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Genetic control of experience-dependent plasticity in the visual cortex

J. A. Heimel*, J. M. Hermans, J.-P. Sommeijer, Neuro-Bsik Mouse Phenomics consortium and C. N. Levelt

Netherlands Institute for Neuroscience, Royal Netherlands Academy of Arts and Sciences, Meibergdreef 47, 1105 BA, Amsterdam, The Netherlands
**Corresponding author: J. A. Heimel, Netherlands Institute for Neuroscience, Meibergdreef 47, 1105 BA Amsterdam, The Netherlands. E-mail: heimel@nin.knaw.nl*

Depriving one eye of visual experience during a sensitive period of development results in a shift in ocular dominance (OD) in the primary visual cortex (V1). To assess the heritability of this form of cortical plasticity and identify the responsible gene loci, we studied the influence of monocular deprivation on OD in a large number of recombinant inbred mouse strains derived from mixed C57BL/6J and DBA/2J backgrounds (BXD). The strength of imaged intrinsic signal responses in V1 to visual stimuli was strongly heritable as were various elements of OD plasticity. This has important implications for the use of mice of mixed genetic backgrounds for studying OD plasticity. C57BL/6J showed the most significant shift in OD, while some BXD strains did not show any shift at all. Interestingly, the increase in undeprived ipsilateral eye responses was not correlated to the decrease in deprived contralateral eye responses, suggesting that the size of these components of OD plasticity are not genetically controlled by only a single mechanism. We identified a quantitative trait locus regulating the change in response to the deprived eye. The locus encompasses 13 genes, two of which – *Stch* and *Nrip1* – contain missense polymorphisms. The expression levels of *Stch* and to a lesser extent *Nrip1* in whole brain correlate with the trait identifying them as novel candidate plasticity genes.

Keywords: BXD, down syndrome, Nrip1, ocular dominance, quantitative trait loci, recombinant inbred, Stch

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Visual experience during postnatal development has a major impact on how neurons in the primary visual cortex respond to visual stimuli later in life. Especially during a sensitive period of development, monocular deprivation (MD) can cause a severe loss of acuity and shift the ocular dominance (OD) toward the undeprived eye. In the last decade, the use of mice has advanced the study of the molecular mechanisms that underlie OD plasticity, greatly improving our understanding of cortical plasticity. Mutant mice with known deficits in other forms of synaptic plasticity or neuronal function have been analyzed for changes in OD plasticity leading to the identification of various important molecular and cellular mechanisms involved. This method of logical induction has been successful, but more systematic approaches are necessary to identify the remaining crucial molecular players. One such approach is a survey of differential genes expression using subtractive libraries or microarrays. Studies of temporal gene expression differences during the critical period (Jiang & Yin 2007; Ossipow *et al.* 2004; Prasad *et al.* 2002) or expression changes induced by MD or enucleation (Majdan & Shatz 2006; Lachance & Chaudhuri 2004; Tropea *et al.* 2006) have led to the identification of unsuspected pathways involved in experience-dependent plasticity. Some genes that play important roles in cortical development and plasticity, however, may not be differentially expressed in the visual cortex and would not surface in these microarray studies. Conversely, many differentially expressed genes may not play any role in OD plasticity. We have therefore taken an alternative systematic approach exploiting genetic and phenotypic differences of recombinant inbred strains derived from crosses of C57BL/6J and DBA/2J mice (Taylor 1978). Optical imaging of intrinsic signal (Grinvald *et al.* 1986) was used to quantify the visual response in the primary visual cortex and the change therein induced by a period of MD. As both parental strains have been sequenced and high resolution maps of all recombinant BXD (C57BL/6J × DBA/2J) strains are available, it is possible to identify genetic loci with differences between C57BL/6J and DBA/2J that correlate with specific quantitative traits (quantitative trait locus, QTL), including OD, response strength and OD plasticity. An added advantage of this approach is that the results can be combined with the growing phenotype database of these strains, including large topographically restricted gene expression databases and behavioral and morphometric datasets (www.genenetwork.org; Chesler *et al.* 2004) to unveil novel interactions between genes and traits. Our study identifies a gene locus that correlates strongly with loss of deprived eye responses. Moreover, we show that reduction of deprived eye responses and strengthening of non-deprived eye responses – the two factors that together determine the OD shift – are not a single genetic trait. Last, we present information on the heritabilities of various vision-related and plasticity related quantitative traits. The latter is important, as most brain research in the normal healthy mouse uses the C57BL/6J inbred strain, while transgenic and knockout mice often have different (mixed) genetic backgrounds.

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Materials and methods

Mice

Male mice from C57BL/6J, DBA/2J and 20 BXD strains (1, 2, 6, 9, 11, 13, 14, 16, 19, 21, 27, 28, 29, 31, 32, 33, 34, 36, 39 and 40) were obtained from Jackson Laboratories (http://www.jax.org/) for the Neuro-Bsik Mouse Phenomics Consortium (see appendix) and bred at Harlan Germany (http://www.harlaneurope.com/). The different strains were bred simultaneously, and litters were randomly selected for imaging. From a single litter, a maximum of two animals were imaged so that data from most strains was acquired over an extended period. Juvenile animals with weights of more than two standard deviations below average were excluded from the analysis. All animal experiments were in compliance with guidelines provided by the Royal Dutch Academy of Sciences and carried out with permission of the Academy's animal experiment committee.

MD and optical imaging of intrinsic signal

At P35, intrinsic signal response to visual stimulation was measured transcranially in the left visual cortex as described previously (Heimel *et al.* 2007). Briefly, mice were anesthetized by an intraperitoneal injection of urethane (http://www.sigmaaldrich.com/; 20% in saline, 2 g/kg). Atropine sulfate (0.05 mg/ml in saline, 0.1 mg/kg) was injected subcutaneously to reduce mucous excretions. The skull was illuminated with 700-nm light. Images were acquired using an Optical Imager 3001 system (http://www.opt-imaging.com/). A monitor covered the mice' visual field from -15 to 75° horizontally and from -45 to 45° vertically. The screen was divided in 2×2 equal patches, and drifting gratings were used to map the retinotopic representation of V1. The representation of the upper nasal screen patch was used to calculate responses for the following OD measurement. For this, computer-controlled shutters alternated visual stimulation of the eyes, while drifting square wave gratings were shown in the upper nasal screen patch. The imaged ocular dominance index was defined as the iODI = (contralateral response − ipsilateral response)/(contralateral response + ipsilateral response). In a subset of the animals, right eyelids were sutured at P28 under isoflurane anesthesia as previously described (Heimel *et al.* 2007). At P35, the eyelid was reopened at the start of the imaging session. All data shown contain data from at least three animals per group. Average number of undeprived animals per strain is 5.3. Average number of successfully measured sutured animals is 4.3. The exact number of successfully imaged mice per group for each strain is given in Table S1. Undeprived animals were measured at P35 plus or minus 1 day. Suturing and imaging of MD animals was performed at exactly P28 and P35, respectively.

Heritability calculation and statistical analysis

Narrow-sense heritabilities were calculated using a custom Matlab (http://www.mathworks.com/) implementation (available at http://www.nin.knaw.nl/~heimel/software/heritability) of a calculation which takes differences in the number of animals per group into account (Lynch & Walsh 1998), given by $h^2 = (A - B)/(A + 2kB - B)$, where $k = (N - 1/N\sum_s n_s^2)/(S - 1)$, $A = [\Sigma_s n_s\ (t_s - T)^2]/(S - 1)$, $B = (\Sigma_s n_s v_s)/(N - S)$. N is the total number of imaged animals, S is the number of strains, T the overall trait average, n_s the number of animals for strain s, t_s the trait average for strain s and v_s the trait variance for strain s. Significance of a non-zero heritability was calculated by performing 1000 permutations of the values, while keeping the number of animals per strain and per group constant. P value is the fraction of heritability values on the shuffled data, which were higher than the heritability of the unshuffled data.

For the analysis of the power by which we could detect a linear relationship between contralateral response gain and ipsilateral loss, we constructed a single model, given by two equations:

$$\Delta\text{contra} = \text{mean}\Delta\text{contra} - g \times \text{SD}\Delta\text{contra} + n_c \times \text{SEM}\Delta\text{contra},$$

$$\Delta\text{ipsi} = \text{mean}\Delta\text{ipsi} + M \times g \times \text{SD}\Delta\text{ipsi} + n_i \times \text{SEM}\Delta\text{ipsi},$$

where mean Δcontra and mean Δipsi are the average measured shift in contralateral and ipsilateral eye responses, SDΔcontra and SDΔipsi are the measured standard deviations of the strain averages and SEMΔcontra and SEMΔipsi are the average measured SEMs for the strain mean shifts in contralateral and ipsilateral eye response. The random variables g, n_c and n_i are normally distributed. To model a situation where the absolute amount of gain and loss vary in the same direction, we took $M = 1$. To model a genetic variation in the balance between gain and loss or a variable level of homeostasis, we took $M = -1$. Using our measured parameters, we generated 10 000 sets of data points for the 13 strains for which we measured the OD shift, and for each set, we calculated the significance of the correlation between the absolute contralateral and the ipsilateral shifts.

Correlations between traits are given by the Pearson correlation coefficient. Student's t-tests are used to test if correlations are different from zero or if two groups or strains are significantly different.

QTL and expression correlation analysis

Linkage mapping of traits to genotypes was performed using a set of 3795 single nucleotide polymorphism (SNP) and microsatellite markers by scripts at http://www.genenetwork.org/ (Chesler *et al.* 2004; Wang *et al.* 2003). At each marker locus, a likelihood ratio statistic (LRS) (Haley & Knott 1992) was calculated by a mixture of simple marker regression, linear interpolation and standard Haley–Knott interval mapping. The significance threshold was set by a permutation test (Churchill & Doerge 1994) at the LRS value that corresponds to a 5% probability of falsely rejecting the null hypothesis that there is no linkage anywhere in the genome. The suggestive LRS threshold is defined as that which yields, on average, one false positive per genome scan (Wang *et al.* 2003). To use all available data, we included parental strains C57BL/6J and DBA/2J in this analysis. *Cis*-regulation of genes at the QTL was studied in the whole brain using the INIA Brain mRNA M430 (January 2006) PDNN dataset (Peirce *et al.* 2006).

Results

Intrinsic signal response strength and OD

We determined the location of binocular primary visual cortex using intrinsic signal optical imaging and measured responses in this region to stimulation of each of the eyes individually in a large number of mice from BXD recombinant inbred strains. The intrinsic signal response strengths to each of the eyes differed considerably between the strains (Fig. 1b). Both the contralateral and the ipsilateral eye response strengths had a non-zero heritability ($h^2 = 0.21$, $P = 0.001$ and $h^2 = 0.19$, $P < 0.001$, respectively; Table 1). Heritability is the proportion of the trait variation in an outbred population that is genetically determined. In a sample of homozygous animals, like our set of recombinant inbred mice, the actual genetic proportion of the trait variation is a little higher. As shown in Fig. 1b, the strain means of the responses to the two eyes were strongly correlated (correlation coefficient = 0.91, $P < 10^{-7}$, df = 19, t-statistic = 9.3), suggesting that most of the heritable component of response strength was shared by the two eyes. To investigate how much of the strain dependence was caused by these common factors and how much by changes in the balance between the eyes or eye specific responses, we need to separate these two factors. For this reason, we calculated the total visual response by adding the two responses. This visual response, shown in Fig. 1c, is expected to reflect more

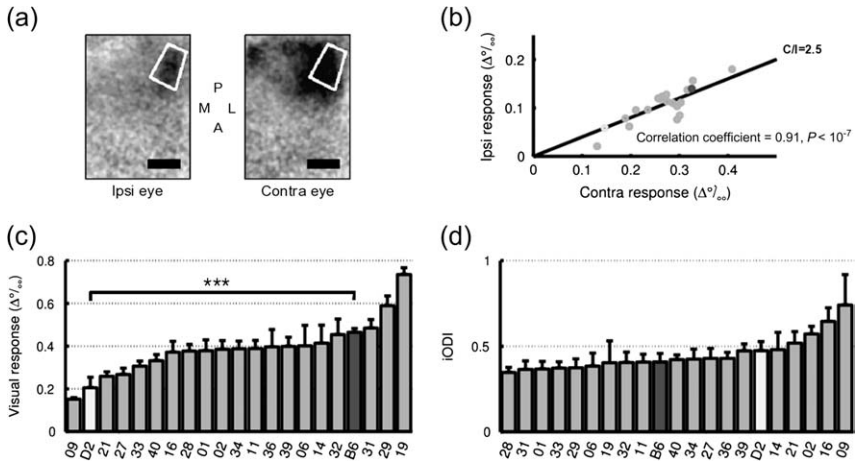

Figure 1: Intrinsic signal response to visual stimuli is strongly strain dependent. (a) Example responses in the left visual cortex of a C57BL/6J mouse with iODI = 0.42. White polygon is drawn based on the retinotopic test and marks the area used for calculating responses. Scale bar is 1 mm. (b) Intrinsic signal responses to visual stimulation of the ipsilateral eye vs. stimulation of the contralateral eye. Dots are strain means. Darker and lighter dot are C57BL/6J and DBA/2J strain means, respectively. Most of the variation in the response to ipsilateral eye stimulation is shared by the response to stimulation to the contra eye. (c) Strain means and standard errors of the sum of the responses to visual stimuli to each eye individually. Parental strains C57BL/6J and DBA/2J are significantly different ($P = 0.0005$, *t*-test, other significance levels are not shown). (d) Strain means of iODI, i.e. (contra – ipsi)/(contra + ipsi) at P35, do not show large heritable differences.

of the underlying visual response strength and to be less dependent on the balance between the eyes' responses. It is significantly heritable ($h^2 = 0.24$, $P < 0.001$). The parental lines C57BL/6J and DBA/2J are among the extreme cases of intrinsic signal response strengths and are significantly different ($P = 0.0005$, df = 9, *t*-statistic = 5.4). DBA/2J has less than half of the C57BL/6J response and less than a third of the best responding strain, BXD-19. Linkage mapping did not show any QTLs. Besides simply adding the eyes' responses, we also computed responses projected to the first principal component along which most of the response variation lies. The first principal component is given by the regression line C/I = 2.5 in Fig. 1b. This is a better method to isolate the visual response strength trait underlying the individual eyes' responses, but this also did not show any robust QTLs. The absence of a QTL for response strength is probably because of the multifactorial nature of the intrinsic signal as many traits, such as neuronal excitability, hemodynamics, skull thickness and phototransduction, can independently affect response strength. As all these traits are expected to have a heritable component but all coded by different genes, total heritability will be high but not dependent on a single QTL.

Table 1: List of trait heritabilities, h^2

Trait	Heritability	Significance (*P* value)
Response to contralateral eye	0.21	0.001
Response to ipsilateral eye	0.19	<0.001
Visual response (both eyes added)	0.24	<0.001
Visual response (first principal component)	0.23	<0.001
Ocular dominance index (iODI)	0.10	0.008
Response to contralateral eye after MD	0.18	0.004
Response to ipsilateral eye after MD	0.13	0.01
Visual response after MD	0.09	0.08
Ocular dominance index after MD	0.33	<0.001

The balance between the response of the two eyes, expressed by the imaged ocular dominance index (iODI), given by (contralateral response − ipsilateral response)/(contra response + ipsilateral response) differed over a considerable range (Fig. 1d), but heritability of this trait in the BXD lines was only 10% ($P = 0.008$). The parental strains have similar imaged iODIs.

Experience-dependent plasticity

We were particularly interested in identifying strain dependencies and/or QTLs modulating OD plasticity during the sensitive period. Mice from 17 BXD strains underwent MD from P28 to P35, covering the peak and the end of the sensitive period of C57BL/6J (Gordon & Stryker 1996). MD causes a reduction in deprived eye responses during the first days followed by a slower enhancement of open eye responses and stabilization or partial recovery of deprived eye responses (Frenkel & Bear 2004; Kaneko *et al.* 2008; Mrsic-Flogel *et al.* 2007). Depriving for one full week thus allows the analysis of both these components of an OD shift. Confirming the strain dependence of the visual response, we found that the total visual response after deprivation strongly correlated with the response level in undeprived mice at P35 (correlation coefficient = 0.82, $P < 10^{-3}$, df = 11, *t*-statistic = 4.8; Fig. 2c). However, 1 week of MD did have a wide-ranging impact on the iODI, and heritability of this trait was strong

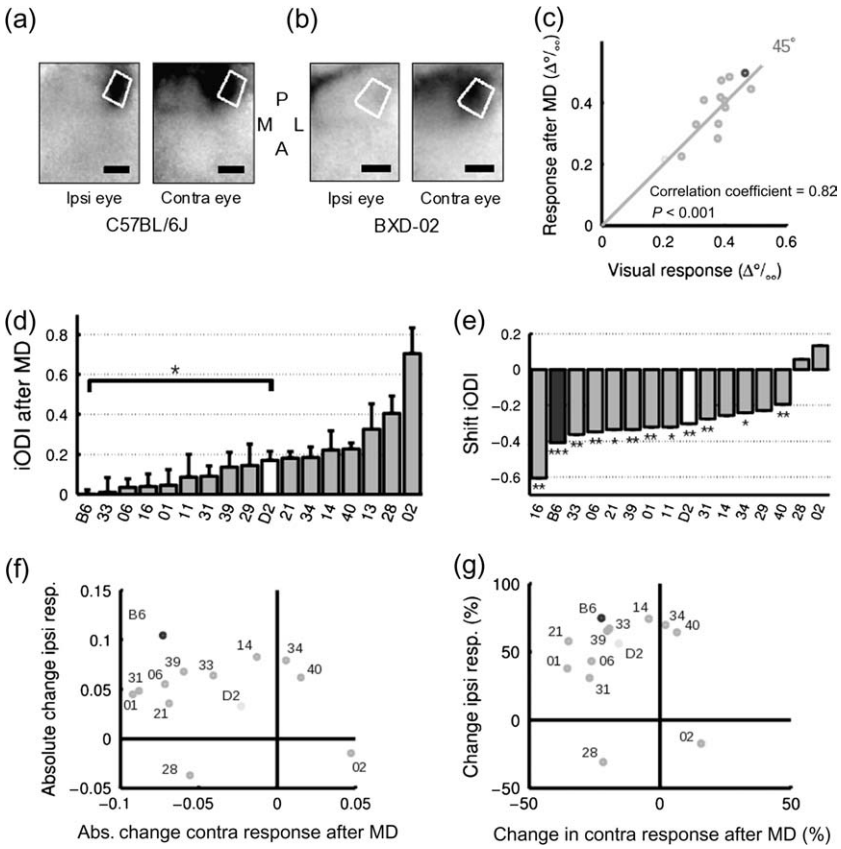

Figure 2: Ocular dominance (OD) shifts are strain dependent. (a, b) Example responses in the left visual cortex after MD of the contralateral eye of (a) a C57BL/6J mouse (iODI = 0.06) and (b) a BXD-02 mouse which did not show a shift toward the undeprived eye (iODI = 0.81). (c) MD of the contralateral eye for 1 week from P28 to P35 changes the summed visual response to both eyes very little in comparison to strain differences in summed responses. (d) There are large strain differences in iODI after MD. Parental strains C57BL/6J and DBA/2J show a significant difference ($P = 0.02$, *t*-test, other significance levels not shown). (e) BXD strains show large differences in ocular dominance shifts (iODI after MD minus iODI without deprivation). Significance levels of two-tailed *t*-test between control and deprived groups are indicated; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Four strains (BXD-02,14,28,29) did not show a significant change in ocular dominance ($P = 0.4$, 0.1, 0.5, 0.09, respectively). (f, g) Response loss for deprived eye and response gain for undeprived eye after MD are regulated by more than one genetic trait. (f) Absolute change in response of contralateral deprived eye was uncorrelated to absolute change in response to undeprived eye (correlation coefficient = −0.16, $P = 0.6$). (g) Relative change in response of contralateral deprived eye was uncorrelated to relative change in response to undeprived eye (correlation coefficient = −0.09, $P = 0.8$).

($h^2 = 0.33$, $P < 0.002$; Fig. 2d). This was already indicated by a significant difference between C57BL/6J and DBA/2J ($P = 0.02$, df = 6, *t*-statistic = 3.3). Twelve of the 16 test strains showed clear OD shifts (Fig. 2e). Four strains showed no significant difference between undeprived and deprived iODI. Two of these showed a downward trend in iODI with MD (BXD-14: $P = 0.11$, *t*-statistic = 1.8, df = 7; BXD-29: $P = 0.09$, *t*-statistic = 2.1, df = 5). The two other strains did not even show a tendency to shift (BXD-28: $P = 0.52$, *t*-statistic = −0.68, df = 7; BXD-02: $P = 0.35$, *t*-statistic = −0.97, df = 10). Five of the six monocularly deprived BXD-02 animals and three of four BXD-28 had an iODI above their undeprived strain average. Thus, while OD per se is only weakly heritable, OD plasticity is strongly influenced by the genetic makeup of different mouse strains.

Loss vs. gain of responsiveness

We next analyzed whether the changes in the responses of the two eyes were independently regulated or whether there was evidence for a genetic factor modulating the level or direction of plasticity. We also assessed whether this would be influenced by the strength of the responses in the undeprived animals, as intuitively one could expect strong responders to show a larger loss of responsiveness after MD. We did not observe any correlation between the initial signal strength and the amount of strengthening of the open eye (correlation coefficient = 0.22, $P = 0.5$, df = 11, *t*-statistic = 0.75) or weakening of the deprived eye responses (correlation coefficient = −0.2, $P = 0.4$, df = 11, *t*-statistic = 0.79). Surprisingly, we found that the changes in responsiveness to the two eyes were completely uncorrelated. Figure 2f,g shows this absence of correlation both in the change in absolute

responses to stimulation of the two eyes (correlation coefficient = −0.16, $P = 0.6$, df = 11, *t*-statistic = 0.55) and in the change in relative responses, computed as the difference in response as percentage of response in undeprived animals (correlation coefficient = −0.09, $P = 0.8$, df = 11, *t*-statistic = 0.31). Thus, these results provide no evidence for a single genetic factor governing either the total amount of change or the balance in the change of deprived and undeprived eye responses. To estimate how likely we were to have missed the presence of either factor because of non-genetic (experimental or environmental) variation, we did a power analysis using the average measured shifts in the contra and ipsilateral responses and the standard error in the strain averages (see *Methods*). We had 66% chance to have detected a single factor setting either the overall level of plasticity or the balance between gain and loss with $P < 0.05$. If loss and gain are linearly related, we had a 97% probability to have measured a correlation that was more significantly different from zero than the correlation that we measured. Given the non-zero heritability of OD shifts, this suggests the presence of more than one heritable trait underlying OD plasticity in the family of BXD strains.

QTL for loss of responsiveness

Last, we analyzed whether we could identify QTLs responsible for OD plasticity. While marker regression for the gain in undeprived eye responses did not show significant QTLs, the reduction of deprived eye responses showed a significant QTL on chromosome 16 from marker rs3656776 at 73.63 Mb to marker rs3680665 at 84.44 Mb (LRS = 21.0 if we include parental strains and standard errors) (Fig. 3a,b). This stretch contains the 13 genes given in Table 2. In order to identify the gene responsible for the trait variation, we assessed whether any of the genes contained missense SNPs or showed allele-dependent changes in expression levels. Although two of these, *Stch* and *Nrip1*, contained a SNP-causing missense mutations, the mutations were both localized to non-conserved regions of the proteins. Therefore, we also analyzed quantitative expression differences using the gene network expression database. In the absence of data on gene expression in the visual cortex during the critical period, we used the adult whole brain dataset (Peirce *et al.* 2006). We found that probes for *Stch* were strongly (anti)correlated with the trait (Fig. 4a–d for all four *Stch* probes in whole brain tissue; probe 1433772: correlation coefficient = −0.64, $P = 0.02$, df = 10, $t = 2.7$; probe 1429502: correlation coefficient = −0.87, $P = 0.0002$, df = 10, *t*-statistic = 5.7; probe 1453172: correlation coefficient = 0.91, $P < 10^{-4}$, df = 10, $t = 6.8$; probe 1430026: correlation coefficient = 0.92, $P < 10^{-4}$, df = 10, $t = 7.2$) and one of six *Nrip1* probes in the whole brain expression database correlated with the trait (correlation coefficient = −0.75, $P = 0.004$, df = 10, *t*-statistic = 3.7). *Stch* probe-binding levels appeared to be bimodal in several tissues analyzed with the same microarray (e.g. brain, eye and kidney). Probe sets 1433772 and 1429502 showed opposite levels compared with probe sets 1453172 and

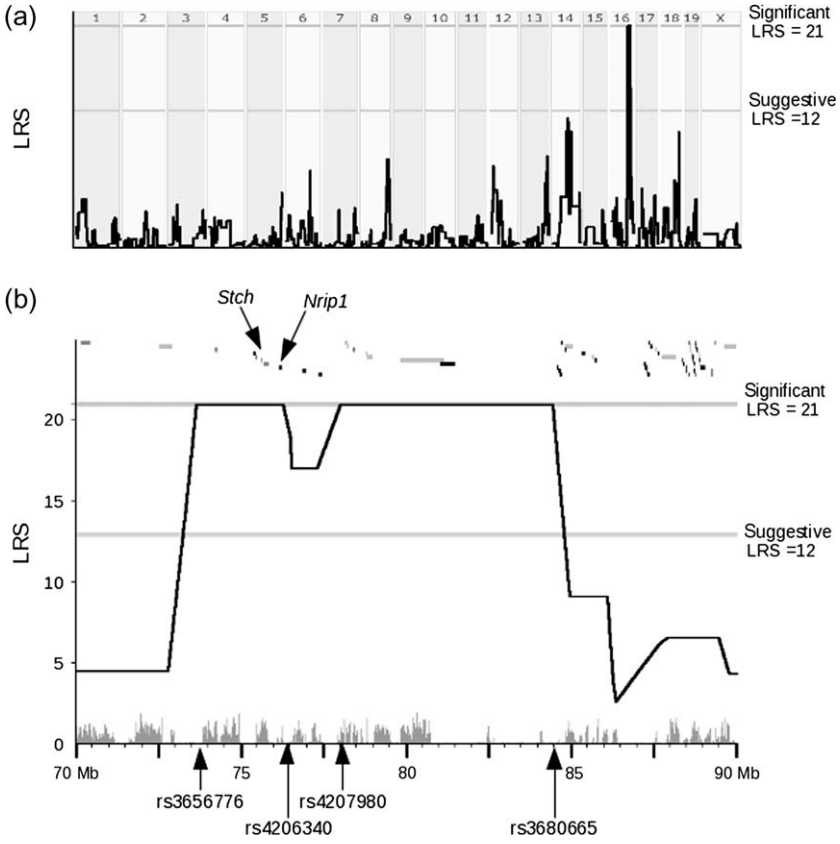

Figure 3: QTL for response reduction after MD. (a) Interval mapping of QTLs for the change in the response to the deprived contralateral eye after MD, from the first base of chromosome 1 on the left to the last base of chromosome × on the right. Black line indicates the LRS. Horizontal lines indicate suggestive and significant LRS thresholds. A significant QTL is present between markers rs3656776 and rs3680665 on chromosome 16. (b) Magnified view of the QTL showing the coding genes at its top and selected markers at the bottom. Positions of Stch and Nrip1 are indicated. Gray bars on *x*-axis indicate SNP-density.

Table 2: Genes on chromosome 16 at the quantitative trait locus for deprived eye shift

Gene	Name	Start (Mb)	Length (kb)	Number of missense SNPs	Correlation	
					r	*P*
Robo2	Roundabout, axon guidance receptor, homolog 2 (*Drosophila*)	74.23	62.03	0	0.32	0.3
Lipi	Lipase, member I	75.42	27.14	0		
Rbm11	RNA-binding motif protein 11	75.48	8.85	0	−0.65	0.02
Stch	Stress 70 protein chaperone, microsome-associated, 60 kDa	75.64	11.76	1	0.92	$<10^{-4}$
Samsn1	SAM domain, SH3 domain and nuclear localization signals 1	75.74	94.54	0	−0.43	0.2
Nrip1	Nuclear receptor interacting protein 1	76.17	82.19	1	−0.76	0.004
Cxadr	Coxsackie virus and adenovirus receptor	78.18	58.09	0	0.27	0.4
Btg3	B-cell translocation gene 3	78.24	16.92	0	−0.41	0.2
D16Ertd472e	DNA segment, Chr 16, ERATO Doi 472, expressed	78.43	33.10	0	−0.42	0.2
Chodl	Chondrolectin	78.81	20.78	0	0.35	0.3
Prss7	Protease, serine, 7 (enterokinase)	78.84	138.09	0	0.40	0.2
AK018881	RIKEN cDNA 1700066C05 gene	79.88	1264.22	0		
Ncam2	Neural cell adhesion molecule 2	81.08	423.53	0	−0.47	0.13

Gene Usp25 is excluded from this table as it is located on a part inside the QTL where a double crossover has reduced the LRS below significance. The columns labeled correlation contain for each gene the correlation coefficient and *P* value for the probe set with the highest correlation in whole brain tissue (expression data from Peirce *et al.* 2006). For the missing values, no probe was available on the AFFYMETRIX chip.

1430026. Strains with high labeling of probe sets 1433772 and 1429502 (and thus low labeling of 1453172 and 1430026), like C57BL/6J, showed large responsiveness losses, while mice with the DBA/2J allele and expression phenotype showed little loss of responsiveness after MD. The archetypical *Stch* sequence BC085181 (available at www.ncbi.nlm.nih.gov) is the only form containing the nucleotide sequences of probes 1433772 and 1429502 and must underlie the correlation of these probes (Fig. 4e). The negative correlation of the other two probes is most likely caused by differential expression of splice variants BC094508 or AK167839. Overall, the expression data suggest that *Stch* and *Nrip1* are the most likely candidate plasticity genes.

Discussion

We quantified visual responses and experience-dependent plasticity in the primary visual cortex in mice of C57BL/6J, DBA/2J and a large number of recombinant inbred strains derived from these strains. We found that the variation in intrinsic signal response to visual stimuli has a large genetic component. C57BL/6J and DBA/2J vary more than twofold in response strength. Many components along the visual pathway from opacity of the cornea to ion channel expression in the cortex can influence the neuronal response. Still other factors, such as baseline glutamate levels, or blood oxygenation and vascularization, determine how neuronal activity is translated into intrinsic signal. Therefore, it is perhaps not surprising that in spite of the considerable heritability we did not locate any QTLs for response strength. Of more relevance is the heritability of the effects of MD. The induction of OD plasticity by MD serves as a model for the onset and treatment of amblyopia (Mitchell & MacKinnon 2002), which is a loss of acuity in one or both eyes in the absence of ocular deficits affecting 2–4% of the world population (Webber & Wood 2005). Moreover, MD is also the most prevalent paradigm to study experience-dependent cortical plasticity *in vivo*.

Traditionally, OD shifts have been expressed as changes in the relative response to the two eyes (Wiesel & Hubel 1963) and the loss of deprived eye responsiveness and gain of response to the undeprived eye have been treated as one process. The response loss, however, starts before the response gain (Frenkel & Bear 2004; Mrsic-Flogel *et al.* 2007) suggesting that these may be separate processes. The loss and gain could be genetically uncorrelated instances of long term depression (LTD) (Heynen *et al.* 2003) and long-term potentiation (Rao *et al.* 2004) related processes. Alternatively, several mechanisms, shown or proposed to be involved in OD plasticity, regulate loss and gain simultaneously. Genetic variation in the metaplasticity of a sliding threshold in the balance of potentiation and depression (Bienenstock *et al.* 1982; Frenkel & Bear 2004) would exhibit itself in our data as a correlation between the changes in deprived and open eye responses, as would a genetic component in the amount of homeostatic gain in the responses of both eyes compensating for the loss of total visual input (Kaneko *et al.* 2008; Maffei & Turrigiano 2008; Mrsic-Flogel *et al.* 2007; Turrigiano & Nelson 2004). Genetic control of a hypothesized mechanism setting the magnitude of the total expressed plasticity (Philpot *et al.* 2007), however, would cause an anticorrelation between the changes in the responses of the two eyes. Our data show neither a correlation nor an anticorrelation in loss and gain. This

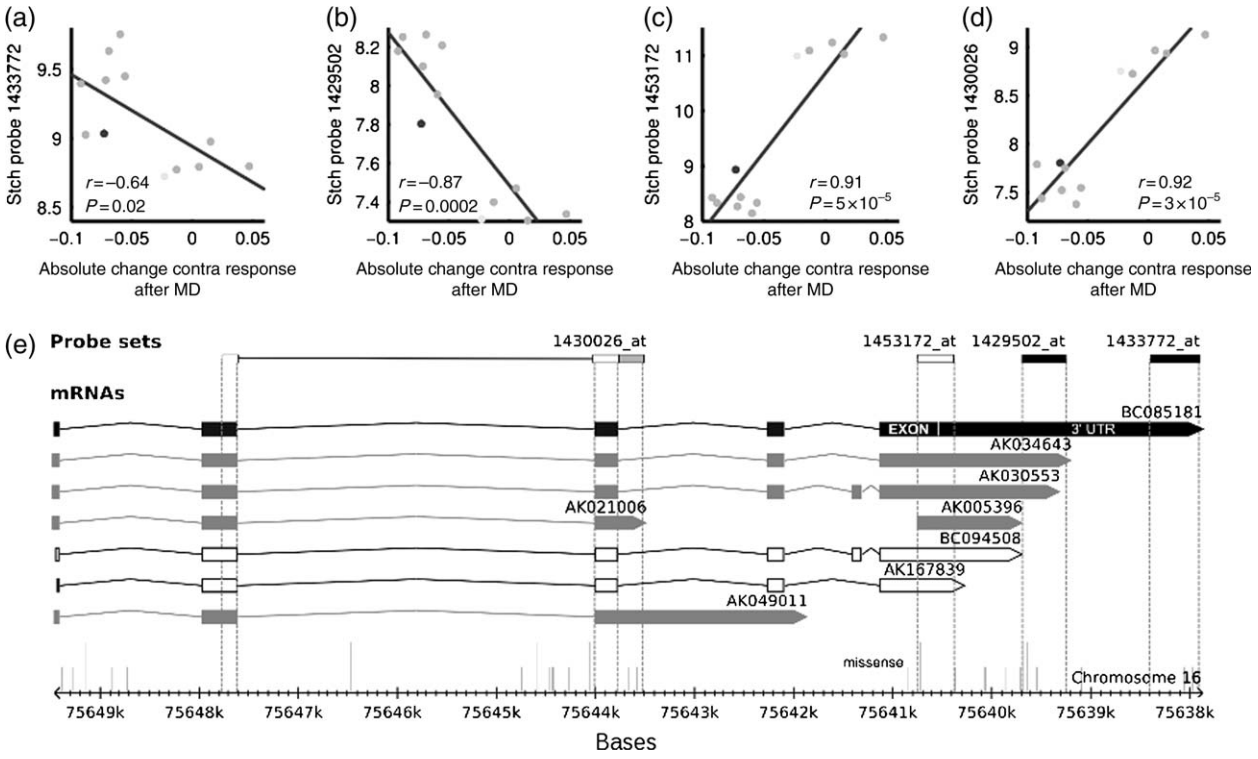

Figure 4: Expression of specific Stch splice variants correlate to loss of responsiveness after MD. (a, b) Whole brain expression of two Stch probe sets anticorrelate to response loss. (c, d) Whole brain expression of two other probes correlate. Expression data from INIA Brain mRNA M430 (January 2006) PDNN on www.genenetwork.org. (e) Genomic location of all known Stch splice variants shown aligned with probes from (a to d). Trait correlation of probes is indicated by black (negative) and white (positive). Putative splice variants underlying these correlations are indicated by the same colors. Probe correlations suggest that loss of responsiveness correlates with increased expression of the BC085181-form and reduced expression of the truncated forms BC094508 or AK167839. All three forms contain the HSP70 domain. BXD SNP locations are shown as gray bars at the bottom. Figure adapted from www.informatics.jax.org.

suggests that genetic variations in more than one of these mechanisms are causing the variation of these two aspects of OD plasticity in BXD strains.

While our screen did not provide candidate genes regulating the increase of undeprived eye responses during the critical period, we did find a significant QTL, containing 13 genes, regulating the reduction of deprived eye responses. Interestingly, this QTL is located on the region of chromosome 16 which is homologous to human chromosome 21 triplicated in down syndrome. Monocular amblyopia, for which MD is a model, has a very high prevalence (8–22%) in the population with down syndrome (Pueschel & Gieswein 1993). The Allen Brain Atlas (www.brain-map.org; Lein *et al.* 2007) shows mRNA expression in the visual cortex of six genes on the QTL: *Robo2*, *Samsn1*, *Nrip1*, *Cxadr*, *Btg3* and *Ncam2*. The Allen Brain Atlas does not show expression of the gene *Stch* anywhere in the adult brain, but other studies show clear expression in adult brain tissue (e.g. genenetwork; Reymond *et al.* 2002) and visual cortex specifically (Okazaki *et al.* 2002). We have confirmed the presence of at least two *Stch* splice variants in the visual cortex with rtPCR (unpublished data). Of the genes present on the QTL, only *Nrip1* and *Stch* contain missense SNPs, which could underlie the phenotypic variation in the absence of expression differences. However, as these mutations are localized to non-conserved regions of these proteins, this is not evident. One of six Affymetrix probe sets shows an anticorrelation between the whole brain *Nrip1* expression and the trait. *Nrip1*, formerly known as *Rip140*, encodes nuclear receptor interacting protein 1, which is an estrogen-receptor-related transcription co-regulator. Estrogen receptors can play a role in synaptic plasticity (Liu *et al.* 2008), but a role of *Nrip1* in plasticity has not been previously documented. As expression of *Nrip1* is also upregulated after dark rearing and short MD (Tropea *et al.* 2006) investigating its potential role in OD plasticity may be worthwhile. Expression of none of the genes on the QTL, including *Nrip1*, was regulated by monocular enucleation (Majdan & Shatz 2006). That study, unfortunately, did not include probes for *Stch* for which there is stronger evidence suggesting that it is the gene underlying the QTL. Two probe sets show that in whole brain tissue *Stch* has the highest correlation with the trait of all genes represented on the Affymetrix microarray, while the other two *Stch* probe sets show strong to moderate anticorrelation. This *Stch* probe level pattern is present in several tissues tested throughout the body and suggests that the DBA/2J and C57BL/6J alleles cause body wide differences in the expression of *Stch* splice variants (Fig. 4e). Interestingly, *Stch* expression in the visual

cortex is downregulated after short- or long-term MD and after dark rearing (Tropea *et al.* 2006), further supporting a role for this gene in OD plasticity. One slight note of caution is that our focus on *Nrip1* and *Stch* is based on whole brain expression in adult BXD mice. This does not exclude the possibility that other genes on the QTL are differentially regulated in visual cortex during the critical period in BXD mice. Other studies, however, on genes specifically expressed in the visual cortex (Leamey *et al.* 2008), during the critical period (Prasad *et al.* 2002; Ossipow *et al.* 2004; Jiang & Yin 2007) or induced by MD or enucleation (Lachance & Chaudhuri 2004; Majdan & Shat 2006; Tropea *et al.* 2006) have not implicated any of the other genes. Therefore, we consider *Stch* the most interesting candidate for a more targeted follow-up study.

The protein 'Stress 70 protein chaperone, microsome-associated, 60 kDa', coded by *Stch*, is a constitutively expressed member of the heat shock 70 (Hsp70) protein family and is induced by calcium increases but not by heat shock (Otterson *et al.* 1994). Proteins of the Hsp70 family are generally regarded as chaperone molecules assisting in protein folding and cellular protection against stress through an ATPase-dependent mechanism. How Stch may alter visual plasticity remains unclear. Possibly, Stch alters clathrin-mediated endocytosis by interacting with Hspa8 (Hsc70), a synaptic protein involved in uncoating clathrin-coated vesicles (Eisenberg & Greene 2007) and regulated by neuronal activity (Jiang & Yin 2007; Kaneko *et al.* 1993; Nedivi *et al.* 1993). This is a tempting explanation as long-term depression requires clathrin-mediated endocytosis (Wang & Linden 2000) and is closely related to the consequences of short-term MD (Heynen *et al.* 2003). However, the primarily microsomal localization of Stch does not quite fit this role of an active ingredient in synaptic plasticity regulation. Alternatively, Stch may in a similar fashion to BiP, another microsomally localized Hsp70 family member, regulate the surface expression of ligand gated ion channels (Wanamaker & Green 2007) or other synaptic proteins. We anticipate that these possible functions of Stch would differ significantly depending on the splice variants being expressed. The short splice variant encodes a truncated Stch protein which only contains the N-terminal leader sequence and ATPase domains, while it misses the C-terminal domain of full-length Stch which is essential for all protein interactions described.

In the last decade, knockout and transgenic mice have been instrumental in advancing our knowledge about experience-dependent plasticity. The big differences in OD plasticity in our set of inbred strains derived from two pigmented mouse strains with, at this age, a normally functioning visual system (Wong & Brown 2007) illustrate that one has to be careful about interpreting data of genetically manipulated mice vs. 'wild type' controls. Most of mouse vision research is performed with C57BL/6J animals, while genetically altered mice are usually made in other backgrounds. Insufficient back-crossing could lead to variable results at best or to misleading results in the worst case. Use of littermate controls may partially overcome this problem. Another point to note is that the strain differences in plasticity could be part of the explanation of the species difference in adult plasticity, which is seen in mice, but not in rats. All studies showing adult plasticity use C57BL/6J (Fischer *et al.* 2007; Heimel *et al.* 2007; Sawtell *et al.* 2003; Tagawa *et al.* 2005) or C57BL/6J × 129/SvJ (Hofer *et al.* 2006). In our sample, C57BL/6J is the strain showing the most significant OD shift as a juvenile. In a discussion on adult plasticity, it is useful to keep in mind that other mice strains may show less adult plasticity.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1: Means, SEMS and animal numbers for all recorded parameters

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Appendix

The Neuro-Bsik Mouse Phenomics consortium is composed of the laboratories of A.B. Brussaard, J.G. Borst, Y. Elgersma, N. Galjart, G.T. van der Horst, C.N. Levelt, C.M. Pennartz, A.B. Smit, B.M. Spruijt, M. Verhage and C.I. de Zeeuw, and the companies Noldus Information Technology (http://www.noldus.com/) and Synaptologics (http://www.synaptologics.com/).