell development and the timecourse of the critical period. This observation of an earlier onset of critical period through accelerated maturation of the inhibitory circuit was also made in BDNF overexpressing mice (Huang et al. 1999).With regard to recent screens for OD plasticity genes (Majdan and Shatz 2006) it might be worth to extend the search for *plasticity factors along the visual pathway.*

2.2.1.3 Activity dependent structural rearrangements in the mouse visual cortex

In this section, I will briefly focus on structural changes occurring after MD. Short-term MD during the critical period leads initially to the weakening of deprived eye responses and then to the strengthening of open eye responses (Antonini et al. 1999, Gordon and Stryker 1996,

Bear 2003). These rapid changes in functional responsiveness are followed by the anatomical reorganization of geniculocortical afferents in the visual cortex after several weeks, as shown by transneuronal labeling and axon arbor reconstruction (Antonini et al. 1999). This study revealed that axonal arbors in the normal binocular visual cortex continue to expand beyond the critical period. After long-term MD (20 days) non-deprived eye fibers were significantly larger in comparison to those in normally reared animals. MD for 40 days lead to a significant growth arrest of deprived eye arbors.

Much more rapid changes in structure have been found at the subcellular level. Inducing four days of MD during the critical period revealed that the loss of functional responses coincided with a loss of spines, dendritic protrusions on dendrites of excitatory pyramidal neurons (Mataga et al. 2004). They could distinguish between deprived eye and open eye connections as the change in spines occurs earlier (after two days) in deprived eye fibers. Serine proteases, such as tissue plasminogen activator (tPA), might be potential candidates linking neuronal activity and structural changes. It was shown that tPA KO mice display no functional decrease in responsiveness of the deprived eye after short- and long-term MD during the critical period (Mataga et al. 2002). Through degradation of extracellular matrix and cell adhesion molecules, tPA could have a permissive function which leads to synapse destabilization and spine elimination after sensory deprivation (Mataga et al. 2004).

2.3 Ocular dominance plasticity in adulthood

In recent years several studies have shown that OD plasticity in mice is not restricted to a critical period but persists into adulthood (Sawtell et al. 2003, Pham et al. 2004, Hofer et al. 2006). OD shifts in adult mice are induced after MD and were confirmed using different techniques such as intrinsic optical imaging (Hofer et al. 2006, Lehmann and Löwel 2008), visually evoked potentials (VEP) (Sawtell et al. 2003) and single-unit recordings (Hofer et al. 2006, Fischer et al. 2007) (Fig. 2.2).

Thus, it is possible to induce OD shifts in adult mice, although MD has to be somewhat longer (Pham et al. 2004). Despite clear indications for adult OD plasticity, the discussion on the extent and manifestation of adult OD plasticity in mice is ongoing. There are hints that some studies were not able to detect OD shifts because of the use of barbiturate anesthesia.

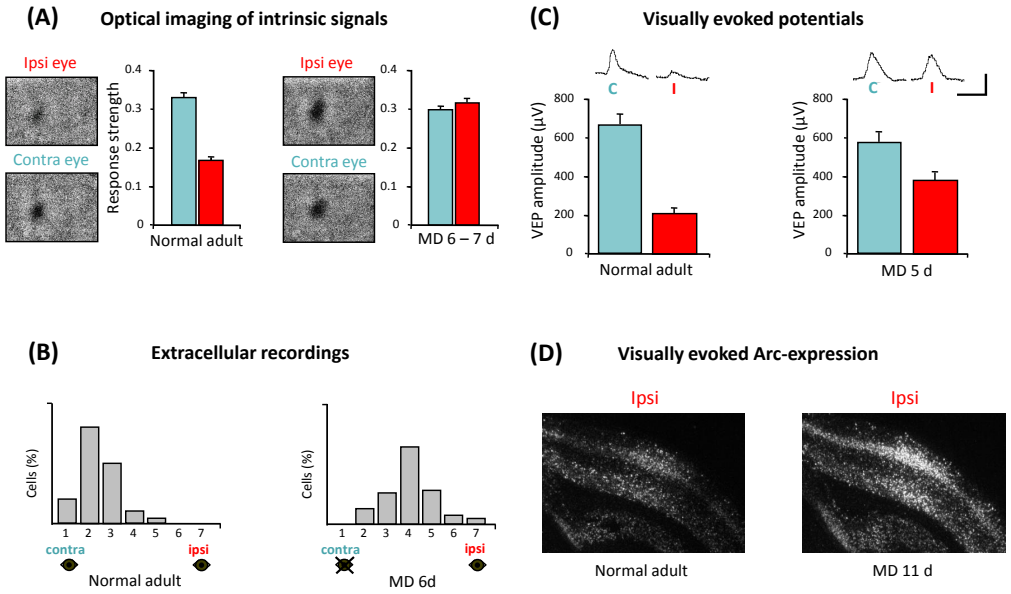

Figure 2.2: OD plasticity in adult mouse is monitored by different techniques. A) Intrinsic optical imaging data. Dark patches correspond to cortical regions activated by a small visual stimulus delivered via the ipsi- or contralateral eye. In comparison to a non-deprived mouse, the ipsilateral eye response increased after six days of contralateral eye MD, indicating an OD shift. The corresponding population response strength analysis is shown for the contra- (blue) and ipsilateral eye (red). B) OD was assessed with extracellular recordings, and cells were grouped following the seven class scheme of Wiesel and Hubel (1963). Six days of contralateral MD induce a strong shift towards the ipsilateral (open) eye. C) Averaged visually evoked potentials after contra- (blue) and ipsilateral eye (red) stimulation. After five days of MD, a shift in OD is visible, consisting of both, an increase in ipsilateral and a decrease in contralateral eye response strength. D) Immediate early gene expression using Arc in situ hybridization in coronal sections of mouse visual cortex after eleven days of contralateral MD. Arc mRNA expression is enhanced after ipsilateral eye stimulation. After Hofer et al. 2006.

Indeed, Pham et al. (2004) showed that barbiturate anesthesia acutely masks adult OD plasticity, in contrast to OD shifts in juvenile mice, which remained unaffected by this type of anesthesia. Adult OD plasticity was further demonstrated in a study in awake mice using VEP-recordings (Sawtell et al. 2003). Thus, it seems that the choice of anesthesia is crucial when investigating experience-dependent plasticity in the adult mouse visual system. These and other observations make it likely that the mechanisms of OD plasticity change during development into adulthood. MD in juvenile mice leads to an initial weakening of deprived eye responses followed by a strengthening of non-deprived eye response (Frenkel and Bear 2004). In adult mice, the MD induced changes in response strength with respect to either eye are less clear. With intrinsic optical imaging a study in our lab measured changes in OD in adult mice (Fig. 2.2A, (Hofer et al. 2006)). They could observe a strengthening in open eye response after six days of MD. This result was additionally confirmed by extracellular recordings (Fig. 2.2B). Frenkel et al. have been also able to observe strengthening of non-deprived eye response after five days of MD using VEP recordings (2006). Furthermore, weakening of deprived eye response was also detected (Fig. 2.2C). Tagawa and colleagues (2005) mapped the representation of the non-deprived eye in the visual cortex of adult mice by inducing the expression of the early gene Arc after brief visual stimulation of the non-deprived eye (Fig. 2.2D). The expression level of Arc mRNA was found to be significantly stronger in animals which were deprived for eleven days in comparison to normally reared adult mice. These studies demonstrate that adult OD plasticity is present but they do not answer which regulators are involved.

There is indication that NMDAR activation is involved in mediating adult OD plasticity. In a recent study, the role of NMDAR in juvenile and adult mice after MD was investigated by intraperitoneal injection of the NMDAR antagonist CPP (Sato and Stryker 2008). Like in juvenile mice, the shift in OD was impaired in adult mice after CPP administration.

Recent studies have investigated the factors which might play a role in restricting OD plasticity in the mature visual cortex. The extracellular matrix (ECM) is a crucial component of the CNS in regulating adult OD plasticity (Pizzorusso et al. 2002). Chondroitin sulphate proteoglycans (CSPGs) are part of the ECM and are known to inhibit axonal growth and sprouting (Fawcett and Asher 1999). CSPGs condense gradually with age in parallel with the decline of critical period plasticity (Pizzorusso et al. 2002). Degradation of CSPGs by in-

fusion of a chondroitinase reactivated full OD plasticity in adult rats (Pizzorusso et al. 2002). Myelin derived components such as the Nogo receptor is also involved in the inhibition of structural rearrangements. In 1988 the lab of M. Schwab made the discovery that Nogo-A has a repellent function on neurite outgrowth in tissue of the adult central nervous system (Caroni and Schwab 1988) and that antibodies against Nogo-A prevented this inhibition. A study asked the question whether the Nogo receptor restricts adult OD plasticity due to its function to inhibit neurite outgrowth (McGee et al. 2005). An OD shift could be induced after short-term MD in adult mice lacking the Nogo receptor.
OD plasticity was also facilitated after reverse suture coupled with enriched environment (Sale et al. 2007), chronique administration of the serotonin reuptake inhibitor Fluoxetine (Vetencourt et al. 2008) or by simply dark rearing the animals for 10 days (He et al. 2006) although the effect was not persistent for a long time (Philpot et al. 2003).
Prior experience itself can also promote OD plasticity in the mature visual cortex (Hofer et al. 2006). Using intrinsic optical imaging it was shown that prior MD in adult mice can facilitate OD plasticity in adult mice after short-term MD. It is important to note that in this study the enhancement of OD plasticity was only induced after repeated MD of the same eye, but not the other eye. Thus the mechanism for the effects of prior experience might differ from those of dark rearing or a wide range of pharmacological manipulations, where cortical plasticity is affected ubiquitously. Facilitated plasticity after prior experience was observed for the first time in the midbrain of barn owls (Knudsen 1998).

2.3.0.4 Experience-dependent structural changes in the adult mouse visual cortex

Are these changes in OD also accompanied by structural rearrangements in the mature visual cortex like it has been shown in the developing visual cortex? Retinal lesion experiments have been the first studies which demonstrated that cortical maps can be remodeled by changes in input pattern in adulthood (Kaas et al. 1990). A study by Darian-Smith (1994) in adult cats demonstrated that there is morphological change of horizontal projection neurons in the visual cortex. Labeling of these long-range neurons in the superficial cortical layers with the anterograde dye biocytin revealed an increase in axonal fibers density and synapse numbers. Electrophysiological recordings after retinal lesions revealed that the structural changes were

associated with topographic remodeling of the cat visual cortex.
MD induced structural changes were also observed beyond the critical period in the visual cortex of mice (Antonini et al. 1999). After long-term MD (20 days) non-deprived eye fibers were significantly larger in comparison to those in normally reared animals. MD for 40 days leads to a significant growth arrest of deprived eye arbors. Structural plasticity in adult mice has been recently observed also on the subcellular level of postsynaptic cells (Hofer et al. 2009). MD for eight days increased the spine density of layer 5 neurons in the binocular visual cortex. Thus, experience dependent plasticity in the mature visual cortex is indeed associated with structural rearrangements. Nevertheless, it is obvious that the degree of such changes as well as of OD plasticity is more prominent and persistent during the critical period in comparison to adulthood.

2.4 Molecular determinants of ocular dominance plasticity

Many investigations of the visual system have addressed the role of neuronal activity. Less attention has been directed towards the molecular factors which are involved in shaping the visual system. The question arises in which way cortical neurons integrate neuronal activity which leads to plasticity dependent gene expression. Three signalling kinases were identified which modulate synaptic strength and which have been linked to the induction of OD plasticity: the extracellular signal-regulated kinase (ERK), protein kinase A (PKA), and the calcium/calmodulin-dependent protein kinase II alpha (CaMKIIα). Changes in synaptic efficacy have been observed after AMPAR and GABAR phosphorylation via PKA and CaMKIIα (Derkach et al. 1999, Esteban et al. 2003). The role of the 2nd messenger effector PKA has been first demonstrated in the primary visual cortex of kittens (Beaver et al. 2001). Pharmakological blockage of PKA prevented the OD shift after MD. Blockade of MD was also observed after infusion of an ERK inhibitor into the visual cortex of rats (Cristo et al. 2001). It was further demonstrated that autophosphorylation of CaMKIIα plays a role in the induction of OD plasticity after MD (Taha et al. 2002). In this study a transgenic mouse line with a point mutation substitute of alanine for threonine rendered CaMKIIα unable to pursue autophospohorylation. These mice displayed no OD shift after MD. The intracellular signalling of these three kinases involve the activation of cAMP-responsive element-binding

protein (CREB), a transcription factor which mediates plasticity relating gene expression. It was shown that patterned vision induces the activation of CREB (Cancedda et al. 2003). Further this study could show that pharmacological block of ERK supressed the CRE mediated gene expression after visual stimulation(Putignano et al. 2007).

In recent years, several genetic screens assessed the expression levels of molecules present during the development of the visual system (Majdan and Shatz 2006, Tropea et al. 2006). One of the first genetic screens revealed that class I major histocompatibility complex (MHC) proteins were expressed in neurons and were regulated by neuronal activity in the developing and mature visual cortex of mice (Corriveau et al. 1998). Recent studies have provided evidence that this family of proteins is indeed not only involved in immune related responses, but also in activity-dependent structural and functional plasticity (Corriveau et al. 1998, Syken et al. 2006, Stellwagen and Malenka 2006, Kaneko et al. 2008). It might seem surprising that immune factors are acting in the healthy brain, but these studies have shown exactely that quite convincingly. In the next section, I will give an outline of the so called "non-immune function" of various molecular players of the immune system in the healthy CNS.

2.4.1 Expression of immune factors in the central nervous system

After brain injuries, molecular factors of the immune system such as cytokines or MHC class I molecules pass the blood brain barrier and can lead to an immune response. After axonal lesions in the brain, it was reported that microglia expressing MHC class I and II molecules function as antigen presenting cells (Streit et al. 1989). These cells are a crucial part of the adaptive immune system (Dangond et al. 1997). Further it was shown that the release of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1β (Il-1β) was significantly increased, in order to maintain the physiological milieu during defense responses after brain injuries including ischemia, head trauma, infections or stroke (Wang and Shuaib 2002). On the other hand, the constitutive expression of these immune factors takes also place in the healthy CNS. In an immune hybridization assay it was demonstrated that MHC class I genes were expressed by neuronal and non-neuronal uninjured cells (Huh

et al. 2000). MHCI protein was detected in diverse neuronal populations such as motor neurons, developing and adult hippocampal and cortical pyramidal cells (Neumann et al. 1997, Corriveau et al. 1998, Huh et al. 2000) and sensory neurons of the vomeronasal organ (Loconto et al. 2003).

2.4.2 Immunosignaling is involved in activity-dependent plasticity in the developing and mature visual system

One of the first studies that focused on the non-immune function of MHC class I molecules, investigated the development of retinogeniculate projections in mice lacking most of the MHC class I genes (Huh et al. 2000). In these mice, retinal afferents failed to segregate into eye-specific layers although normal retinal activity was present. This finding indicates that MHC class I molecules might be involved in bridging neural activity and developmental network changes. The role of MHC class I molecules in synaptic plasticity was also investigated in the hippocampus of adult mice (Huh et al. 2000). It was demonstrated that NMDAR dependent LTP was enhanced in MHC KO mice in comparison to WT mice, whereas basal synaptic transmission remained unaffected. In contrast, LTD was abolished in these KO mice, pointing to a role for MHC class I molecules in synaptic depression.

Moreover, a recent study showed that proteins of the complement cascade, an important part of the innate immune system, are involved in the elimination of inappropriate synapses during the development of the retinogeniculate pathway (Stevens et al. 2007). Mice lacking the complement proteins C1q and C3 showed impaired segregation of retinogeniculate fibers into eye specific layers in the dLGN. Around P30, each dLGN neuron is normally innervated by one or two RGCs in the mouse. In contrast, in C1q KO mice four or five RGCs synapse onto single dLGN cells. This finding has been interpreted to indicate that C1q might act as an elimination marker that could tag synapses for removal in the developing retinogeniculate pathway. Further, this study showed that C1q was upregulated and localized to adult RGC synapses in a glaucoma mouse model, pointing to a role of the complement system in mediating synapse loss during neurodegenerative diseases . Another recent study revealed that the cytokine TNF-α, which mediates the upregulation of MHC class I protein (Neumann et al.

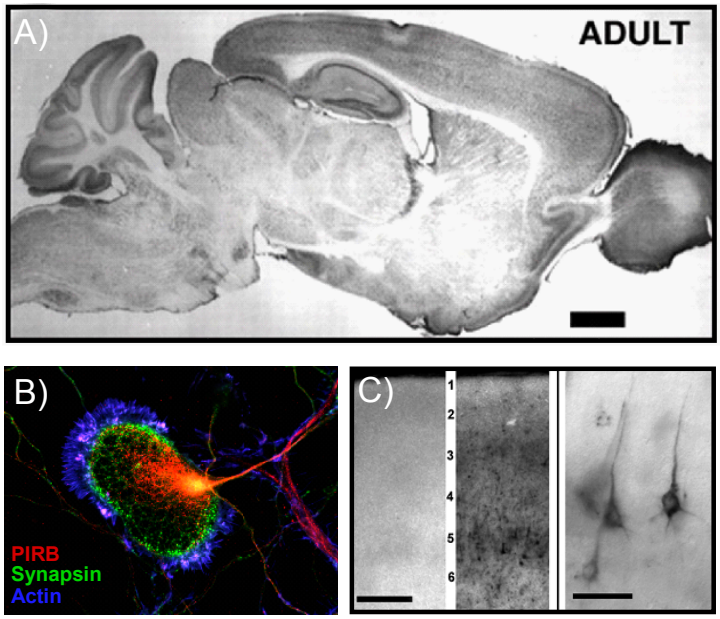

Figure 2.3: Expression of PirB in the mouse brain. A) Sagittal section of adult mouse brain stained with PirB specific antibodies. Scale bar, 1mm. Note high staining levels in several layers of the cortex. B) Growth cone of a cultured cortical neuron immunostained for PirB, Synapsin and Actin. Scale bar, 10 μm. C) Soluble PirB binds to pyramidal neurons in a section of mouse visual cortex. Cortical layers are indicated. Scale bar, 250μm (left and middle panel); 50μm (right panel). From Syken and Shatz (2006).

1997), is involved in OD plasticty in the mouse visual system (Kaneko et al. 2008). The study showed that TNF-α KO mice, which were deprived during the critical period for six days, displayed a normal decrease of deprived eye response comparable to WT mice. Importantly, the increase in non-deprived eye response strength was impaired in these mice. A previous study using dissociated hippocampal cell cultures from TNF-α KO mice, demonstrated that homeostatic plasticity was absent after prolonged activity blockade, whereas NMDAR-dependent LTP and LTD were not impaired (Stellwagen and Malenka 2006). This might indicate that TNF-α is involved in homeostatic synaptic scaling during development and plasticity of mouse visual cortex. Despite the variety of observed phenomena, this overview has hopefully illustrated that molecules of the immune system and in particular MHCI and its receptors are involved in shaping the visual system in the healthy CNS.

Very recently it was also shown that the MHC receptor paired-immunoglobulin-like receptor B (PirB) which is expressed in the mouse brain (Fig. 2.3) is involved in OD plasticity in juvenile and adult mice (Syken et al. 2006). With the technique of Arc in-situ hybridisation Syken and colleagues showed that OD plasticity is enhanced in PirB KO mice after different periods of monocular enucleation during and after the critical period in comparison to WT mice. But there are limitations to Arc in-situ hybridisation. To detect the signal of the ipsilateral eye in the binocular zone it is necessary to remove the contralateral eye. Since the Arc in-situ technique is restricted in detecting on OD plasticity of only one eye, I have used intrinsic optical imaging to study the role of PirB on plasticity of either eye.

In my thesis, I aim to understand the non-immune function of PirB for OD plasticity in juvenile and adult mice. PirB is a very interesting candidate molecule since it might be involved in bridging short-term synaptic changes with long-term structural remodeling (Syken et al. 2006).

3 Material and methods

Animal experiments were performed on mixed background mice (C57Bl/ six x SV/ 129J) and were carried out in accordance with the guidelines of the local government (Regierung von Oberbayern) and the Max Planck Society.

3.1 Plasticity paradigm

3.1.1 Eyelid suture

Monocular deprivation (MD) for two to seven days was performed on juvenile (P26-P30) or adult mice (P90-P120). I used a completely reversible anesthesia regime, as the recovery time is shortened and in case of emergency such as hypothermia or depression of the respiratory and cardiovascular system, the anesthesia can be antagonized (Henke et al. 2004). Mice were anesthetized with a mixture of Fentanyl (0.05 mg/kg), Medetomidin (0.5 mg/kg) and Midazolam (5.0 mg/kg). Animal weights were in the range of 11-15 g for juvenile mice and 23-32 g for adult mice. During the procedure the eye was continuously rinsed with eye fluid (Oculotect) to protect from impurities and drying out. The non-deprived eye was protected with eye cream (Isoptomax) during surgery. First, the hair around the eye lid was trimmed with a spring scissor. Next, the margin of the eye lid was cut and a small drop of eye cream was put onto the eye. The eyelid was sutured shut with two to three mattress stitches using 6-0 silk (Ethicon). Each stitch was sealed with three knots, with the first knot left loose to avoid necrotic damage to the skin. For longer MD periods, the tips of the mice claws were cut to prevent the mouse from scratching the wound. Immediately after surgery and again one day later, mice were injected intraperitoneally with 0.2 mg/kg Chloramphenicol and 0.1 mg/kg Buprenorphin. After injection of the specific antagonists Naloxon (1.2 mg/kg), Flumazenil (0.5 mg/kg) and Atipamezol (2.5 mg/kg) the mouse was fully awake after a few

minutes. For longer deprivation times, the eyelid was checked after four to five days of MD. For mice short-term deprived for only two to three days, the duration of MD varied by less than two hours.

3.1.2 Eye reopening

Eye reopening was carried out either at the beginning of an imaging experiment under halothane anesthesia or in chronic experiments under the same anesthesia as used during eyelid suture. After longterm MD, the two eye lids merge and therefore the eye had to be cut open with a spring scissor. The cut was accomplished with some restriction to the temporal side as there are some blood vessels localized. The reopened eye was covered with ophthalmic cream (Isoptomax) to prevent corneal damage or cloudiness. On the following days, the reopened eye was checked to ensure that the eye lid did not merge again.

3.2 Optical imaging of intrinsic signals

Intrinsic optical imaging (IOI) is a non-invasive technique, which monitors changes in reflected light from activated cortical regions upon sensory stimulation. The advantage of this technique is the relatively non-invasive, yet precise spatial mapping of neuronal populations (in the range of 50-100μm). Thus this technique is suitable to map activity in the cortex upon stimulation.

3.2.1 Sources of intrinsic signals

Several studies have demonstrated that there is a strong coupling between neuronal activity and hemodynamic changes (Fox and Raichle 1986, Frostig et al. 1990, Kleinfeld et al. 1998, Devor et al. 2003). Experiments revealed that the intrinsic signal consists of at least three components (Frostig et al. 1990, Malonek and Grinvald 1996)). The first component results from increased oxygen consumption in the activated region of the brain. The concentration of deoxygenated hemoglobin increases, which leads to an increase in absorption and a decrease in reflectance of the tissue. The second component is a change in blood flow and volume by dilation of blood vessels in response to local neuronal activity, leading to an overall increase

of light absorption after activation. The third response component originates from activity-dependent changes in light scattering. Ion and water flux across membranes leads to changes in cell volume. Expansion of blood vessels and neurotransmitter release also contribute to the light scattering component. A recent study has addressed the importance of astrocytes linking metabolic processes and neural activity (Gurden et al. 2006). It was postulated that glutamate uptake, by astrocytic glutamate transporters (GLT1, GLAST), induces light scattering due to cell swelling and blood flow, resulting in intrinsic optical signals.

3.2.2 Surgery

To investigate changes in response strength in the binocular visual cortex resulting from MD, I used intrinsic optical imaging (Grinvald et al. 1986). Mice were preanesthetized in a chamber with a gaseous mixture of nitrous oxide/oxygen (1:1) and 1.7 % halothane for ten minutes followed by 2.2% halothane for ten minutes. After the breathing rate had dropped sufficiently, Mice were intubated with a plastic tube, which was connected to a blunt cannula. For juvenile mice I used an intubation tube with an inner diameter of 0.86 mm (for adult mice the tube was 0.58 mm wide) and an approximately length of 2.8cm. The intubation tube was connected to a mouse ventilation system (HSE Harvard MiniVent). Mice were ventilated at the following rates: Adult mice: Stroke volume=300μl, Strokes/min=150-160; Juvenile mice: Stroke volume= 260μl, Strokes/min=140-150. The gas mixture consisted of 1.5% halothane, 40% nitrous oxide and 60% oxygen. The mice were placed on a heated blanket which was feedback controlled (temperature=37 C) to maintain the animals' body temperature. Mice were then injected subcutaneously with 20 μl atropine (0.1 mg/ml) diluted in glucose solution (1:10). The glucose solution injection was repeated every two h during the experiment to prevent dehydration of the animal. The heartbeat was continuously monitored during the experiment. The eyes were protected with eye cream (Isoptomax) during surgery. The position of the head was loosely adjusted with earbars. After application of local anesthetics (xylocain gel 2%) to the head, the scalp was unilaterally removed. The skull was washed with saline to remove remaining hair and a headbar was attached rostrally with superglue (Pattex). Warm Agarose (2% diluted in saline) was added to the bone over the visual cortex and sealed with a coverslip (10x10mm). The cortex was evenly illuminated with

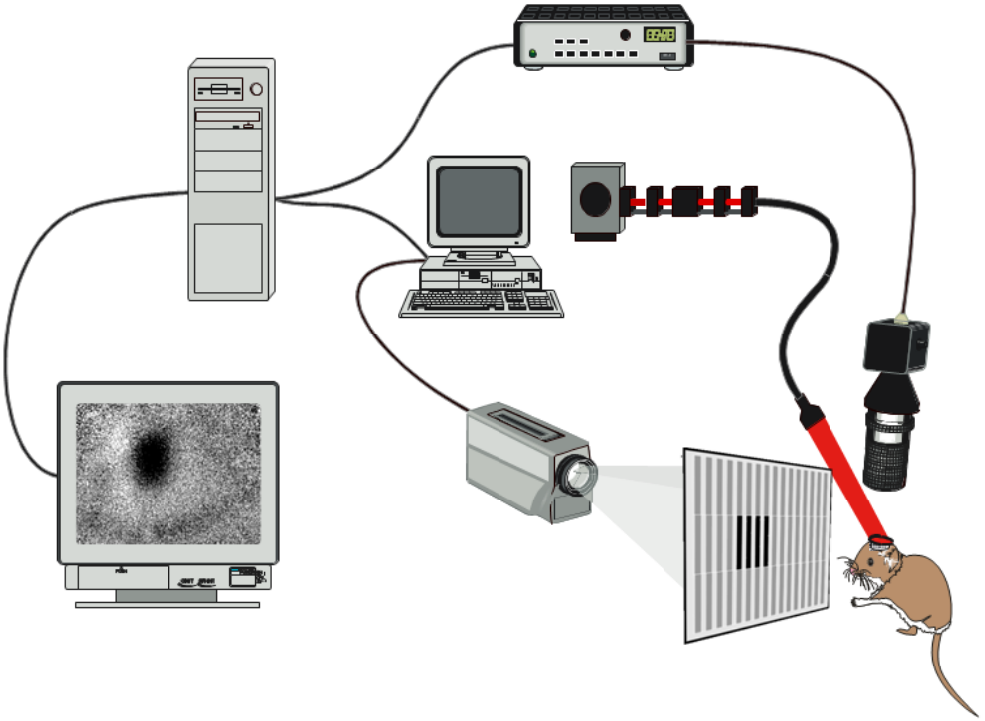

Figure 3.1: Schematic of an intrinsic optical imaging in vivo setup. The anesthesized mouse is located on a heating blanket while visual stimuli are projected with a video projector onto a tangent screen in front of the mouse. The visual cortex is illuminated with monochromatic light (λ=707nm) and changes in reflectance are imaged with a CCD camera. The signals are digitized with an analog/digital converter, and send to the data acquisition computer.

monochromatic light at 546nm and a blood vessel image of the cortical surface through the closed skull was recorded with a slow scan CCD camera (12 bit, 384 x 288 pixel, ORA 2001, Optical Imaging Inc.) to determine the position of the mouse visual cortex. After focusing 250-300 μm below the cortical surface which is sufficient to blur surface vasculature, illumination was switched to monochromatic light at 707nm to image intrinsic optical signals.

3.2.3 Visual stimulation and data acquisition

In Fig.3, a scheme of an IOI setup in vivo is shown. A video projector displayed visual stimuli on a plastic screen with a minimum distance of 17cm in front of the mice. Visual stimuli were square wave drifting gratings (side length=16deg, spatial frequency=0.03cyc/deg, temporal

frequency=2cycl/sec), which changed their orientations every 0.6 sec. Computer controlled shutters allowed for independent stimulation of the two eyes. First, visual stimuli were randomly presented in twelve different positions of the mouse visual field (Fig. 4.1C) to map the visual field representation of the ipsilateral eye in order to determine the exact position of the binocular visual cortex. Each cortical region was responsive to a visual stimulus depending on its position in the visual field. Next I mapped the representation of either eye in the binocular visual cortex. Thus four adjacent visual stimuli, which had elicited the strongest response in the ipsilateral eye mapping experiment to ensure that I recorded activity in the binocular visual cortex (Fig. 4.1C: position 2a,b and 3a,b). For analysis I used response maps from two central stimuli (Fig. 4.1C: position 2a,b) which elicited maximal activity in the binocular visual cortex.

Images were blank-corrected by subtracting baseline images which were recorded without visual stimulation to correct for uneven illumination. For each stimulus presentation epoch, data acquisition time was divided into thirteen frames (each frame time=600msec), which could then be analyzed separately. The images were first-frame corrected by subtracting the first three frames recorded before the onset of visual stimulation (Bonhoeffer et al. 1995). We chose an interstimulus time of ten to twelve seconds to account for the slow decay time of the intrinsic signal.

The single condition activity maps were calculated by clipping (1.5%) and high-pass filtering the first-frame corrected maps. Averages of ten to twelve single condition activity maps yielded an averaged activity map. To determine the region containing the most responsive pixels, single condition activity maps were thresholded (average background + 0.8 STD). The artefact free non responsive area was subtracted from the region of interest (ROI). The resulting pixel values within the ROI and above threshold, were integrated to determine the overall strength of activation.

4 Results

4.1 Intrinsic signal imaging in the mouse binocular cortex

4.1.1 Mapping the binocular visual cortex

As a prerequisite to assess the relative strength of the representation of both eyes in the binocular visual cortex in control mice and after monocular deprivation, it was necessary to map the binocular visual cortex. Since the exact position of the binocular visual cortex may vary slightly from mouse to mouse, this procedure was carried out in every animal, before the actual measurements began.

As an example, I present optical imaging data from an adult C75Bl6 mouse (age P90, Fig. 4.1A). In order to assess the extent of the binocular visual cortex, stimuli were shown at various positions (Fig. 4.1C) to either eye in the mouse visual field. Depending on stimulus position, different regions of the visual cortex were activated, reflecting the retinotopic organization of the visual cortex: Showing a stimulus in the central visual field induces activity which is displayed as dark patch in the lateral region of the visual cortex, while presentation of a peripheral visual stimulus leads to activation of medial visual cortex. Upper visual field stimulation activates posterior visual cortex and lower visual field stimulation evokes activity in the anterior visual cortex.

As shown in Fig. 4.1A, projections from the ipsilateral eye (red) are restricted to the binocular visual cortex, in contrast to projections from the contralateral eye (blue), which also innervate the monocular visual cortex. The intensity and the area of the activity maps of both eyes differ with the overall response strength derived from the contralateral eye being stronger in comparison to the ipsilateral eye. Further the ipsilateral representation in the periphery is getting weaker towards the region of the monocular visual cortex. In this example both eyes' activity maps derived from the binocular region are superimposed since the

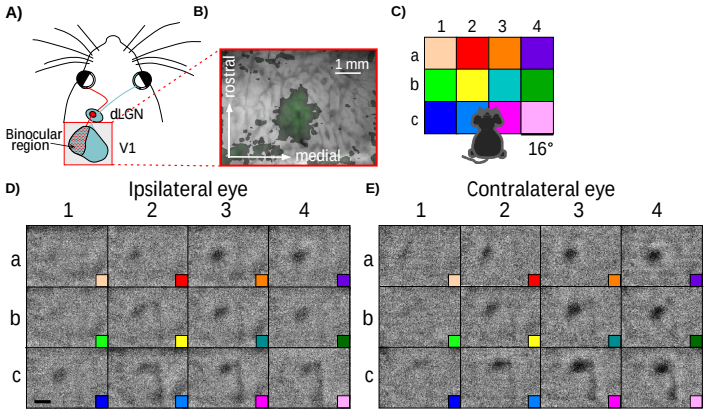

Figure 4.1: Mapping mouse binocular visual cortex. A) Schematic of the mouse visual system. Projections from the ipsi- and contralateral eye representing the binocular visual field are kept separate in the LGN, but merge in the binocular region of the visual cortex (red and blue). The monocular visual cortex (blue) receives only input from the contralateral eye. B) Blood vessel image of the visual cortex acquired with a CCD camera through the closed skull. An activity map (green translucent area) of the binocular region was overlaid on the blood vessel image. C) Color coded arrangement of the twelve stimulus positions in the visual field used to map the binocular visual cortex. D), E) Activity maps obtained in response to the twelve different stimulus positions (depicted in C) presented to the ipsi- and contralateral eye. Scale bar, 1mm.

optical axes of both eyes are aligned. Thus, in order to map the location of the binocular visual cortex, it would be in principle sufficient to stimulate only the ipsilateral eye (Fig. 4.1D). However in strabismic mice, both eyes do not cover the same binocular visual field and the activity patches of the contra and ipsilateral eye do not cover the same binocular visual area. Thus mapping of the contralateral eye (Fig. 4.1E) in addition to the ipsilateral eye (Fig. 4.1D) allowed to test whether the optical axis of both eyes were aligned.

4.1.2 Analysis of OD plasticity in adult C57Bl6 mice

While there is no doubt that MD induces OD shifts in mice during the critical period (P19-32), there is ongoing discussion in the field whether visual deprivation leads to functional changes also in adult mice (Sawtell et al. 2003, Pham et al. 2004, Morishita and Hensch 2008). To address this issue in a systematic fashion and to extend earlier findings from

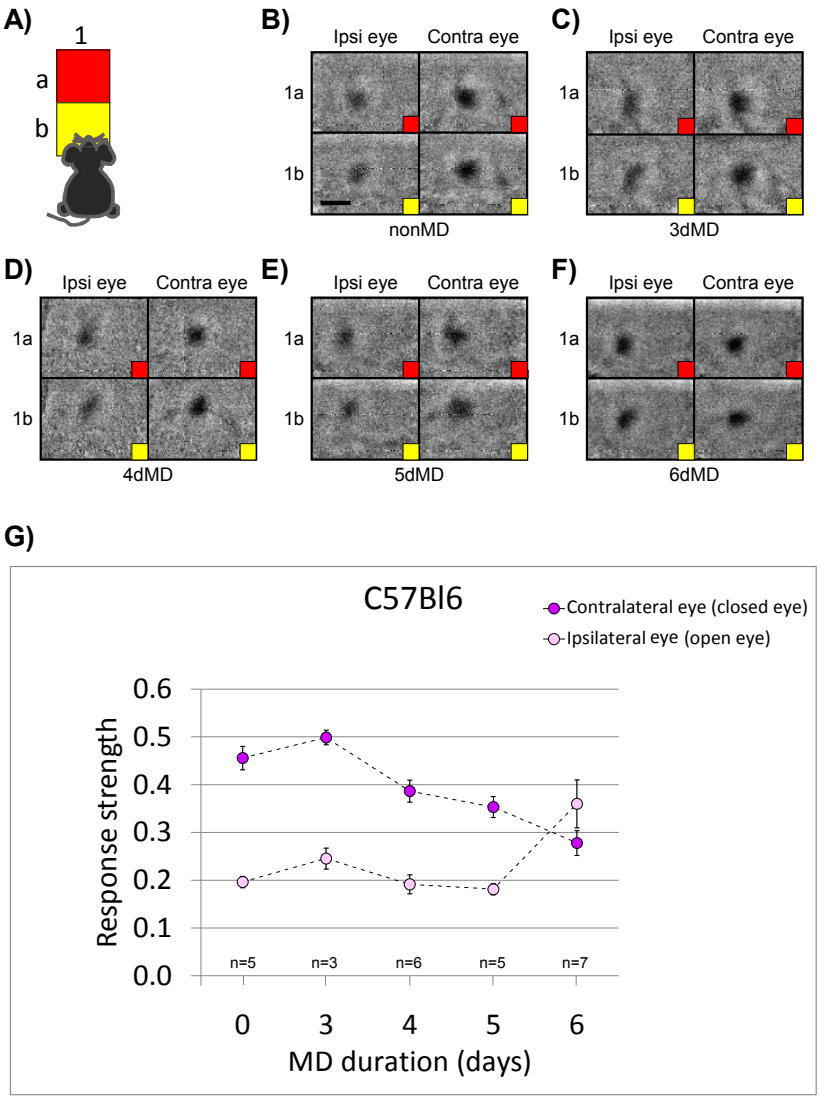

Figure 4.2: OD plasticity in adult C57Bl6 mice. A) Schematic depicting the two stimulus positions in the central visual field which were used to assess the strength of eye representation. B) Activity maps elicited by stimulation of the ipsi- and contralateral eye in the binocular visual cortex of a non-deprived mouse. Scale bar, 1mm. C)-F) Activity maps elicited by ipsi- and contralateral eye stimulation after MD of three, four, five and six days. G) Change in each eye's response strength with increasing MD durations. Response strength is measured as $\sum \frac{\Delta R}{R}$ (a detailed description can be found in the Methods chapter). Error bars indicate SEM.

our lab (Hofer et al. 2006), I investigated OD plasticity in adult C57Bl6 mice (P90) after increasing durations of MD. To compare the maximal response strength originating from

ipsi- and contralateral eye stimulation, I presented two visual stimuli in the central visual field (Fig. 4.2A). The positions of those stimuli were determined after the initial mapping procedure described in the previous paragraph. In Fig. 4.2B four activity maps of a non-deprived C57Bl6 mouse are shown. As expected, the response strength of the ipsilateral eye is weaker in comparison to the contralateral eye. As is evident from the activity maps of deprived mice (Fig. 4.2C-F), MD leads to a weakening of closed (contralateral) eye response strength and an increase of open (ipsilateral) eye response strength over time. This change in response strength is visible both, by the size and intensity of the activity patches. During MD, the area of the contralateral eye patch becomes smaller and its intensity gets weaker. In contrast, the activity patch of the ipsilateral eye increases in size and intensity.

The timecourse of both eyes' response strength also differs with MD duration (Fig. 4.2G). Weakening of closed eye response strength progresses steadily and precedes ipsilateral eye strengthening. A significant drop in contralateral (closed) eye response strength after five days of MD (0.35 ± 0.2, n=5) in comparison to non-deprived mice (0.46 ± 0.03, n=5) can be observed (nonMD vs 5dMD, $p<0.05$, t-test). Six days of MD of the contralateral eye leads to a significant increase (nonMD vs 6dMD, $p <0.05$, t-test; n=7) in ipsilateral (open) eye response strength (0.36 ± 0.05, n=7) in comparison to non-deprived mice (0.19 ± 0.01, n=5). Now we have learned that deprivation in adult C57Bl6 mice leads to changes in the response strength of the closed and the open eye. The overall OD shift incorporates the changes of both eyes' response strength, and can be conveniently expressed by calculating the ratio of contralateral to ipsilateral eye's response strength (contra/ipsi ratio). In Fig. 4.3A+B, the individual response strength curves of the ipsi- and contralateral eye are plotted again. Fig. 4.3C plots the resulting contra/ipsi ratio over MD duration. In non-deprived mice the ratio is high (2.42 ± 0.06, n=5) reflecting the dominant input of the contralateral eye. Deprivation of the contralateral eye for six days leads to a significant drop in contra/ipsi ratio (nonMD vs 6dMD: $p<0.005$, Mann-Whitney U test; n=7). This overall drop in the contra/ipsi ratio is due to the combined effects of the decrease in closed eye response strength and the increase in open eye response strength (Fig. 4.3A+B). In summary, my data demonstrate that OD plasticity is clearly present in adult C57Bl6 mice aged 90 days. In comparison to juvenile mice, which were deprived during the critical period, OD shift in adult mice develop slower (Fig. 4.3).

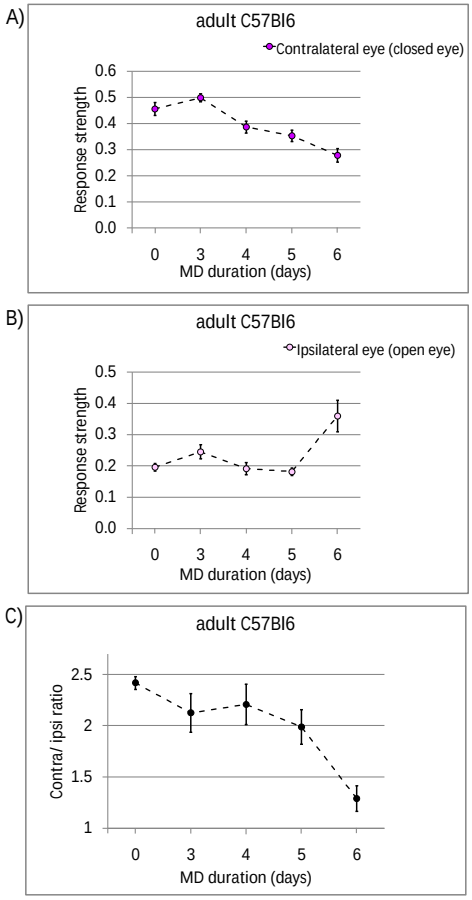

Figure 4.3: OD shifts after MD in adult C57Bl6 mice. A) Contralateral eye response strength is plotted against MD duration. There is a significant decrease in closed eye response strength in comparison to non-deprived mice after five days of deprivation. B) Ipsilateral eye response strength is plotted against MD duration. A significant increase in open eye response strength can be observed after six days of deprivation in comparison to non-deprived mice. C) The overall shift in OD is expressed by calculating the ratio of contra- to ipsilateral eye response. After six days of deprivation, there is a significant shift in OD in comparison to non-deprived mice. Error bars indicate SEM.

4.2 Investigation of OD plasticity in juvenile PirB knockout (KO) mice

While the data above as well as earlier studies (Hofer et al. 2006, Frenkel et al. 2006) demonstrate clear OD plasticity in adult mice, there is general agreement that OD shifts are

strongest during the critical period (P19-32). It is thought that the critical period is regulated by several factors and that its closure is achieved by the establishment of a mature state where plasticity is more limited (Morishita and Hensch 2008). Focusing on factors which play a role in critical period plasticity, the lab of Carla Shatz has found a candidate protein involved in critical period plasticity: the MHCI receptor PirB (Syken et al. 2006). Using Arc in-situ hybridization technique, the authors showed that OD plasticity is enhanced in PirB KO mice during the critical period. Interestingly, they observed an expansion of ipsilateral projections into the monocular zone of the visual cortex in PirB KO mice. However, the use of Arc in situ hybridization as a readout technique for OD shifts has certain limitations. To detect a clear Arc signal in the binocular zone induced by ipsilateral eye activation, it is necessary to remove the contralateral eye. Thus, in a given animal, the method does only allow assessing the activity level of one eye, but not the other. As usually both eyes change their response strength after MD (see above, (Sawtell et al. 2003, Hofer et al. 2006, Lehmann and Löwel 2008) and can do so with different time-courses, I was curious to learn whether the enhanced plasticity in PirB KO mice is caused by changes in only one of the two inputs, or both. Intrinsic optical imaging is ideally suited to address this question.

4.2.1 Response strength analysis of juvenile PirB KO mice after short- and long-term MD

In this section I will focus on OD plasticity in juvenile WT and PirB KO mice after short-term (two to three days) and long-term (five days) MD. Fig. 4.4A depicts the arrangement of visual stimuli in the central visual field. In Fig. 4.4B+D, activity maps of non-deprived WT and PirB KO mice at the peak of the critical period (P28-P32) are shown. In both genotypes the activity patch of the contralateral eye is larger and darker in comparison to the area that was activated upon ipsilateral eye stimulation. After three days of contralateral eye deprivation, the map of the ipsilateral (open) eye is darker and bigger whereas the area of the contralateral patch has shrunken. Fig. 4.5 depicts the changes in response strength as a function of different deprivation times in juvenile WT mice. In non-deprived WT mice, the response strength of the contralateral eye is two to three times higher (0.67 ± 0.04, n=7) in comparison to the ipsilateral eye (0.26 ± 0.02, n=7) which is in accordance with the literature

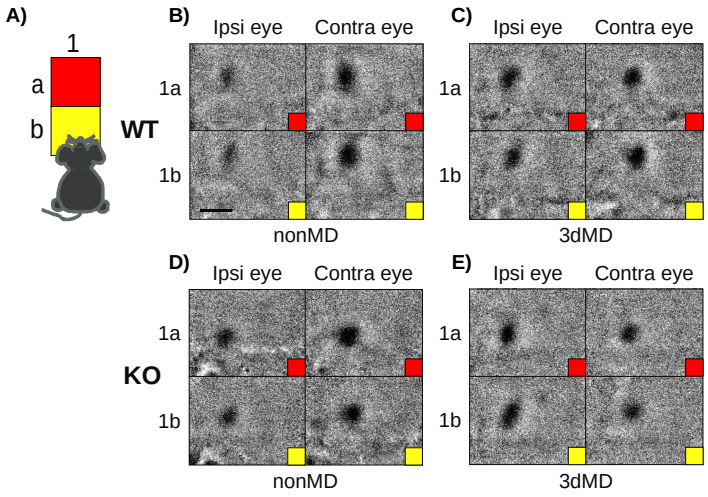

Figure 4.4: Comparison of short-term OD plasticity in WT and PirB KO mice during the peak of the critical period. A) Schematic of stimulus positions. B)-E) Examples of activity maps of non-deprived and three day deprived WT and PirB KO mice. B)+D) In non-deprived mice in both genotypes, the map of the contralateral eye is larger in comparison to the patch of the ipsilateral eye. C)+E) Three days of MD lead to slightly smaller activity patches of the contralateral (closed) eye in both,WT and PirB KO mice. Further, ipsilateral eye patches are larger and darker after a three day MD in both genotypes. Scale bar, 1mm.

(Frenkel and Bear 2004, Hofer et al. 2006). Three days of deprivation of the contralateral eye leads to a significant drop (nonMD vs 3dMD, $p < 0.005$, t-test; n=7; Fig. 4.5) in closed eye response strength (0.45 ± 0.05, n=6) in comparison to non-deprived WT mice (0.67 ± 0.04, n=7). Long-term deprivation for five days induces a further decline in closed eye response strength (nonMD vs 5dMD, $p<0.001$, t-test; n=7). The response strength of the ipsilateral eye is significantly increased (nonMD vs 5dMD, $p<0.001$, t-test; n=7) after five days of deprivation (0.44 ± 0.02, n=5) in comparison to the non-deprived baseline state (0.26 ± 0.02, n=7).

Thus I observed a pattern of eye response strength changes consisting of a rapid drop in closed eye response and a delayed strengthening in open eye response which is in accordance to the changes observed by Gordon and Stryker (1996) and Frenkel et al. (2004) in juvenile mice.

PirB KO mice differed in several respects from their WT counterparts. In comparison to

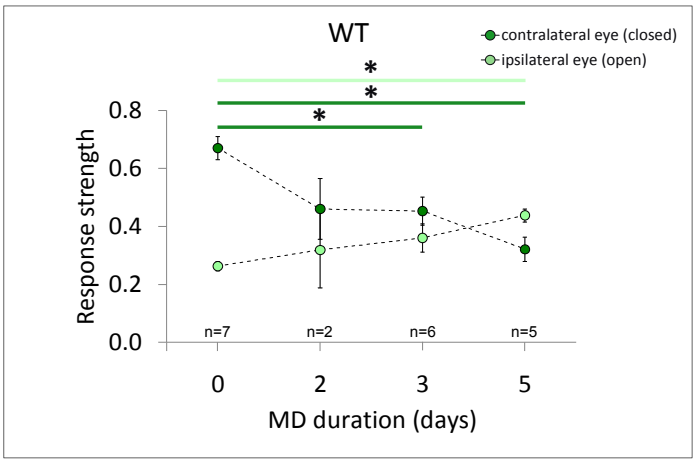

Figure 4.5: Changes in eye response strength after MD in juvenile WT mice. Individual responses of contralateral (dark green) and ipsilateral eye (light green) are plotted against different MD periods. There is a significant decrease in response strength of the contralateral eye after three days of deprivation in comparison to non-deprived mice. A five day MD leads to a further decline in contralateral eye response strength and an increase in ipsilateral response strength. Error bars indicate SEM.

WT mice, I observed a more rapid decline in closed eye response strength in PirB KO mice. After 2 days of deprivation a significant decrease (nonMD vs 2dMD: $p<0.01$, t-test; n=6; Fig. 4.6) in closed eye response strength (0.35 ± 0.09, n=3) was detected in comparison to the non-deprived PirB KO mice (0.69 ± 0.05, n=6). Closed eye response continuously decreases after five days of deprivation (3dMD vs 5dMD: $p<0.005$ t-test; n=8). Also, in contrast to juvenile WT mice, I did not observe a significant increase of ipsilateral (open) eye response strength, even after five days of deprivation in juvenile PirB KO mice.

In summary, the response strength analysis during the peak of the critical period shows that PirB KO mice display a more rapid and stronger drop in closed eye response strength, and at the same time lack the strengthening of open eye responses after longer MD durations seem in WT mice.

Next I will address the question whether the response strengths of both eyes are considerably different in PirB KO and WT mice after deprivation. In Fig. 4.7A a response strength analysis of the contralateral eye of both genotypes is shown. With increasing MD

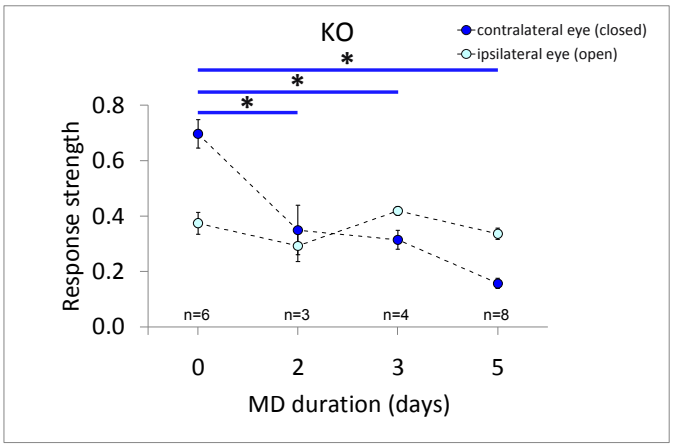

Figure 4.6: In juvenile PirB KO mice the decline in closed eye response strength is stronger and more rapid. An analysis of response strength of the contralateral (dark blue) and ipsilateral eye (light blue) is shown as a function of increasing MD periods. Two days of deprivation lead to a significant decrease in the contralateral (closed) eye response strength in comparison to non-deprived mice. After three and five days of deprivation, the contralateral eye response strength continues to decline. Error bars indicate SEM.

duration the closed eye response strength declines in WT as well as in PirB KO mice. Comparing deprived eye response strength of both genotypes, I found that the overall PirB KO response strength decreases significantly faster and stronger in comparison to the WT response strength (0d-5dMD, PirB-/- vs WT: $p<0.05$, two-way ANOVA, Tukey post hoc tests; n=8). Regarding the ipsilateral eye, both genotypes exhibited similar response strengths. Note that the response curve during short-term MD (two to three days) overlaps in both genotypes. After five days of deprivation, I observed a significant increase in open eye response strength in juvenile WT mice in comparison to PirB KO mice (5dMD, PirB-/- vs WT: $p<0.005$, t-test; n=8). Thus the decline in contralateral eye response strength is stronger and more rapid in juvenile PirB KO mice in comparison to WT mice.

4.2.2 Comparison of OD shifts in juvenile WT and PirB KO mice

The preceding section has demonstrated that MD leads to distinct changes of eye response strength in both genotypes. In order to assess the overall amount of OD plasticity in both

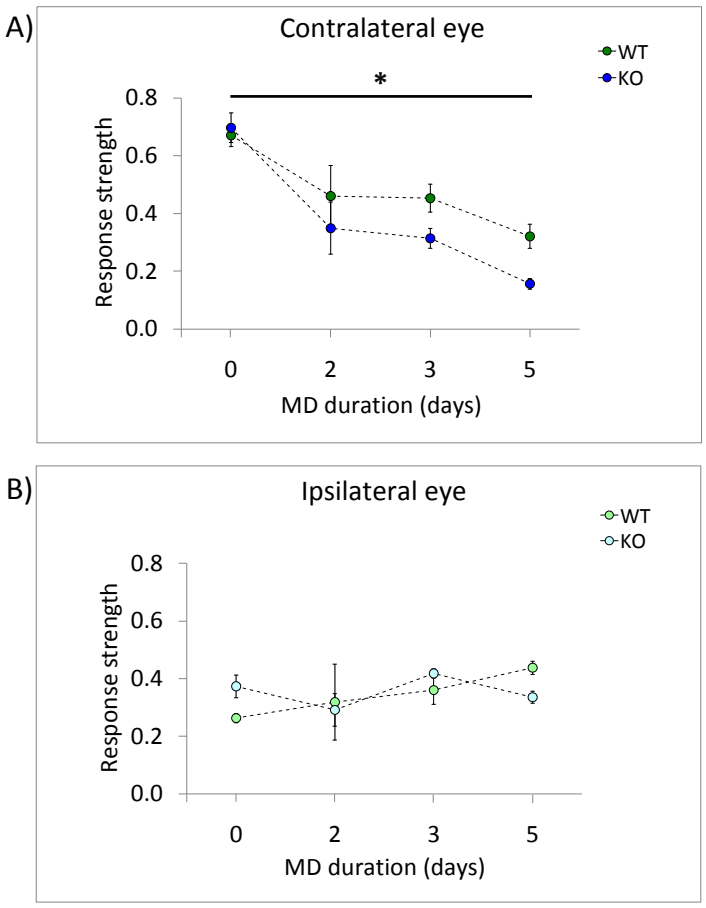

Figure 4.7: Direct comparison of eye response strengths between juvenile WT and PirB KO mice. A) Contralateral eye response strengths of both genotypes are plotted against different MD duration. Note stronger and more rapid drop in contralateral eye response strength in PirB KO mice. B) Ipsilateral eye response strengths of both genotypes. No systematic differences between both genotypes are present. Error bars indicate SEM.

genotypes it is useful to analyze the changes in contra/ipsi ratios as a function of increasing MD durations. Fig. 4.8 compares contra/ipsi ratios of WT mice and PirB KO mice. The non-deprived baseline state between WT mice (2.58±0.2, n=7) and PirB KO mice (1.96±0.1, n=6) differs significantly (nonMD, PirB-/- vs WT: $p<0.01$; n=7, Mann Whitney U test). This is due to the significantly higher ipsilateral eye response strength of juvenile PirB KO mice (0.37 ± 0.04, n=6) in comparison to juvenile WT mice (0.26 ± 0.02, n=7) which is depicted in Fig. 4.7. In both genotypes, there is a significant drop in the contra/ipsi

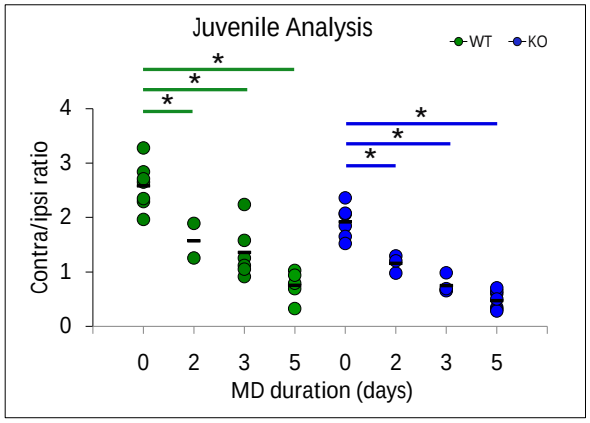

Figure 4.8: OD shift analysis of juvenile WT and PirB KO mice. Contra/ipsi ratios of juvenile WT (green) and PirB KO mice (blue) are plotted as a function of MD duration. Black bars indicate the mean response strength. Deprivation of WT and PirB KO mice induces a continuous, significant drop of the contra/ipsi ratio with increasing MD duration.

ratio after two (WT: $p<0.05$; PirB-/-: $p<0.05$, Mann-Whitney U test), three (WT: $p<0.01$; PirB-/-: $p<0.01$, Mann-Whitney U test) and five days (WT: $p<0.001$; PirB-/-: $p<0.001$, Mann-Whitney U test) of deprivation in comparison to non-deprived mice. Thus, while the response strengths of both eyes change in a distinct fashion in WT and KO mice, the overall amount of OD plasticity is comparable.

4.3 OD plasticity in adult PirB KO mice

It has been postulated that different mechanisms underlie OD plasticity in juvenile and adult mice, and that adult plasticity is more limited than critical period plasticity (Sawtell et al. 2003, Pham et al. 2004, Morishita and Hensch 2008). The Arc in-situ hybridization data of Syken et al. (2006) indicate larger OD shifts in adult PirB KO mice (P100). However, these results were again based on the observation of an expanded ipsilateral eye projection in the visual cortex after eleven days of monocular enucleation, and could not make any statements on the response strength of the contralateral eye. I therefore used intrinsic optical imaging to test the degree of OD plasticity in mature PirB KO mice (P90), allowing us to measure *both* eyes' response strength also after *shorter* durations of MD.

4.3.1 Eye response strength in adult WT and PirB KO mice after MD

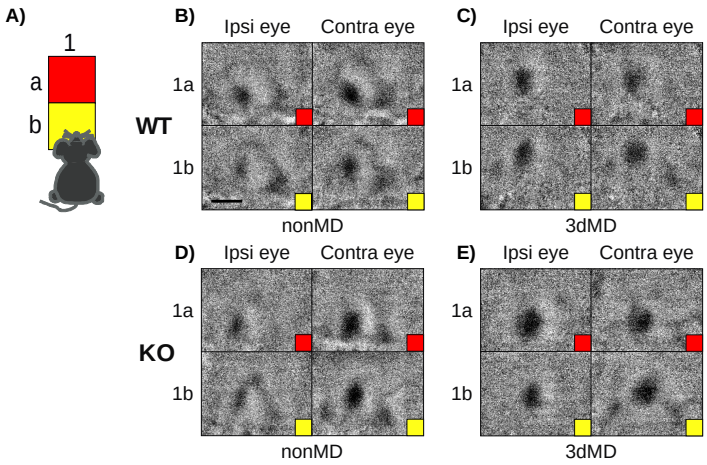

Figure 4.9: OD plasticity is present in adult WT and PirB KO mice (P90). A) Stimulus arrangement in the central visual field. B-E) Examples of activity maps of non-deprived and three days deprived WT and PirB KO mice. B)+D) In non-deprived WT and PirB KO mice, contralateral eye activity patches are stronger. C)+E) Three days of MD leads to OD shifts in both genotypes. Scale bar, 1mm.

In Fig. 4.9, activity maps derived from adult, non-deprived WT mice and PirB KO mice (P90) are shown. In both genotypes, the adult visual cortex receives its dominant input from the contralateral eye, as expected and similar to the juvenile state. Thus, the activity patches of the contralateral eye are larger and darker in both genotypes, in comparison to

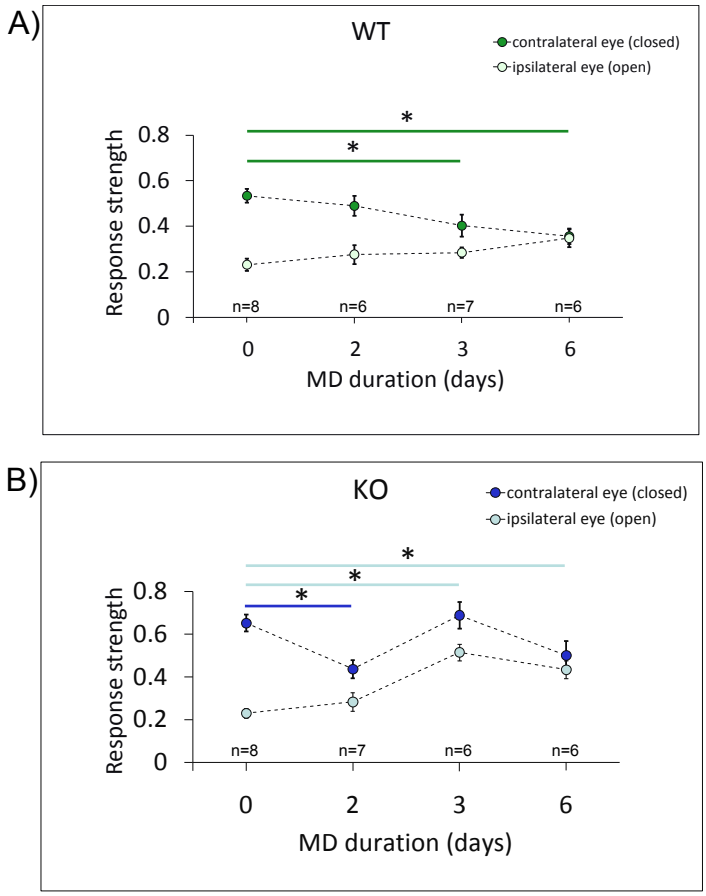

Figure 4.10: Changes in eye response strength after increasing MD durations. (A) A significant change in adult WT mice was first seen after three days as a drop in closed eye response strength, which was even more prominent after six days of deprivation. The increase in ipsilateral response is not significant. In adult KO mice, a rapid drop in deprived eye response strength occurred after two days of MD. A parallel increase in deprived and non-deprived eye responsiveness developed after three days of MD. Error bars indicate SEM.

the area activated by the ipsilateral eye. After three days of MD, clear changes in patch size and intensity are obvious in both, WT and PirB KO mice (Fig. 4.9C+E). Both genotypes exhibited clear adult OD plasticity, but the underlying changes in eye response strength that lead to the overall OD shift were different. To analyze adult OD plasticity in detail, individual eye responses were plotted as a function of increasing MD periods (Fig. 4.10). The main MD effect in WT mice was a steady decline in closed eye response strength over

time which was significant after three (nonMD vs 3dMD: $p < 0.05$, t-test; n=7) and six days (nonMD vs 6dMD: $p < 0.01$, t-test; n=6), in combination with a steady, weak increase in open eye strength which is not significant even after six days of MD. In contrast, PirB KO mice showed a rapid and strong drop in closed eye response strength after only two days of MD, comparable to the drop in response strength of juvenile KO mice (Fig. 4.6A). Remarkably, this rapid drop was followed by a fast increase of closed and open eye strength (PirB-/-, nonMD/ 3dMD: 0.51±0.04; $p<0.0001$, t-test; n=8). After six days of MD responses for both eyes weakened again.

In order to test the hypothesis that adult PirB KO mice display enhanced OD plasticity I directly compared the responses of the two eyes of both genotypes (Fig. 4.11). The clearest difference was seen after a three day MD, when both, deprived and non-deprived eye stimulation lead to stronger responses in PirB KO mice (deprived eye : $p<0.01$, t-test; n=7; non-deprived eye: $p<0.005$, t-test; n=7). Overall the response strength of the contralateral eye in adult PirB KO mice is significantly stronger after MD in comparison to adult WT mice (0d-5dMD, PirB-/- vs WT: $p<0.001$, two-way ANOVA, Tukey post hoc tests; n=7). The same increase holds true for the ipsilateral response strength in adult PirB KO mice. (0d-5dMD, PirB-/- vs WT: $p<0.01$, two-way ANOVA, Tukey post hoc tests; n=7). The data comply with the overall trend that the OD shift in WT mice is primarily due to deprived eye weakening (Fig. 4.10A), while open eye strengthening causes the shift in PirB KO mice (Fig. 4.10B), but these effects are convoluted by the strong increase in both eyes' response strength after three days of MD in the PirB KO mice.

4.3.2 Overall OD shifts in adult WT and PirB KO mice

In the preceding section, the eye response strength analysis revealed differences between adult WT and PirB KO mice. Next, I computed the overall OD shift in both genotypes. In Fig. 4.12, the contra/ipsi ratios are plotted as a function of MD duration. Note that the baseline value (non-deprived) in KO mice (PirB-/-: 3.23±0.03) is significantly higher than in WT mice (WT: 2.33±0.02, n=8, PirB-/- vs WT: $p<0.01$, Mann-Whitney U test; n=8). This is predominantly due to the higher contralateral eye response strength of non-deprived PirB KO mice (Fig. 4.11A). A deprivation period of just two days led to a significant reduction

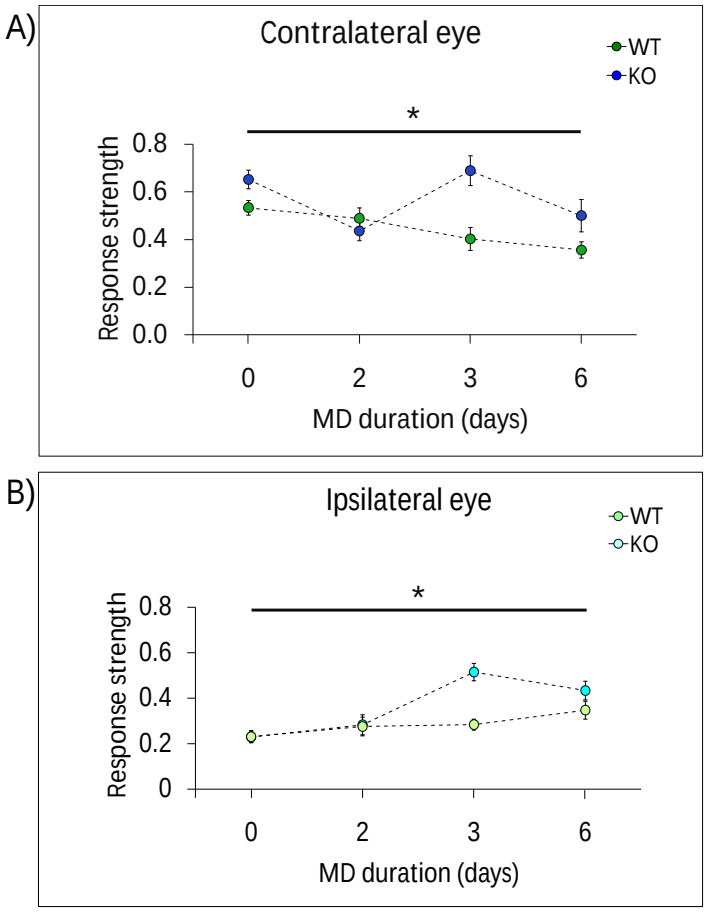

Figure 4.11: Individual eye responses in KO mice are increased after MD. The contralateral (A) and ipsilateral (B) eye response strengths are stronger in PirB KO mice in comparison to WT mice with increasing MD durations. Error bars indicate SEM.

in the contra/ipsi ratio of PirB KO mice (2dMD, PirB-/- vs WT: $p<0.01$, Mann-Whitney U test; n=8). In the preceding section, the eye response strength analysis revealed differences between adult WT and PirB KO mice. Next, I computed the overall OD shift in both genotypes. In Fig. 4.12, the contra/ipsi ratios are plotted as a function of MD duration. Note that the baseline value (non-deprived) in KO mice (PirB-/-:3.23 ± 0.03) is significantly higher than in WT mice (WT: 2.33 ± 0.02, n=8, PirB-/- vs WT: $p<0.01$, Mann-Whitney U test). This is predominantly due to the higher contralateral eye response strength of non-deprived PirB KO mice (Fig. 4.11A). A deprivation period of just two days led to a significant reduction in the contra/ipsi ratio of PirB KO mice (2dMD, PirB-/- vs WT:

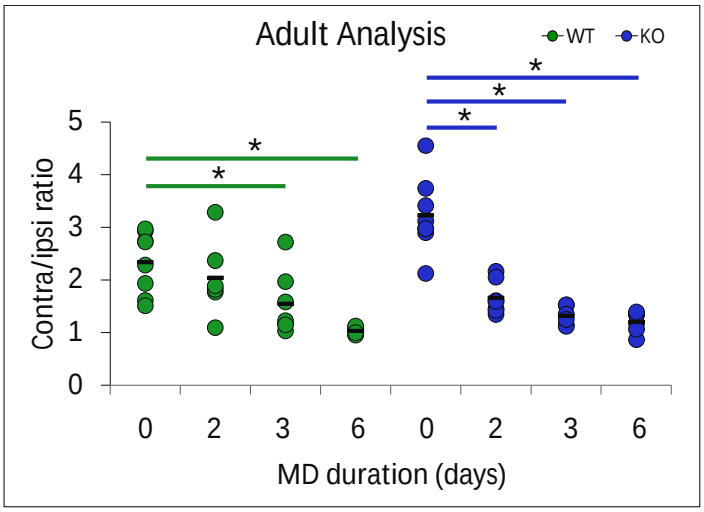

Figure 4.12: Rapid OD shift in adult PirB KO mice. Contra/ipsi ratio from individual mice (WT in green, PirB KO in blue) are plotted against distinct MD periods. Black horizontal bars denote mean values.

p<0.01, Mann-Whitney U Test; n=8). In WT mice a considerable shift in OD was only observed after three days of deprivation (3dMD, PirB-/- vs WT: p<0.05, Mann-Whitney U test; n=8) . Six days of deprivation resulted in clear OD shifts in both PirB KO (6dMD, PirB-/- vs WT: p<0.005, Mann-Whitney U test; n=8) and WT mice (6dMD, PirB-/- vs WT: p <0.005, Mann-Whitney U test; n=8). Slightly longer deprivation (seven days) did not result in further OD shifts (data not shown).

In summary, MD alters the strength of the representation of both eyes in a differential fashion in adult WT and PirB KO mice, and the KO mice exhibit overall more rapid OD plasticity in comparison to their adult WT counterparts.

4.4 Investigation of metaplasticity in juvenile PirB KO mice

A recent study in our lab has demonstrated that the degree of MD plasticity was enhanced in adult mice when those mice experienced a prior MD (Hofer et al. 2006). This effect was seen irrespective of whether the first MD was induced during the critical period or in adult mice. As the data described above indicate that PirB might limit plasticity, I here ask the question whether PirB might be involved in this priming effect of a prior MD. To this end I deprived the contralateral eye of PirB KO mice during the critical period for three days, reopened the eye, and nine weeks later induced a second MD of the contralateral eye for another three days (Fig. 4.13A for a schematic of the experimental paradigm).

4.4.1 Response strength analysis of PirB KO mice after prior experience

In Fig. 4.13C+E, activity maps of WT and PirB KO mice which were deprived for three days in adulthood (P90-100) are shown. These maps show a strong activated region for both eyes after three days of deprivation in comparison to non-deprived WT and PirB KO mice (Fig 3.9B+D). In Fig. 4.13D+F activity maps of both genotypes are illustrated which had undergone three days of MD during the critical period and, after several weeks of binocular vision, a second three day MD in adulthood. Maps of repeatedly deprived WT mice illustrate stronger patches in comparison to the maps of three days deprived WT mice (Fig 3.13 C+D). This increase in patch size and intensity after prior MD from both eyes is not as pronounced in PirB KO mice.

Analysis of each eye's response strength revealed a significant increase in ipsilateral eye response strength in WT mice after repeated MD (WT, 3dMD/ repeated MD: $p< 0.01$, t-test; n=7; Fig. 4.14A), while the contralateral eye response strength did not change significantly after repeated MD. A different effect can be observed in PirB KO mice after prior MD (Fig. 4.14B). While ipsilateral eye response strength of experienced PirB KO mice did increase in comparison to non-deprived KO mice, there was no additional effect of prior deprivation. In fact repeated MD induced a significant drop in ipsilateral eye response strength in PirB KO mice in comparison to single MD (PirB-/-, 3dMD/ repeated MD: $p<0.05$, t-test; n=7). There was no significant change in contralateral eye response strength after a second MD in PirB KO mice. In Fig. 4.15, the overall shift in OD of WT mice (green) and PirB KO mice

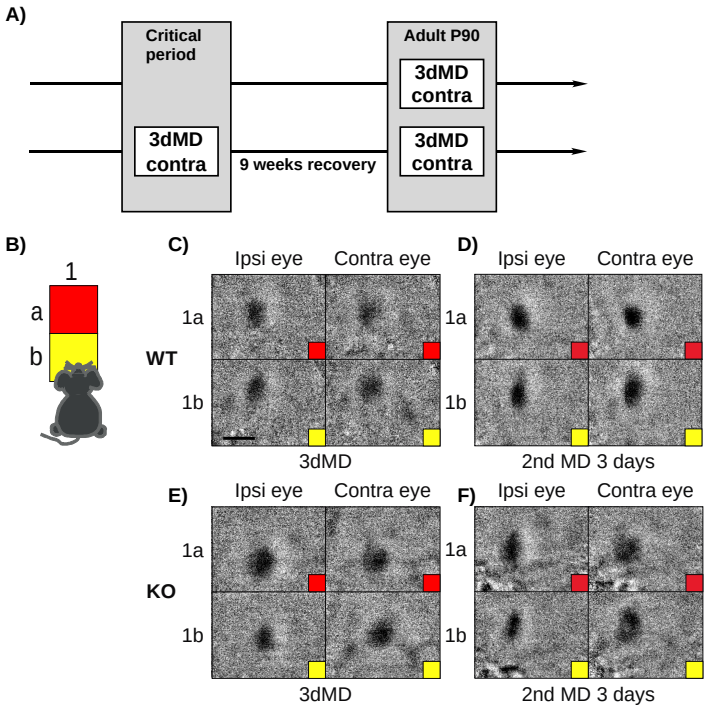

Figure 4.13: Assessing metaplasticity in PirB KO mice. A) Schematic of the experimental paradigm. B) Visual stimulus arrangement. C-F) Examples of activity maps of three day deprived and repeatedly deprived PirB and WT mice. C+D) Activity map of prior deprived adult WT mice is stronger in both eyes in comparison to three days deprived adult WT mice. E)+F), activity maps of KO mice after contralateral eye MD for three days during the critical period and after a second three day MD in adulthood (P90) display similar activity maps in comparison to adult WT mice. D)+F) Activity maps of WT mice are darker and stronger after repeated MD in comparison to those of PirB KO mice. Scale bar, 1mm.

(blue) is illustrated. Comparing the contra/ipsi ratios of three days deprived (1.55±0.2, n=7) and prior deprived WT mice (1.16 ± 0.06, n=6) revealed that there is a significant shift in OD (WT, 3dMD vs 3+3dMD: $p < 0.05$, Mann-Whitney U test; n=6). Importantly, PirB KO mice do not show this effect of prior MD. Would a shorter second MD in adulthood point to further occlusion of metaplasticity in PirB mice? To address this question, I deprived juvenile mice for three days during the critical period and then again as adults for two days. In Fig. 4.16, the response strength analysis of WT (Fig. 4.16A) and PirB KO mice (Fig. 4.16B) is illustrated. WT mice did not display a priming effect after shorter repeated MD

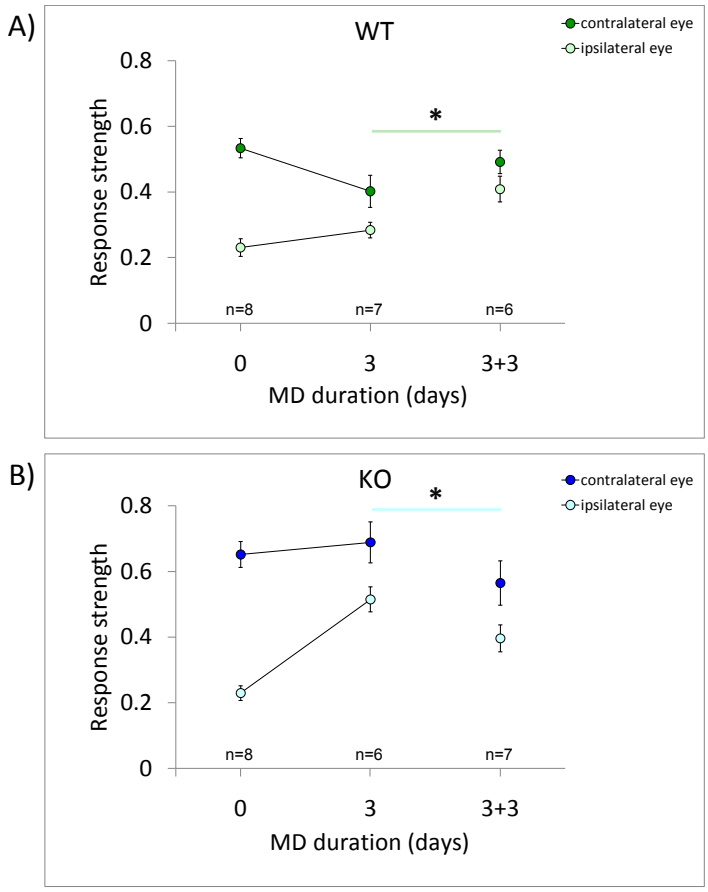

Figure 4.14: Prior MD induces differential effects in PirB KO mice and WT mice. A) Comparison of each eye's response strength in non-deprived WT mice, after a single three day MD, and after a second three day MD (3+3). Note significant increase in ipsilateral (open) eye response strength after repeated MD (0.40 ± 0.03, n=7) in comparison to a single three day MD (0.28 ± 0.02, n=7). The increase of contralateral (closed) eye response strength is not significant. B) Response strength analysis of PirB KO mice. After repeated MD, the response strength of the ipsilateral (open) eye is significantly *decreased* (0.39 ± 0.03, n=7) in comparison to three days deprived KO mice (0.51 ± 0.04, n=7). Error bars indicate SEM. As with WT mice, the change in contralateral eye response strength is not significant.

(3+2) indicating that two days of MD in adult mice is too short to induce an MD shift. The effect in PirB KO mice was also absent which is not surprising as the WT mice did not display plasticity for this deprivation time.

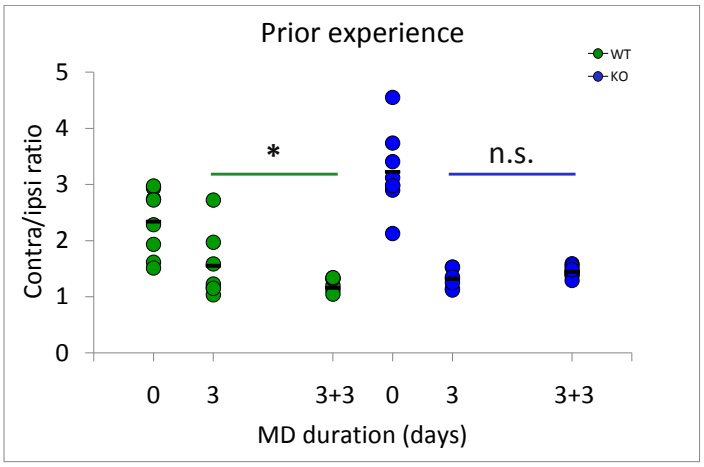

Figure 4.15: The effect of prior experience is occluded in PirB KO mice. The contra/ipsi ratios of WT (green) and PirB KO mice (blue) are plotted as a function of MD history. Note significant difference between naïve, three days deprived and repeated deprived WT mice. In contrast, repeated experienced PirB KO mice show no further shift.

In summary I have observed that PirB KO mice display no enhancement of plasticity by prior MD, suggesting a potential role for PirB in the priming effect described by Hofer et al (2006).

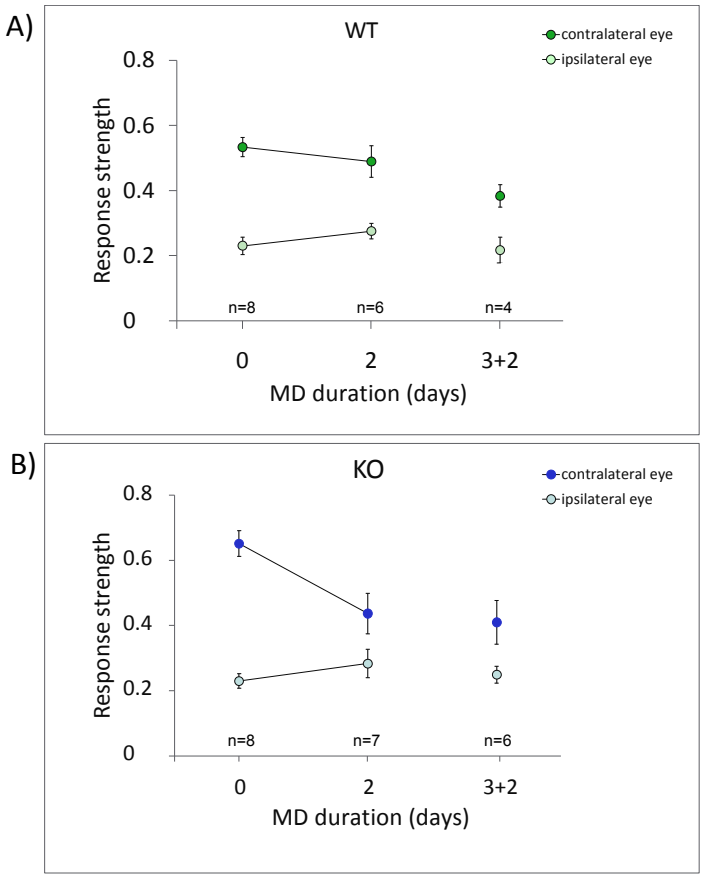

Figure 4.16: Shorter repeated deprivation time reveals no further metaplasticity in PirB KO mice and WT mice. In the response strength analysis I analyzed whether three days of MD during the critical period and two days of MD in adulthood induces metaplasticity in PirB KO mice. There were no apparent changes in both genotypes (A+B) after shorter, repeated MD. Error bars indicate SEM.

4.5 Response strength analysis of adult PirB KO mice after peripheral visual stimulation

Shatz and colleagues have found that vision of non-deprived PirB KO mice is indistinguishable from WT mice. After MD, however, PirB KO mice showed a strong expansion of the ipsilateral eye projection beyond the border of the normal binocular visual cortex in comparison to WT mice. Based on these results I wanted to test whether enhanced responses to ipsilateral eye stimulation could be detected in deprived PirB KO mice with intrinsic optical

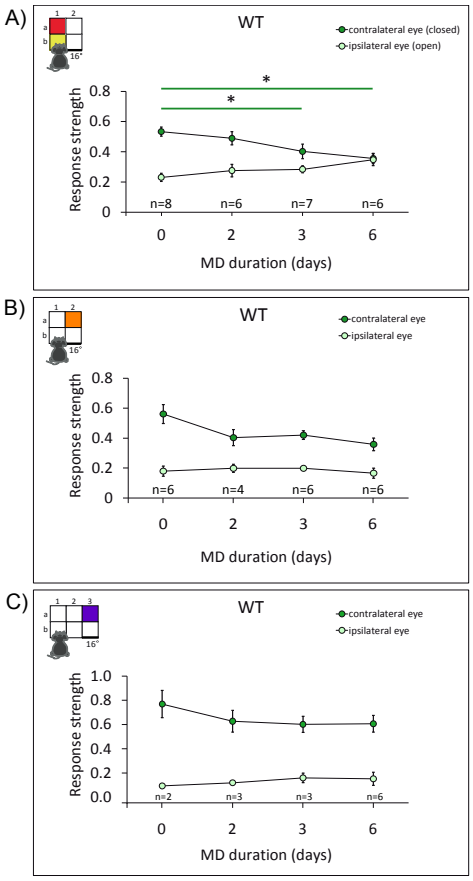

Figure 4.17: Response strength in adult WT mice (P90) is unaltered after peripheral visual stimulation. A) As a reference, the response strength analysis for central visual field stimulation is shown. B) In contrast to central visual field stimulation (A), peripheral stimulation (16 degrees distant from the central visual stimulus) reveals no significant activity changes upon independent eye stimulation. C) Stimulation even further into the peripheral visual field (32 degrees from central stimulus) also reveals no increase in ipsilateral response strength in comparison to the analysis which is shown in A).

imaging after stimulation at the border of the binocular visual field into the periphery. I analysed the response strength in adult WT mice after central (Fig. 4.17A) and peripheral visual stimulation (Fig. 4.17B+C). In comparison to response strength changes induced by MD after central visual field stimulation, there was no significant change in response strength of WT mice after presenting stimuli in the peripheral visual field (Fig. 4.17B). Note that with further peripheral stimulation, the contralateral eye evoked activity increased, whereas the

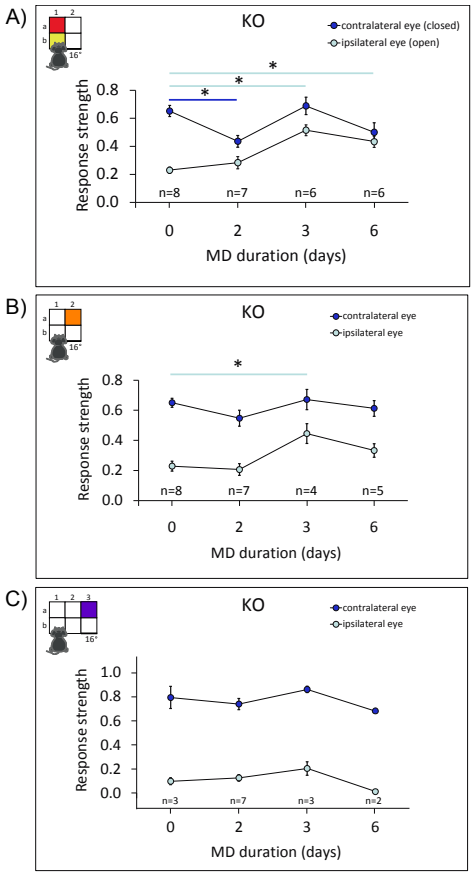

Figure 4.18: Ipsilateral eye response strength of adult PirB KO mice (P90) is enhanced after peripheral visual stimulation. A) A response strength analysis of the central visual field is shown. B) Peripheral visual stimuli which were 16 degrees apart from the central visual field were shown. A significant increase of ipsilateral response strength after three days of MD can be observed in comparison to non-deprived PirB KO mice after peripheral visual stimulation (3dMD: 0.44 ± 0.07, n=4; non-deprived mice: 0.23 ± 0.03, n=8). C) Advanced peripheral stimulation (32 degrees distant to central visual field stimulation) shows no further changes in ipsilateral response strength after MD.

ipsilateral eye driven activity decreased (Fig. 4.17C). This is not surprising, as the peripheral visual stimulus was presented outside the binocular visual field towards the monocular visual field where there is no ipsilateral representation. Thus, in line with the Shatz lab, I detected no apparent MD induced changes in response strength in adult WT mice after peripheral visual stimulation.

Next, I focused on the peripheral visual field of adult PirB KO mice and subsequent changes in activity after MD (Fig. 4.18B). I observed a significant increase in ipsilateral response strength after three days of deprivation in comparison to the non-deprived state (PirB-/-, nonMD vs 3dMD: $p<0.0005$, t-test; n=8). Further peripheral visual stimulation led to a decline in ipsilateral response strength and an increase in contralateral eye response strength (Fig. 4.18C) similar to the findings in adult WT mice (Fig. 4.17C).

I was also interested whether I would observe OD shifts in the context of peripheral visual

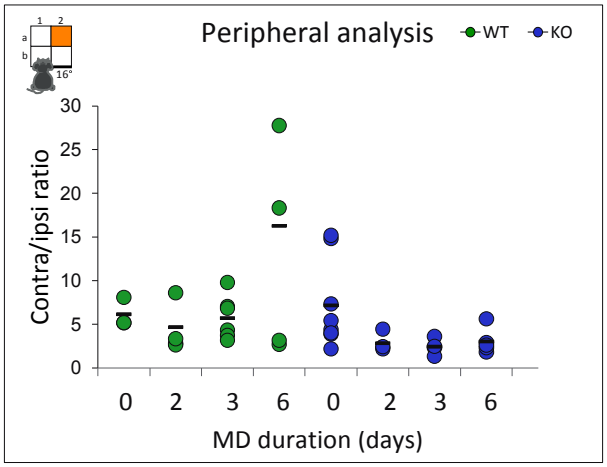

Figure 4.19: Absence of OD shift after peripheral visual stimulation. OD shift analysis of adult WT (in green) and PirB KO mice (in blue) after peripheral visual stimulation. The contra/ipsi ratio is plotted against different MD periods. Stimuli which were 16 degrees in distance to the central stimuli presentation, were shown. There is no apparent change in OD in the peripheral visual field of adult WT and PirB KO mice.

stimulation in adult WT mice (in green) and in PirB KO mice (in blue) after MD (Fig. 4.19). According to the findings of the Shatz lab, there was no differences in the contra/ipsi ratio of non-deprived WT and PirB KO mice. Focusing on deprivation effects, I detected no apparent changes of contra/ipsi ratio in dependence of deprivation periods in both genotypes. Note that the measure of contra/ipsi ratio in the context of periphery is difficult as in some cases the ipsilateral eye response strength decreases below the detection threshold with further peripheral visual stimulation. Thus the contra/ipsi ratio in the peripheral anal-

ysis reaches higher values in comparison to the central visual field analysis in the preceding sections about juvenile and adult mice.

In summary, analysis of peripheral visual field stimulation indicates that the enhanced response strength after ipsilateral eye stimulation which I observed in adult PirB KO mice (Fig. 4.18B) is in accordance with the findings of Syken et al, who found an expanded ipsilateral eye projection in the visual cortex of adult PirB KO mice using arc in situ.

5 Discussion

In this study, I investigated whether genetic deletion of the MHCI receptor PirB leads to enhanced OD plasticity. I was able to detect OD plasticity of PirB KO mice during development and adulthood after relatively short MD periods. During the critical period, PirB KO and WT mice displayed comparable OD shifts (Fig. 4.8). A focus on the response strength kinetics of the individual eyes revealed a heterogenous picture. In juvenile control mice, I observed a weakening of the deprived eye responsiveness which was followed by a strengthening in the non-deprived eye (Fig. 4.5). This result is in accordance with the literature. In juvenile PirB KO mice I assessed an accelerated closed eye depression after two days and no open eye potentiation even after five days of MD (Fig. 4.6). Additionally OD shifts were also present in adult WT and transgenic mice (P90). I explored that the OD shift is more rapid in adult PirB KO mice (Fig. 4.12). Regarding the eye specific plasticity I found a steady decline in closed eye response strength in WT mice and a non-significant increase in open eye strength (Fig. 4.10). PirB KO mice displayed a rapid closed eye depression and a subsequent potentiation of closed and open eye response strength (Fig. 4.10).
Additionally I investigated OD plasticity in adult deprived mice (C57Bl6) which is still under debate to occur. Thus I will relate these results in adult plasticity to the recent findings in the literature in the next chapter.

5.1 OD plasticity in adult C57Bl6 mice

It has generally been assumed that OD plasticity in the visual cortex is strictly limited to a critical period early in live. Recent findings in the mouse have questioned this dogma, but the results on adult OD plasticity are highly variable. Some labs showed clear evidence for

OD plasticity in adult mice, but other labs have not been able to measure any shifts with their respective technique. In this section, I will address the question why there is such a high variability of results and I will evaluate recent reports as well as my own data along four criteria: measurement technique, potential species differences between rats and mice, impact of genetic background, and age-dependence of OD plasticity.

Clear OD plasticity in adult mice was initially detected with VEP recordings in the Bear lab (Sawtell et al. 2003). This finding was confirmed by other labs (Hofer et al. 2006, Fischer et al. 2007, Lehmann and Löwel 2008) and is also supported by my results. Critics of adult OD plasticity argue that so far only certain measurement techniques (such as VEP recordings (Sawtell et al. 2003), intrinsic optical imaging (Hofer et al. 2006) and immediate early gene expression, (Tagawa et al. 2005)) have been able to detect adult OD plasticity, whereas such as extracellular single-unit recordings have failed in demonstrating adult OD shifts (Morishita and Hensch 2008). It was postulated that, only extracellular recordings, which measure mainly suprathreshold events are sufficient in detecting changes in visual acuity (which are typically seen after MD) and are therefore adequate for investigating OD plasticity (Morishita and Hensch 2008). However, a combined optical imaging and extracellular recording study has clearly shown that adult OD plasticity can be reliably measured with both techniques (Hofer et al. 2006).

What about the argument that visual acuity has not been detected with other techniques than single-unit recordings? A recent study investigated visual acuity in a behavioral paradigm using a virtual optomotor system which was first introduced by Douglas and Prusky (2004) in combination with intrinsic optical imaging (Lehmann and Löwel 2008). The authors detected an increase in visual acuity of the open eye in juvenile *and* adult mice (P25-P90) which underwent MD. Thus, it is possible to assess changes in visual acuity following deprivation not only with single-unit recordings but also with a combination of techniques such as intrinsic optical imaging or behavioural paradigms.

Many experiments have shown that OD plasticity is largely restricted to the critical period in higher mammals such as cats and monkeys. In contrast, a discrepancy on the extent of adult OD plasticity does exist in rodents. Several studies investigating OD plasticity in adult rats have been unable to detect changes in OD after MD (Fagiolini et al. 1994, Pizzorusso et al. 2002). Using VEP recordings, it was impossible to show changes in response strength

of the deprived and the open eye in adult rats (Fagiolini et al. 1994), while the same method revealed OD shifts in adult mice (Frenkel et al. 2006). Single-unit recording also failed to detect OD shifts in adult rats (Pizzorusso et al. 2002). But there are also studies which indicate that visual cortex in adult rats remains plastic. One is based on enhanced VEPs in the visual cortex (Heynen and Bear 2001). LTP of field potentials was induced in the visual cortex and resulted in enhanced response strength in adult rats. While the current data point to differences in OD plasticity between rats and mice in adulthood, one should also consider that fewer labs work with rats and only a subset of techniques has been used to assess adult OD plasticity. Thus it would be worthwhile to start investigations of OD shifts using intrinsic optical imaging to extent the range of studies focusing on adult rats.

As a next parameter potentially important for adult OD plasticity, I will address the role of genetic background in mice. Levelt and his colleagues identified specific gene loci contributing to OD plasticity and compared them in different imbred strains of C57Bl6 and DBA/2J mice (Heimel et al. 2008). They could demonstrate that C57Bl6 mice (P35) showed pronounced OD shifts, which were significantly reduced in strains with mixed background. Transgenic mice often have a mixed genetic background, and thus it is important to consider which controls are chosen for comparison. As I am also working with transgenic mice, I will take these findings into account.

When does the critical period end? Based on findings which reported a gradual decline rather than a sudden end of plasticity (Fischer et al. 2007, Hofer et al. 2006), it was suggested that the critical period could outlast P35 and might be extend until P60 (Morishita and Hensch 2008). A recent study has directly addressed the age-dependence of OD plasticity in adult mice (Lehmann and Löwel 2008). The authors found prominent plasticity below P110 which was assessed with intrinsic optical imaging. OD plasticity was diminished after long-term MD in mice which were older than P110. Thus, while this detailed study lends further support to the presence of OD plasticity in adult mice, it also shows that there is an age limit to it.

I recapitulate that I have been able to detect OD plasticity in adult mice (P90) with intrinsic optical imaging. In contrast to juvenile mice where OD plasticity can be induced after short-term MD, I detected a clear OD shift in adult mice after six days of MD (Fig. 4.3). Other labs have shown that this prolonged MD period was also used in combination with other

techniques to induce reliably adult OD plasticity (Hofer et al. 2006, Frenkel et al. 2006, Fischer et al. 2007).

Starting with a publication from the Bear lab (Sawtell et al. 2003), it has become obvious that the overall OD shift is composed of two components, namely an increase in open eye response strength and a decrease in that of the closed eye. While this might sound trivial, up to that paper only very few studies have actually analyzed the two eyes independently. Most papers have used single cell recordings, which, due to the relatively small sample size, hardly allow making statements on the absolute response level across animals (see however Gordon and Stryker, 1996). Instead, for each recorded neuron, a ratio was formed, comparing contra- and ipsilateral eye response. Since the publication by Sawtell and colleagues, more and more studies have used techniques that allow for assessing the individual changes in response strength of both eyes (*IOI:* (Hofer et al. 2006, Kaneko et al. 2008) this study; *two-photon calcium imaging:* (Mrsic-Flogel et al. 2007); *VEPs:* (Frenkel and Bear 2004, Frenkel et al. 2006)). Since it is becoming very obvious that the mechanisms leading to the response strength changes of the deprived and non-deprived eye might be very different, future studies on OD plasticity should be designed such that both eyes cortical representation can be determined independently. The section on juvenile OD plasticity (see 4.2, Figure 4.5) clearly illustrate the importance of this concept. Focusing on the kinetics of either eye after MD, I observed a steady depression of the closed eye which was significantly different after five days of MD (Fig. 4.2). Closed eye depression after long-term MD in adult mice has been also reported before using Arc in situ hybridization (Tagawa et al. 2005) or VEP recordings (Frenkel et al. 2006). In addition I detected a strong open eye potentiation after six days of MD in adult mice. This increase in open eye response strength is also in accordance with the literature (Sawtell et al. 2003, Hofer et al. 2006, Fischer et al. 2007).

5.2 Response strength analysis in juvenile and adult PirB KO mice

The main aim of this study was to test whether OD plasticity in PirB KO mice is enhanced during development into adulthood. While the Shatz lab found indeed evidence for enhanced plasticity in PirB KO mice (Syken et al. 2006), limitations of their technique did only allow them to assess changes in the representation of the non-deprived eye. Using intrinsic signal

imaging, we were able to also test the strength of the deprived eye . Several labs have observed that short-term deprivation of juvenile mice during the critical period leads to a rapid decrease in closed eye response strength, which is followed by an enhanced responsiveness of the open eye after longer MD durations (Frenkel and Bear 2004, Hofer et al. 2006, Kaneko et al. 2008). Our findings on eye specific changes in normal juvenile mice after different periods of MD are basically in accordance with these reports: closed eye response strength has already declined after two days, whereas open eye strengthening proceeds more steadily and slowly (Fig. 4.5). In principle, all these findings are in accordance with a model put forward by Bear and colleagues (Heynen et al. 2003), which states that the initial closed eye weakening is brought about by an LTD-like process, triggered by the decorrelation of deprived eye input activity due to eye closure. Whether the subsequent open eye strengthening is mediated by an LTP-like process that is promoted by a shift of the modification threshold as featured by the BCM model ((Bienenstock et al. 1982) see also introduction), or whether it is caused by NMDA receptor independent homeostatic synaptic scaling (Turrigiano and Nelson 2004, Mrsic-Flogel et al. 2007) is currently unclear.

In contrast, the findings in the literature on individual eye response strength changes during adult OD plasticity are more diverse. There are several studies which found that only open eye response strength changes in adult mice after MD (Fischer et al. 2007, Sawtell et al. 2003, Hofer et al. 2006). Other studies also observed closed eye depression in adult mice (Tagawa et al. 2005, Frenkel et al. 2006). In line with these results, in the present study adult WT mice showed a steady decline in closed eye response strength with increasing MD duration, along with open eye strengthening (Fig. 4.10A). Further we cannot rule out that our PirB KO and the respective WT mice express a phenotype of heterogenous OD plasticity as they are derived from a mixed background (C57BL6 and SV129/2J) (Heimel et al. 2008). An earlier study from the Bear lab has already pointed out that visual acuity differences are present with respect to the mouse strain (Muhammad et al. 2007, Soc. Neurosci Abstracts, No. 130.9).

5.2.1 PirB KO mice display enhanced OD plasticity in comparison to WT mice throughout life

In this section, I will put my results in context to the Shatz lab's findings (Syken et al. 2006) and I will discuss new observations on the representation of the deprived eye in juvenile and adult PirB KO mice. The major result of the study by Syken et al. was the detection of an expanded ipsilateral eye representation into the previously monocular visual cortex in contralaterally deprived juvenile and adult PirB KO mice. This expansion was assumed to reflect an increased strengthening of the non-deprived eye in the KO mouse. I used a functional analysis in adult PirB KO mice, which were deprived for three or five days, and found an enhanced response strength of the ipsilateral eye. This increase in comparison to WT mice, which show only a very modest increase in open eye response strength, was evident after central (Fig. 4.10B) as well as more peripheral visual field stimulation (Fig. 4.18B). The peripheral visual field stimulation was specifically carried out to test for the strength of the ipsilateral eye representation in the cortical region where Syken et al. observed the expansion of the ipsilateral eye input in PirB KO mice using arc in situ hybridisation. Thus, in adult mice my results, fully support the data from Shatz and colleagues, which were obtained with a very different method.

In contrast to Syken et al., however, I was unable to detect enhanced ipsilateral eye response strengthening in juvenile PirB KO mice after MD; in fact, ipsilateral response strength in non-deprived PirB KO mice is of similar magnitude as after five days of deprivation in the central visual field (Fig. 4.6) . The reason for this could be that ipsilateral eye response strength in non-deprived PirB KO mice has already a relatively high level, significantly above that of WT mice. But also systematic differences could account for potential mismatches between us and them. Syken et al. predominantly focused on OD plasticity after monocular enucleation. In their study they used eleven days MD to reliably assess OD plasticity. Still the effects after monocular enucleation were stronger in contrast to the MD effects assessed with Arc in situ hybridization ((Syken et al. 2006) and personal communication).

The major advantage of the method used in this study, intrinsic signal imaging, in comparison to the Arc in situ technique employed by Syken et al. is that it also allows for measuring responses of the contralateral eye (to detect the Arc signal derived from the ipsi-

lateral eye, the contralateral eye has to be removed). In juvenile PirB KO mice, I observed a stronger and more rapid drop in closed eye response strength in comparison to WT mice (Fig. 4.7A). Adult PirB KO mice also exhibited OD plasticity on a shorter time scale (two days) in comparison to WT mice (Fig. 4.10A and B). However, the kinetics of deprived eye response strength in adult KO mice were very peculiar, in that response strength increased again after three days of MD, to decline with yet longer MD durations. Thus, in some sense, adult PirB KO mice exhibit juvenile like OD plasticity (compare Fig. 4.5 and Fig. 4.10B). The somewhat paradoxical increase in closed eye response strength after a three day MD in adult PirB KO mice occurs in parallel to a rapid increase of non-deprived eye responses. I speculate that the rapid closed eye weakening is due to LTD, which might be caused by uncorrelated presynaptic activity in the retina and LGN (Bear 2003, Sjöström et al. 2003). The subsequent parallel increase in closed and open eye response strength might be explained by the BCM theory ((Bienenstock et al. 1982), for further details see introduction section 2.2.1). Deprivation of the contralateral eye leads to a considerable reduction in electrical activity in the binocular visual cortex. This leads to the global adjustment of the plasticity threshold, which facilitates potentiation of the open and the deprived eye. This speculation is based on increasing numbers of studies pointing to a mechanism that share competition based (Bienenstock et al. 1982, Bear 2003) and homeostatic based experience dependent plasticity (Turrigiano and Nelson 2004, Mrsic-Flogel et al. 2007). On the other hand, this parallel increase in response strength could also be caused by a direct change in the ability to undergo LTP in the KO mice. My colleague Maja Djurisic investigated LTP in vitro in the hippocampus of adult PirB KO mice, and found it to be enhanced (Djurisic et al. 2007, Soc. Neurosci Abstracts, No. 131.18). Based on these ideas and the current findings it is obvious that the mechanism of enhanced plasticity in PirB KO mice is not clear yet. Furthermore, a recent study pointed to an additional role of PirB as a receptor of myelin based inhibitors (Atwal et al. 2008), which will be discussed in the last section (see 5.3).

5.2.2 Metaplasticity is occluded in PirB KO mice

A recent study from our lab showed that prior experience facilitates the induction of OD shifts in adult mice (Hofer et al. 2006). Would further enhanced plasticity be present in

mice which lack PirB under a paradigm that amplifies plasticity? In contrast to WT mice (Fig. 4.15A), prior experience of PirB KO mice did not lead to further enhanced response strength. Adult PirB KO mice which were single deprived for three days have already displayed a significant OD shift and could not be enhanced with prolonged MD of six days (Fig. 4.12). As these mice are lacking a restriction factor which plays a role in modulating plasticity in the adult CNS upon visual experience, the level of plasticity in PirB KO mice might be saturated after the second deprivation period. Along the results of Hofer et al., (2006) prior experience 'leaves a lasting trace' in the binocular visual cortex of juvenile mice and repetition of this experience (here: MD) in the future leads to accelerated OD plasticity in adult mice. Recently they addressed their functional findings in a structural analysis (Hofer et al. 2009). There they observed that new spines were formed during deprivation, persisted after eye reopening and became functional again after the second deprivation of the same eye in adulthood. In their study they hypothesized that the persistent spines encode for the trace of prior experience. What would be the outcome of a structural analysis in PirB KO mice? The observation of this saturation effect in primed PirB KO mice could either point to no spine gain after the initial MD which seems to be unlikely. One might rather speculate that the number in persistent spines is lower and that a second MD would lead to new spine gain. This speculation would be in line with the postulated role of PirB in consolidating functional and structural plasticity.

5.3 PirB as a substrate for OD plasticity in juvenile and adult mice

There is more and more evidence that experience-dependent changes during development are linked to structural plasticity (Antonini et al. 1999, Hofer et al. 2009). Could PirB be involved in bridging functional plasticity and subsequent anatomical rearrangements? Apart from its function as an MHCI receptor, PirB was recently also recognized as a receptor for myelin inhibitory proteins (Atwal et al. 2008). In 1988, Schwab and colleagues discovered that Nogo-A, a myelin derived component, has a suppressive function on neurite outgrowth in tissue of the adult CNS (Caroni and Schwab 1988), and that antibodies against Nogo-A prevents this inhibition (Chen et al. 2000). After the discovery of the Nogo receptor NgR, a

KO mouse lacking this receptor was created, which showed acute growth cone expansion in the peripheral nervous system despite the action of myelin inhibitory proteins (Zheng et al. 2003). Surprisingly, neurite outgrowth on myelin was still inhibited in the central nervous system of NgR KO mice, which pointed to another modulator limiting axonal outgrowth. In a very recent expression cloning study, Atwal et al. (2008) detected PirB as a candidate molecule mediating axonal growth. Investigation of cultured mouse cerebellar granule cells of PirB KO mice revealed that there was a partial disinhibition of neurite outgrowth on a substrate containing myelin and Nogo. Would the synchronous block of PirB and NgR lead to complete disinhibition of myelin inhibitory proteins? Atwal et al. investigated whether neurite outgrowth from cultured neurons of NgR KO mice in the presence of anti-PirB antibodies would be unaffected due to the blocking effects of myelin inhibitory proteins. Indeed, they found that inactivating both receptors in this fashion was sufficient to allow outgrowth of neurites even in the presence of myelin. Thus, it seems that the concerted action of PirB and NgR profoundly limits axonal outgrowth in the central nervous system. Limitation of axonal growth might not only play a role under conditions of brain injury.

PirB and NgR are also involved in limiting plasticity of synaptic connections in the visual cortex of mice, as this and other studies (McGee et al. 2005; Syken et al. 2006) show. Similar to our findings and those of Syken et al. (2006) in PirBKO mice, adult NgR KO mice displayed rapid OD plasticity after short-term MD (McGee et al. 2005). What is the physiological role of growth inhibiting factors in the healthy adult CNS? Anatomical studies indicate that the adult visual cortex contains continuously changing neuronal networks, which depend on visual experience (Antonini et al. 1999, Hofer et al. 2009). As OD plasticity is less pronounced in adult mice, it is likely that this effect is at least partially mediated through the repulsive action of myelin inhibitory proteins. It is still an open question whether PirB or NgR are involved in structural plasticity such as acute axonal growth or even in the formation of new connections. The finding that PirB acts as a receptor for myelin inhibitory proteins sheds a new light on the field of axonal regeneration and adds more questions to the inhibitory role of these receptors on structural plasticity in the central nervous system. It will be interesting to pursue experiments on OD plasticity and axonal regeneration in NgR-PirB double-KO mice. A better understanding of the role of both molecules and their

potential interactions might even lead to novel approaches for therapeutic interventions in order to promote axonal regeneration in paraplegic patients .

Apart from these potential future applications, the results presented in this thesis support the concept that proteins of the immune system also play important roles for development and plasticity in the healthy nervous system.

6 Abbreviations

LGN	lateral geniculate nucleus
SC	superior colliculus
nACHR	nicotinergic acetylcholine receptor
TTX	tetrodotoxin
MD	monocular deprivation
BDNF	brain-derived neurotrophic factor
GAD	glutamic acid decarboxylase
GABA	gamma-aminobutyric acid
LTP	long-term potentiation
LTD	long-term depression
NT	neurotrophins
NMDAR	N-methyl-D-aspartate receptor
BCM	Bienenstock-Cooper-Munro
tPA	tissue plasminogen activator
VEP	visually evoked potentials
ECM	extracellular matrix
ERK	extracellular signal-regulated kinase
PKA	protein kinase A
CaMKIIα	calcium/calmodulin-dependent protein kinase II alpha
CREB	cAMP-responsive element-binding protein
MHCI	class I major histocompatibility complex
TNFα	tumor necrosis factor alpha
RGC	retinal ganglion cells
PirB	paired-immunoglobulin-like receptor B
IOI	intrinsic optical imaging

7 Curriculum vitae

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Journal papers

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the mouse retina." (Eur J Neurosci, 2005 Dec;22(11):2735-43)

Poster

- Thomas Ladewig and Miriam Mann. "Calcium Buffering in Retinal Ganglion Cells". German Physiology Congress 2004
- Miriam Mann and Mark Hübener. "Developmental function of PirB restricts adult ocular dominance plasticity". FENS, 2008
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