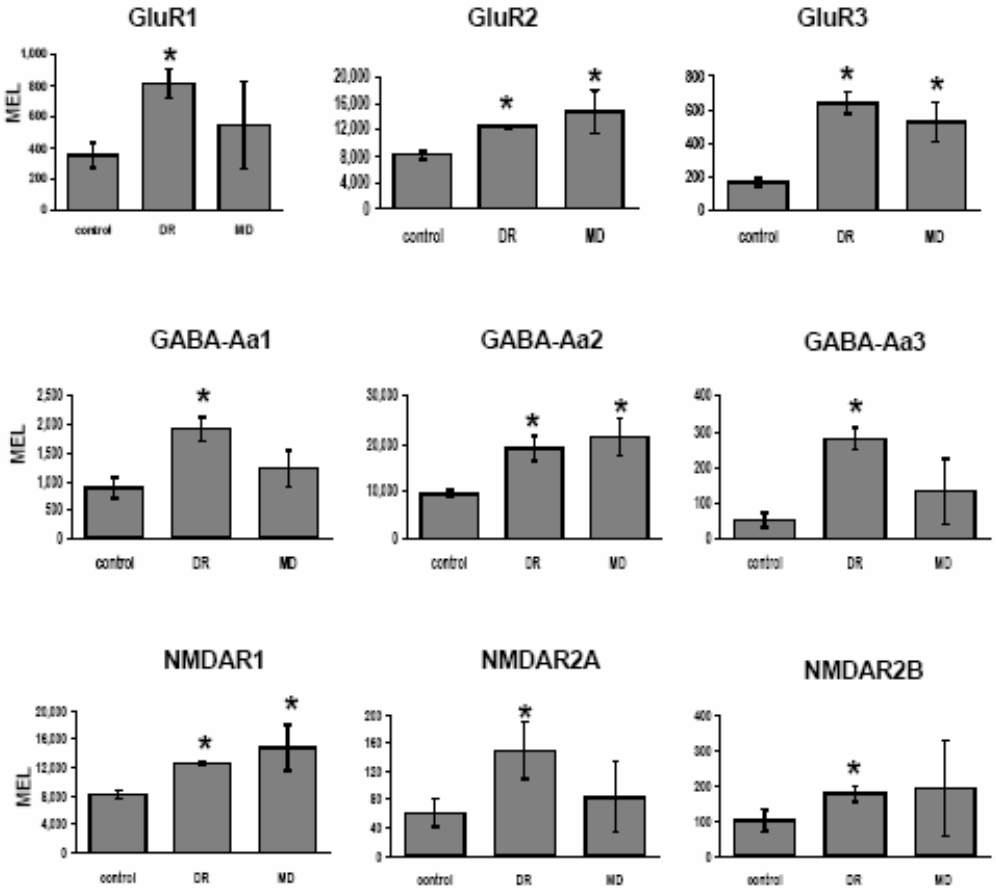
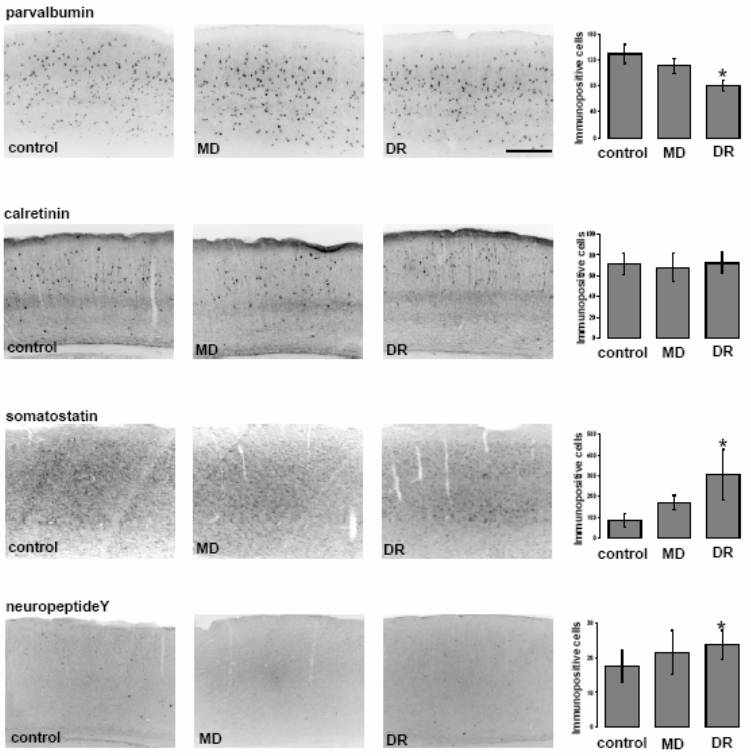
Supplementary Fig. 1



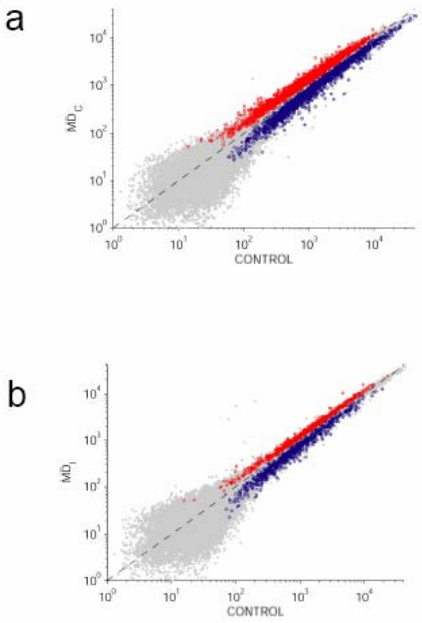
Graphic representation of the Microarray Expression Levels (MEL) for specific GABAergic and Glutamatergic receptors. A star indicates that the difference in expression between the marked group and control is significant ($P \leq 0.05$).

Supplementary Fig. 2



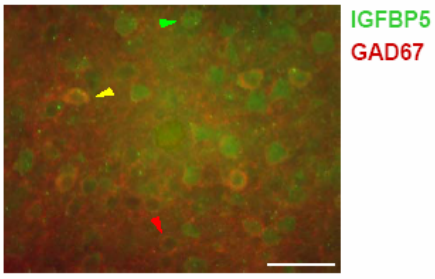
Immunostaining for several interneuron markers in three different conditions: control (Con), Monocular Deprivation (MD) and Dark Rearing (DR). From top to bottom: parvalbumin, calretinin, somatostatin, neuropeptide Y. The number of cells immunopositive for each marker in the different conditions is reported on the right: only parvalbuminergic neurons show a reduction in DR versus control (see also text **Fig. 5a**, which is from a different animal). For the other markers the number of positively stained cells is unchanged or even increased, as suggested by the microarray analysis (text **Fig. 2b**). Scale bar = 70 µm, and applies to all panels.

Supplementary Fig. 3



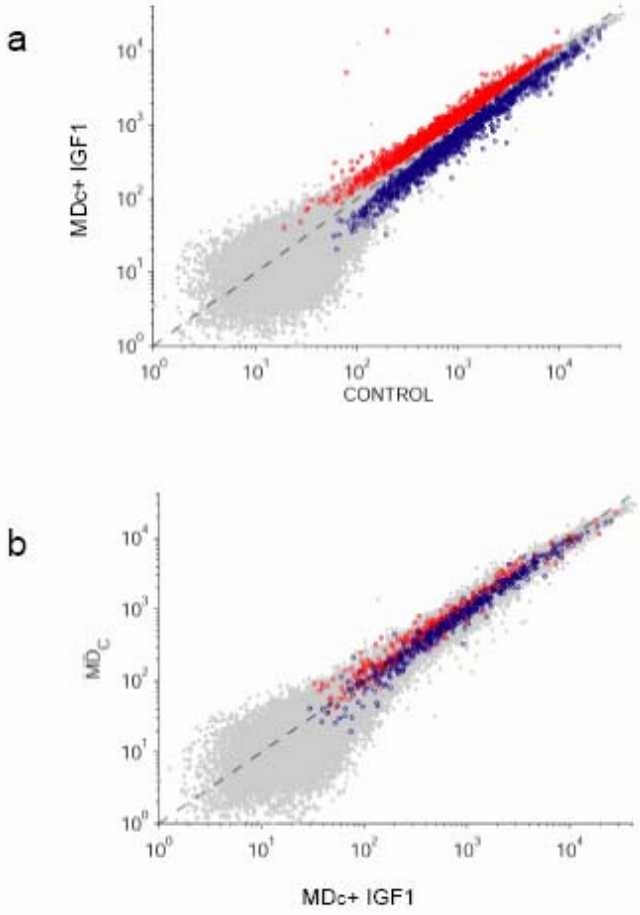
Comparison of gene expression in: (**a**) Short-term MD (4 days, P23-27) – expression in contralateral cortex versus control; (**b**) Short-term MD (4 days, P23-27) – expression in ipsilateral cortex versus control. In (**a**) genes that are significantly up-regulated in contralateral cortex ($P \leq 0.01$) are shown in red, while genes that are significantly down-regulated are represented in blue. In (**b**) genes that are significantly up-regulated in ipsilateral cortex ($P \leq 0.01$) are shown in red, while genes that are significantly down-regulated are represented in blue. Gene expression is shown on a logarithmic scale. The dashed white line corresponds to identity (y=x). Over 60% of the genes that change expression in ipsilateral cortex also do so in the contralateral cortex. Comparison of genes that are upregulated in one cortex versus those that are downregulated in the other indicates that there is little overlap between the two sets of genes.

Supplementary Fig. 4



Double staining for IGFBP5 (green) and GAD67 (red) in visual cortex of a P28 mouse. Yellow arrow shows an overlap between the two colors suggesting that IGFBP5 is present in GABAergic neurons; however the presence of cells immunopositive for IGFBP5 but not for GAD67 (green arrow) and vice versa (red arrow) shows that IGFBP5 is present in other cell classes as well. Scale bar= 17 µm.

Supplementary Fig. 5



Comparison of gene expression in (**a**) Short-term MD+IGF1 versus control; (**b**) Short-term MD versus Short-term MD+IGF1. In (**a**) genes significantly upregulated (*P*<0.01) in the deprived cortex after administration of IGF1 are shown in red, while significantly down-regulated genes are shown in blue. In (**b**) genes significantly up-regulated in the deprived cortex alone are shown in red, while genes that are up-regulated in the deprived cortex after administration of IGF1 are shown in blue. Gene expression is shown on a logarithmic scale. The dashed white line corresponds to identity (y=x).

Supplementary Table 1

Representation of the biological function categories at level 3 of the Gene Ontology (GO) database. For each category, the genes that are up-regulated in the four different conditions are shown: MD>C (number of genes significantly different : 200; genes present at GO level 3: 86), C>MD(number of genes significantly different: 431; genes present at GO level 3: 139) , DR>C (number of genes significantly different : 1730; genes present at GO level 3: 662)and C>DR (number of genes significantly different : 950; genes present at GO level 3: 340) .

biological processes	MD>C	C>MD	DR>C	C>DR
reproduction	2	5	11	8
cell communication	26	21	209	60
feeding behaviour	1	0	0	0
metabolism	52	97	362	226
morphogenesis	10	6	67	23
death	5	5	26	13
cell differentiation	3	0	13	7
regulation of gene	1	1	2	4
homeostasis	1	1	10	2
extracellular structure	1	0	1	0
secretion	2	0	6	0
regulation of cellular processes	2	4	13	4
organismal physiological processes	8	4	0	0
cellular physiological processes	42	54	0	0
response to stimuli	3	8	31	13
aging	0	1	0	0
learning and memory	0	1	3	1
pigmentation	0	1	0	1
regulation of biological processes	0	1	0	0
behavioural fear response	0	0	1	1
cell activation	0	0	3	1
cell motility	0	0	18	4
membrane fusion	0	0	2	0
response to stress	0	0	25	13
pattern specification	0	0	4	2
sex determination	0	0	1	1
sex differentiation	0	0	1	0
respiratory gas exchange	0	0	1	3
lactation	0	0	1	0
rhythmic	0	0	2	3
locomotor	0	0	6	1
feeding behaviour	0	0	2	1
chemosensory behaviour	0	0	2	0
circulation	0	0	4	0
cell growth and maintenance	0	0	269	114
cell death	0	0	26	13
genetic transfer	0	0	1	1

Supplementary Table 2

Representation of the number of genes significantly (P<0.01) up- and down- regulated across different conditions.

	up-regulated	down-regulated
DR versus CONTROL	1730	950
MD (16 days) CONTRA versus CONTROL	200	431
MD (4 days) CONTRA versus CONTROL	2008	1740
MD (4 days) IPSI versus CONTROL	667	709
MD (4 days) +IGF1 versus CONTROL	1719	1435
MD (4 days) CONTRA versus MD + IGF1	353	437

Supplementary Table 3

Representation of the top Gene Sets enriched in DR (left column) and MD (right column) versus control. The Gene Sets are ranked according to their Normalized Enrichment Score. Gene Sets that are enriched in both conditions are outlined in yellow. A star indicates that at least one probe of the correspondent Gene Set has been confirmed with RT-PCR.

	DR>C	NES	MD>C	NES
1	Channel_passive_transporter ★	27.3	egfPathway ★	16.4
2	Metabolism	25.6	igf1Pathway ★	9.7
3	mapkPathway ★	22.6	EGF_receptor_signaling_pathway	9.5
4	Vesicle_coat_protein	21.6	pdgfPathway ★	8.7
5	chr14q31	21.0	Embryogenesis_and_morphogenesis	8.0
6	ghPathway	20.0	Helicase_activity ★	7.9
7	chr8p12	18.8	tpoPathway ★	7.6
8	Secretory_vesicles ★	18.6	nfatPathway ★	7.5
9	chr20p12	17.8	Monocyte_AD_pathway	7.0
10	Apoptosis_regulator_activity	17.6	arfPathway	6.8
11	Protein_amino_acid_phosphorylation	17.4	JAK_STAT_cascade ★	6.7
12	chr4q12	17.3	Differentiation_in_PC12 ★	6.6
13	rarrxrPathway	17.1	Channel_passive_transporter ★	6.4
14	ATPase_activity	17.0	tcrPathway ★	6.2
15	chr5q33 ★	16.8	Transmembrane_RPTP	6.0
16	insulinPathway	16.8	ghPathway ★	5.8
17	Neurotransmitter_secretion ★	16.6	Inositolphosphatidylinositol_kinase_activity	5.6
18	edg1Pathway	16.6	keratinocytePathway	5.6
19	egfPathway	16.5	at1rPathway ★	5.6
20	RAS_protein_signal_transduction	16.5	gleevecPathway ★	5.6
21	Telomerase_dependent_telomere_maintenance	16.4	ngfPathway	5.5
22	Endoplasmic_reticulum ★	16.0	il2rbPathway	5.5
23	par1Pathway	15.6	Cancer_related_testis ★	5.5
24	ngfPathway	15.4	Adrenergic	5.4
25	at1rPathway ★	15.3	il7Pathway	5.3
26	Cancer_related_testis	15.3	il2Pathway ★	5.3
27	erk5Pathway ★	15.2	Dag1	5.3
28	JNK_MAPK_pathway	15.1	G_alpha_5_pathway ★	5.2
29	chr15q22	15.0	PTEN_pathway	5.2
30	Ngvm_c8	15.0	cblPathway	5.1
31	arenrf2Pathway ★	14.9	B_cell_receptor_complexes	5.0
32	Microtubule_binding_activity	14.9	p53_signalling	5.0
33	arfPathway	14.7	arenrf2Pathway ★	4.9
34	Potassium_ion_transport ★	14.5	chr20p12	4.8
35	mtorPathway	14.4	pitx2Pathway	4.8
36	crebPathway ★	14.3	igf1rPathway	4.8
37	gleevecPathway	14.3	hdacPathway ★	4.7
38	Protein_amino_acid_dephosphorylation	14.3	ccr5Pathway ★	4.7
39	myosinPathway	14.3	Insoluble_fraction	4.6
40	pdgfPathway	14.1	Granule_cell_survival ★	4.4
41	Ngvm_c32 ★	14.0	35_cyclic_nucleotide_phosphodiesterase_activity	4.4
42	Microtubule_associated_complex	14.0	hivnefPathway	4.3
43	Neuronal_transmission ★	13.9	GPI_anchored_membrane_bound_receptor	4.2
44	erkPathway	13.6	Positive_regulation_of_transcription	4.2
45	CD40_pathway_map ★	13.6	tnfr1Pathway	4.2
46	Wnt_Signaling	13.6	Neuronal_transmission ★	4.2
47	Ion_transporter_activity	13.5	Transmembrane_RTK_signalling	4.1
48	Calmodulin_binding_activity ★	13.3	Synaptic_transmission ★	4.1
49	GPCR_pathway	13.1	spryPathway	4.1
50	chr2p22	13.1	Golgi	4.0

	C>DR	NES	C>MD	NES
1	Neuropeptide_hormone	-17.0	20S_core_proteasome_complex	-5.3
2	Gas_exchange	-14.3	Ribosome	-4.6
3	Scavenger_receptor	-13.1	Circulation	-4.0
4	Serine_type_endopeptidase	-12.8	NADH_dehydrogenase	-4.0
5	Enzyme_binding_activity	-12.6	NADH_dehydrogenase_ubiquinone_activity	-3.8
6	Spliceosomal_subunit	-10.1	Endopeptidase_activity	-3.6
7	chr4q21	-9.1	Structural_constituent_of_ribosome	-3.2

Supplementary Table 4

Representation of the top Gene Sets enriched in control versus the deprived conditions. The Gene Sets are ranked according to their Normalized Enrichment Score.

Supplementary Information

Microarray Data

The complete microarray data are available at:

http://web.mit.edu/msur/www/Tropea.html

The website also contains:

- A list of genes examined for RT-PCR, including their microarray expression levels, p-values, and associated information.
- The list of gene sets used for the Gene Set Enrichment Analysis (GSEA) and the probes in each set.

Supplementary Methods

Significance analysis of microarrays

We applied a method for the Significance Analysis of Microarrays to assess changes in gene expression[1], implementing the method in MATLAB (The Mathworks, Natick, MA). The method allows the comparison of the expression level of each gene under two conditions (eg., MD vs control, or DR vs control). Under the null hypothesis that there are no changes in expression, the output is a probability of observing the given differences by chance (obtained by shuffling the data from the two conditions). The results of this analysis were compared against those obtained by setting a fixed threshold on the minimum intensity of each gene and a minimum

ratio of expression between the two conditions. The correlations between replicates were calculated as correlation coefficients (c.c.) for all conditions: control (c.c.= 0.99 ± 0.002), MD 16 days (c.c.= 0.9 ± 0.05) , MD 4 days contralateral (c.c.= 0.99 ± 0.001), MD 4 days ipsilateral (0.99 ± 0.005), MD 4 days contralateral plus IGF1 (c.c.= 0.99 ± 0.004).

GO annotations

For the first set of experiments, we retrieved the Gene Ontology (GO) annotations for each of the genes (http://www.geneontology.org/). Mapping of each Affymetrix probe to gene names was done using the annotations from Affymetrix (http://www.affymetrix.com). GO provides information about the molecular function of a given gene (e.g. nucleic acid binding, ion transporter activity, etc.), the biological processes in which is involved (e.g. cell growth, cell communication) and the cellular location (e.g. nucleus, cytoplasm, etc.). For each of these organizing principles, GO provides a list of different categories to which each gene may be assigned. We used FatiGO[2,3] to identify categories for biological functions that are over- or under- represented in the different protocols of visual input deprivation.

Detailed description of the Gene Set Enrichment Analysis (GSEA)

GSEA considers even small variations in all the mRNA probes of a group of genes, thereby assessing the enrichment of the whole gene set, and is relevant for detecting modest but coordinated changes in the expression of groups of functionally related genes. Such an analysis has particular value when an increase in the activity of several genes in a set could be more important than the strong activation of a single gene in a molecular cascade. Furthermore, the genes in the set typically share some functional or structural properties. Different gene sets have different sizes (for example, the gene set 'Channel-passive-transporter' has 238 probes,

while the 'IGF1 pathway' has 46 probes), and all the probes corresponding to a single gene are reported in each gene set. We followed a recent description of the method[4]; a more detailed description has now appeared[5].

Let ${}_S\mu_i$ denote the mean expression level across samples of probe *i* (*i*=1,…,*N* where *N* is the total number of probes) in condition *S* (where *S* = *DR*, *MD* or *control*) and let ${}_S\sigma_i$ denote the standard deviation across samples. For a given probe *i*, we define the signal to noise ratio (SNR) of the deprivation condition with respect to the control. For example, for dark rearing, the SNR was defined as $_{DR}SNR_i = \frac{{}_{DR}\mu_i - {}_{control}\mu_i}{{}_{DR}\sigma_i - {}_{control}\sigma_i}$. Probes were ranked according to the SNR value yielding an ordered list $L=\{g_1,\ldots,g_N\}$.

Given a set ***G*** containing N_G probes we are interested in assessing whether *the set of probes* is significantly over- or under- represented in one of the deprivation conditions with respect to the control condition (irrespective of whether the expression of the individual probes changed significantly or not). A representative example illustrating the algorithm is shown in Figure 4A. We define the following two cumulative distribution functions: $P_{hit}(i)$=proportion of genes in the set ***G*** that show a rank less than *i* ($P_{hit}(i) = \frac{\#[g_{(j\le i)} \in G]}{N_G}$) and $P_{miss}(i)$ = proportion of genes *outside* the set ***G*** that show a rank less than *i* ($P_{miss}(i) = \frac{\#[g_{(j\le i)} \notin G]}{N - N_G}$). The running enrichment score is defined as $RES(i)=P_{hit}(i)-P_{miss}(i)$ (Figure 4A, top) and is derived from the position or rank of the genes in the set (Figure 4A, bottom). The enrichment score ES is the maximum deviation from 0 of *RES(i)*. If the genes in the set are highly enriched in the deprivation condition and appear first in the ordered list *L*, then P_{hit} will grow faster with *i* than P_{miss} for initial values of *i* and this will lead to a high positive ES value. Conversely, if the genes in the set are under-

expressed in the deprivation condition and do not appear at the beginning of the list *L*, then P_{miss} will grow faster with *i* than P_{hit} and this will lead to a high negative ES score. If the genes in the set are randomly distributed, then the ES will show a value close to 0. The statistical significance of a particular value of ES is assessed by comparing it with the null distribution obtained by randomly shuffling the condition labels (deprivation and control) for each probe (using 1,000 permutations).

The procedure just described is repeated for each gene set, obtaining an enrichment score and an enrichment probability value for each set. It is possible to define a set of genes based on several different criteria. In our case, we studied sets of genes defined by common functional or structural properties in 3 specific biological databases: BioCarta (http://www.biocarta.com/), GenMapp (http://www.genmapp.org/) and GO (http://www.geneontology.org/). When a large number of gene sets is considered as in the present case, care should be taken because of the multiple comparisons involved and therefore the increased likelihood that one comparison will yield a significant result by chance. The multiple comparisons question was addressed here by controlling the Family Wise Error Rate[6]. To compare enrichment scores across gene sets, the enrichment scores are normalized by centering and scaling the ES using the mean and variance of each data, gene set pair. Throughout the text and in Supplementary Tables 3 and 4 we show the normalized enrichment scores (NES) for the gene sets enriched in dark rearing or monocular deprivation relative to control, or vice versa.

Description of procedures for optical imaging

The skin was excised and the skull exposed over V1. A custom-made attachment was used to fix the head and minimize movements. The cortex was covered with agarose solution (1.5 %) and a glass cover slip. During the imaging session the animal's body temperature was kept

constant with a heating blanket and the EKG monitored constantly. The eyes were periodically treated with silicone oil and the animal allowed to breathe pure oxygen. Red light (630 nm) was used to illuminate the cortical surface, and the change of luminance was captured by a CCD camera (Cascade 512B, Roper Scientific) during the presentation of visual stimuli (STIM, Optical Imaging). Custom software was developed to control the image acquisition and synchronization between the camera and stimuli. An elongated horizontal or vertical white bar (9°x72°) over a uniformly gray background was drifted continuously through the up-down or peripheral-central dimension of the visual field. After moving to the last position, the bar would jump back to the initial position and start another cycle of movement – thus, the chosen region of visual space (72°x72°) was stimulated in periodic fashion (9 sec/cycle). Images of visual cortex were continuously captured at the rate of 15 frames/sec during each stimulus session of 25 mins. Four sets of stimuli (upward, downward, leftward, rightward) were randomly presented to either eye monocularly or both eyes simultaneously.

A temporal high pass filter (135 frames) was employed to remove slow noise components, after which the temporal Fast Fourier Transform (FFT) component at the stimulus frequency (9 sec^{-1}) was calculated pixel by pixel from the whole set of images. No spatial averaging was done. The amplitude of the FFT component was used to measure the strength of visually driven response for each eye, and the ocular dominance index was derived from each eye's response (R) at each pixel as ODI = (Rcontra – Ripsi)/ (Rcontra + Ripsi). The binocular zone was defined as the region with equivalent driving from both eyes.

For monocular deprivation, animals were anesthetized with avertin (0.016 ml/g) and the eyelids of one eye sutured (at P11-12 for 15-16 days for microarray analyses and at P20-22 for 7 days for imaging experiments). Before imaging, the suture was removed and the deprived eye re-opened. Only animals in which the deprivation sutures were intact and the condition of the

deprived eye appeared healthy were used for the imaging session. For IGF1 treatment, a solution containing GPE, the functional peptide of IGF1, was prepared as described[7] : 300 μg of GPE was injected intra-peritoneally daily for the entire period of deprivation. For DR animals (aged P27-30), the procedure was the same described above, with the exception that the animals were anesthetized in darkness and not exposed to light until deeply anaesthetized; in these mice only the binocular response was evaluated and compared to that in control animals.

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