Inhibitory neurons regulate the adaptation of neural circuits to sensory experience, but the molecular mechanisms by which experience controls the connectivity between different types of inhibitory neuron to regulate cortical plasticity are largely unknown. Here we show that exposure of dark-housed mice to light induces a gene program in cortical vasoactive intestinal peptide (VIP)-expressing neurons that is markedly distinct from that induced in excitatory neurons and other subtypes of inhibitory neuron. We identify Igf1 as one of several activity-regulated genes that are specific to VIP neurons, and demonstrate that IGF1 functions cell-autonomously in VIP neurons to increase inhibitory synaptic input onto these neurons. Our findings further suggest that in cortical VIP neurons, experience-dependent gene transcription regulates visual acuity by activating the expression of IGF1, thus promoting the inhibition of disinhibitory neurons and affecting inhibition onto cortical pyramidal neurons.

To explore how sensory experience affects gene expression in VIP neurons, we examined this process in the visual cortex of adult mice that were housed in standard conditions, in complete darkness (that is, dark-housed), or dark-housed and then exposed to light for increasing amounts of time (Fig. 1a). Light deprivation for as little as 12 h drives robust gene expression after light exposure, and increasing durations of dark-housing accentuate the gene induction response (Extended Data Fig. 1a) irrespective of the phase of the circadian rhythm (Extended Data Fig. 1b). To purify RNA selectively from VIP-expressing and other inhibitory neuron subtypes, we generated mice that were heterozygous for alleles of either Vip-cre, Sst-cre or Pvf-cre, and were also heterozygous for the Rpl22-HA (RiboTag) allele, which expresses a haemagglutinin (HA)-tagged ribosomal protein specifically in Cre-expressing neurons (Fig. 1a). For purposes of comparison, we also purified ribosome-bound RNA from excitatory and inhibitory neurons, labelled by Emx1-cre or Gad2-cre.

By quantitative real-time PCR (qPCR), we find that messenger RNAs for cell-type-specific marker genes are highly enriched in the appropriate samples (Extended Data Fig. 1c) and that light exposure induces the expression of early-response genes in each Cre line (Extended Data Fig. 1d). To quantify experience-induced gene expression at a genome-wide level, we performed RNA-seq on RNA isolated from the dark-housed/light-exposed RiboTag-mice (Supplementary Table 1) (Fig. 1c, d and Extended Data Fig. 2a, b). This analysis identified genes which exhibited reproducible changes in expression levels in response to visual stimulation in at least one Cre line (n = 602; see Supplementary Table 2 and Methods) and thus allowed us to ask how levels of these experience-regulated genes are correlated across the different neuronal subtypes compared to non-regulated genes (n = 13,678) (Fig. 1d–i). We found that the expression of experience-regulated genes is remarkably dissimilar across different neuronal subtypes when compared to genes that are not regulated by sensory experience (irrespective of differences in the number or expression levels of experience-regulated genes; Fig. 1f–i and Extended Data Fig. 3a–d). While unique subsets of experience-responsive genes were identified in each neuronal subtype (Fig. 2a, b and Extended Data Fig. 3e, f), VIP neurons are the most responsive to sensory stimulation (Fig. 1d, e) and possess an experience-induced gene expression program that is markedly distinct from the other neuronal subtypes analysed (Fig. 1h, i). This suggests that in VIP neurons the experience-dependent gene program may have a unique function in adapting the cortex’s neural circuits to sensory experience.

We hypothesized that experience-regulated genes that are specifically expressed and selectively regulated in VIP neurons are likely to have important functions in regulating the synaptic connectivity onto VIP neurons. Thus, we first identified the mRNAs that are specifically enriched in each subtype (Extended Data Fig. 4a; Methods) and cross-referenced these genes with the list of experience-regulated genes (Extended Data Fig. 4b). This analysis identified 31 genes that are both cell-type-specific and experience-regulated, 11 of which are specific to VIP neurons (Supplementary Table 4). Notably, secreted molecules are significantly over-represented in this gene set (GO-term ‘Secreted’ P = 0.002) and each type of neuron has its own set of cell-type-specific experience-regulated secreted factors, including four experience-induced secreted molecules that are specific to VIP neurons (Igf1, Crh, Prok2, Fbn2; Fig. 2b, Supplementary Table 4).

We next performed fluorescent in situ hybridization (FISH) on sections of visual cortices of dark-housed/light-exposed mice to quantify the percentage of cells that co-express an inhibitory subtype marker and the respective secreted factor (Fig. 2c–f). Of the four secreted factors, Igf1 is the one factor that is expressed in the vast majority of VIP neurons, and whose expression is highly enriched in these neurons (Fig. 2d). We were unable to reliably identify Fbn2-expressing cells, and Prok2 was expressed nearly exclusively in a sparse subpopulation of VIP neurons (Fig. 2f), consistent with the low expression level of these genes in the RiboTag-seq experiments (Extended Data Fig. 4c). While the FISH analysis revealed that in the cortex Crh is highly enriched in VIP neurons compared to PV and SST neurons (Fig. 2e), this gene is also expressed in Pvf/Sst/Vip-negative cortical interneurons. Since Igf1 is the sole experience-induced secreted factor that is selectively expressed in most VIP neurons, we focused our subsequent analysis on Igf1.

Previous reports have suggested that Igf1 is synthesized in the cortex, but the function of cortical-derived Igf1 was unknown. Global disruption of the Igf1 gene results in abnormally small animals with smaller brains that contain smaller neurons with dendrites that are less branched and contain fewer synapses, and the effects of Igf1 on brain development and function are due at least in part to Igf1 that is produced by non-neural tissues and then enters the brain. To investigate specifically VIP neuron-derived Igf1, we crossed Vip-cre mice to both Igf1 conditional-knockout mice and Cre reporter mice. Disruption of Igf1 specifically in VIP neurons had no effect on the...
thickness of the cortical layers, on the number and layer distribution of VIP neurons, or on the size of VIP neuron cell bodies at postnatal day 21 (that is, P21) (Extended Data Fig. 5a–d). To test whether VIP neuron-derived IGF1 affects excitatory and/or inhibitory inputs to VIP neurons, we recorded miniature inhibitory or excitatory postsynaptic currents (mIPSCs or mEPSCs) in VIP neurons in acute visual cortex slices; we found that conditional deletion of Igf1 in VIP neurons leads to a significant reduction in mIPSC frequency (Fig. 3a) but not amplitude (Fig. 3b). Since conditional deletion of Igf1 had no effect on the frequency or amplitude of excitatory mEPSCs on VIP neurons (Fig. 3c, d), these findings suggest that VIP neuron-derived IGF1 specifically enhances inhibitory synaptic input onto VIP neurons.

To test whether IGF1 functions cell-autonomously to regulate inhibitory input onto the cell from which it is expressed, we used a virus-based approach to acutely knockdown Igf1 expression in only a few VIP neurons. We generated short hairpin RNA (shRNA) constructs against Igf1 (Extended Data Fig. 6a, b), injected low titre AAVs expressing the shRNA and Cre-dependent enhanced green fluorescent protein (eGFP) into the visual cortex of P14–15 Vip-cre mice, and recorded mIPSCs and mEPSCs one week later (P20–P22) in eGFP-positive VIP neurons that are surrounded by non-infected VIP neurons (Fig. 3e). This sparse and acute knockdown of Igf1 in VIP neurons using either of two distinct shRNAs against Igf1 resulted in a marked reduction in mIPSC frequency and amplitude as compared to VIP neurons infected with a control shRNA (Fig. 3f, g), but had no significant effect on mEPSCs (Fig. 3h, i). These effects are not due to altered VIP neuron morphology (Fig. 3g) and Extended Data Fig. 6c), indicating that VIP-neuron-derived IGF1 acutely promotes inhibition onto VIP neurons in a cell-autonomous manner.

To determine if VIP-neuron-derived IGF1 regulates inhibitory inputs onto other types of cortical neurons, we adopted a protocol that leads to widespread infection of neurons in the cortex (see Methods). Injecting AAVs into Vip-, Pp- or Sst-cre mice to label these cells with eGFP while knocking down Igf1 in VIP neurons, we recorded mIPSCs from each cell type and found that early knockdown of Igf1 in the cortex decreases mIPSCs frequency in VIP neurons, but does not affect mIPSCs onto
PV, SST, or pyramidal neurons (Extended Data Fig. 6e–l). Furthermore, ELISA-based analysis of IGFI levels in the blood of mice whose cortices were injected with these viruses demonstrated that removing IGFI from VIP neurons did not alter the level of serum-derived IGFI (Extended Data Fig. 6d). While we formally cannot exclude that serum-derived IGFI contributes to inhibition onto VIP neurons, this finding indicates that the decrease in mIPSCs in VIP neurons that express Igf1 via secreted factors is due at least in part to reduced Igf1 expression in VIP neurons. Thus, VIP-neuron-derived IGFI regulates the inhibitory inputs onto the VIP neuron in which it is produced, probably via local release from VIP neurons. Consistent with this idea, we find that the Igf1 splice variant expressed by VIP neurons encodes an isoform of IGFI containing a heparin binding domain (Igf1.4; Extended Data Fig. 7a) that may limit the diffusion of IGFI and facilitate its local action.16

We next overexpressed IGFI in VIP neurons by injecting Vip-cre mice with an AAV construct that drives expression of an epitope-tagged version of IGFI together with EGFP in a Cre-dependent manner (Fig. 3k and Extended Data Fig. 7b, c) and assessed the effect on mIPSCs and mEPSCs (Fig. 3l–o). We find that when overexpressed in VIP neurons, IGFI selectively promotes inhibition onto VIP neurons, as it has no effect on mEPSCs in these cells. Likewise, ectopic expression of IGFI in SST and excitatory neurons (by intracortical injections into Sst- or Emx1-cre mice, respectively; Extended Data Fig. 7d–g) leads to a similar increase in mIPSC frequency in these cells. These findings raise the possibility that the selective expression of Igf1 in VIP neurons is required for the proper organization and function of cortical circuits, as aberrant IGFI expression could enhance inhibition indiscriminately within cortical circuits by signalling through IGFI receptors that are ubiquitously expressed in these neurons (Extended Data Fig. 7h–j).

The change in mIPSC frequency upon Igf1 knockdown in VIP neurons could reflect a change in the presynaptic probability of release and/or a reduction in inhibitory synapse number and/or strength. By paired-pulse stimulation we find that Igf1 knockdown in VIP neurons does not significantly alter the probability of release of vesicles from either inhibitory (Fig. 4a) or excitatory (Extended Data Fig. 7k) terminals that synapse onto VIP neurons. To test evoked inhibition, we co-injected Vip-cre mice with either Igf1 or control shRNA AAVs and an AAV encoding the excitatory light-activated ion channel ReachR17. Performing paired voltage clamp recordings from eGFP-positive VIP neurons and neighboring pyramidal cells to control for variation in stimulation intensity, we found that the strength of light-evoked inhibition onto VIP neurons is decreased when Igf1 expression is knocked down in VIP neurons (Fig. 4b). These experiments suggest that the primary site of IGFI action is post-synaptic and indicate that experience-dependent activation of Igf1 expression increases the number and/or strength of functional inhibitory synapses that form on VIP neurons. To test whether this IGFI-dependent decrease in inhibition alters the frequency of action potentials in these neurons, we performed cell-attached recordings from eGFP-labelled VIP neurons expressing control or Igf1 shRNAs. We find that VIP neurons lacking IGFI fire action potentials at a significantly higher rate than controls (Fig. 4c). Given that VIP neurons disinhibit cortical circuits, it seems likely that this decreased firing of VIP neurons might alter how the cortex responds to sensory experience. To begin to investigate this possibility, we next assessed the effect of knocking down Igf1 expression on visual cortex plasticity.

Cortical inhibition regulates ocular dominance (OD) plasticity18 and visual acuity19, and hyper-activation of VIP neurons drives a form of adult cortical plasticity20. To determine whether knocking down Igf1 expression in VIP neurons affects visual cortex function, we injected control or Igf1 shRNA AAVs into the binocular zone of visual cortices of P18 Vip-cre mice and recorded visual-evoked potentials between P28 and P32 (Fig. 4d and Extended Data Fig. 8a). Stimulation of the contralateral or ipsilateral eye with gratings at low spatial frequency elicited robust visual-evoked potentials both under control and Igf1 knockdown conditions (Extended Data Fig. 8b–d). Furthermore, the ratio between the contralateral and ipsilateral eye’s response (C/I ratio) was similar in the presence or absence of Igf1 (Extended Data Fig. 8e), indicating that basic visual cortex function is not obviously disrupted
Figure 3 | IGF1 promotes inhibitory inputs to VIP neurons in a cell-autonomous manner. a–d, Bar graph and cumulative distribution of the frequency and inter-event intervals of mIPSCs or mEPSCs recorded from IGF1 wild-type (WT) or conditional-knockout (cKO) VIP neurons (mIPSC frequency, $P = 0.046$; amplitude, $P = 0.3$; mEPSC frequency, $P = 0.44$; amplitude, $P = 0.9$, Mann–Whitney U-test). e, Example image of sparsely infected VIP neurons upon injection of AAV-shRNA-hUbc-Flex-eGFP into mice expressing tdTomato in all VIP neurons (white box indicates the magnified area; arrows indicate infected VIP neurons; arrowheads indicate non-infected VIP neurons). f–i, Bar graph and cumulative distribution of mIPSC/mEPSC frequency, inter-event interval and amplitude recorded from VIP neurons sparsely infected with control or IGF1 shRNAs (mIPSC frequency: shRNA 1, $P = 0.05$; shRNA 2, $P = 0.042$; mIPSC amplitude: shRNA 1, $P = 0.004$; shRNA 2, $P = 0.001$; mEPSC frequency: shRNA 1, $P = 0.13$; shRNA 2, $P = 0.07$; mEPSC amplitude: shRNA 1, $P = 0.77$; shRNA 2, $P = 0.44$. Mann–Whitney U-test). j, Sholl analyses of VIP neurons infected with control or IGF1 shRNA ($P = 0.76$, two-way repeated-measures ANOVA). k, Expression of epitope-tagged IGF1.4 in VIP-neurons. Cortices of P19 Vip-cre+/+ mice were injected with AAVs driving Cre-dependent expression of SSHE-IGF1.4-Myc-F2A-eGFP (top) or F2A-eGFP (bottom) and stained at P02 for HA (red) and eGFP (green) (Scale bar, 10 µm). l–o, Bar graphs and cumulative distribution plots showing mIPSC/mEPSC amplitude and frequency/inter-event interval in VIP neurons infected with a control AAV or an AAV over-expressing (OE) IGF1. mIPSC: amplitude, $P = 0.05$; frequency, $P = 0.02$; mEPSC: amplitude, $P = 0.55$; frequency, $P = 0.86$. Mann–Whitney U-test. a–d, f–j–l–o, Numbers inside bars indicate the number of cells recorded; *$P < 0.05$ by Mann–Whitney U-test.

upon IGF1 knockdown. Remarkably, when we assessed visual acuity of the contralateral eye by increasing the spatial frequency of the gratings presented, the mice injected with AAVs expressing IGF1 shRNA exhibited significantly increased visual acuity as compared to mice injected with control AAVs (Fig. 4g).

To test whether the effect of IGF1 knockdown is experience-dependent, we next monocularly deprived mice for a brief period of time, beginning at the peak time of ocular dominance plasticity (that is, at P26–28, Fig. 4g). After four days of monocular deprivation, we recorded visual-evoked potentials from the visual cortex contralateral to the deprived eye and quantified the C1 ratio upon stimulation at low spatial frequency as well as the visual acuity upon stimulation of the deprived eye. Brief monocular deprivation led to a reduction in the C1 ratio in mice injected with AAVs expressing either control or IGF1 shRNA; this is a consequence of the reduction in the contralateral response (Extended Data Fig. 8b–e)18. Notably, when we tested visual acuity after brief monocular deprivation, both IGF1 and control shRNA injected mice exhibited similar levels of amblyopia (that is, loss of visual acuity) in the deprived eye (Fig. 4e–g), despite the higher visual acuity in the IGF1 shRNA injected mice that were not monocularly deprived (Fig. 4e–g). These findings indicate that VIP neuron–derived IGF1 regulates visual acuity in an experience-dependent manner and may function as a sensory-dependent brake on cortical plasticity. The observation that in response to sensory experience IGF1 in VIP neurons functions inhibition, taken together with the previous finding that experience induces BDNF in excitatory neurons to regulate excitatory–inhibitory balance21,22, suggests a model in which each type of neuron within a cortical circuit expresses a unique set of experience-induced secreted factors that control specific synaptic inputs onto the neuron and plasticity within a neural circuit23.
Figure 4 | VIP-neuron-derived Igf1 regulates VIP neuron function and regulates visual acuity in an experience-dependent manner. a, Paired-pulse recordings from VIP neurons infected with control or Igf1 shRNA (P = 0.96, two-way ANOVA). b, Left, average traces of light-evoked IPSCs (eIPSC) from paired recordings of VIP neurons infected with control or Igf1 shRNA (green traces) and neighbouring pyramidal neurons (Pyr., black traces). Right, quantification of eIPSC amplitude of the VIP neuron after infection with AAVs expressing control or Igf1 shRNA, normalized to the eIPSC amplitude of the paired pyramidal neuron (P = 0.01, Mann–Whitney U-test). c, Average firing rate of VIP neurons infected with Igf1 or control shRNA (P = 0.04, Mann–Whitney U-test). b, c, Numbers inside bars indicate the number of cells recorded. d, Schematic of the schedule for monocular deprivation (MD) experiments. e, f, Representative traces of visually evoked potential (VEP) amplitude as a function of spatial frequency (cycles per degree (c.p.d.)) in the contralateral visual cortex of mice that received bilateral injections of AAVs expressing Igf1 or control shRNA into their visual cortices and were subjected to monocular deprivation or not. g, VIP-neuron-derived Igf1 restricts visual acuity in an experience-dependent manner. Visual acuity in mice injected with AAVs expressing Igf1 or control shRNA with or without monocular deprivation (P24–P28; control shRNA no MD, n = 5; control shRNA MD, n = 5; Igf1 shRNA no MD, n = 5; Igf1 shRNA MD, n = 7; *P < 0.05; **P < 0.0001; NS, not significant; one-way ANOVA with Tukey’s post hoc test).

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to Online Content are excluded from this list.

Received 30 June 2014; accepted 29 January 2016.

Published online 9 March 2016.

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**METHODS**

No statistical methods were used to predetermine sample size. **Visual stimulation.** For calibrating the duration of the dark housing period before light exposure, C57Bl6 wild-type mice were housed in a standard light cycle until they were placed in constant darkness for varying amounts of time before analysis at postnatal day 56. At P56, all mice were either sacrificed in the dark (dark-housed condition) or light-exposed for 1, 3, or 7.5 h before being sacrificed. The eyes of all animals were enucleated (for the dark-housed condition, enucleation was performed at the end of the stimulus period) before dissection of the visual cortex in the light.

For RiboTag-experiments, mice were reared in a standard light cycle and then housed in constant darkness for two weeks starting from P42; at P56, all mice were either sacrificed in the dark (dark-housed condition) or light-exposed for 1, 3, or 7.5 h before being sacrificed. Additional cohorts of mice for the ‘standard’ condition were housed in a standard light cycle until P56 when they were euthanized. The eyes of all animals were enucleated (for the dark-housed condition, enucleation was performed in the dark) before dissection of the visual cortex in the light.

**RNA isolation, reverse transcription, qPCR analysis.** Total RNA was extracted with TRIzol reagent (Sigma) according to the manufacturer’s instructions, and RNA quality was assessed on a 2100 BioAnalyzer (Agilent); all RNAs were treated with DNaseI (Invitrogen) before reverse transcription. For the cloning of riboprobes, total RNA was extracted from whole adult C57Bl6 wild-type mouse brains and cdNA was prepared using SuperScript II kit (Life Technologies). For real-time quantitative PCR experiments aimed at calibrating the duration of the dark housing period, total RNA was extracted for each sample from the visual cortices of one animal. For real-time quantitative PCR experiments aimed at testing the efficacy of shRNA constructs directed against *Igf1*, total RNA was isolated from two pooled 24 wells of cultured cortical neurons for each condition. For qPCR experiments, RNA was reverse-transcribed with the High Capacity cDNA Reverse Transcription kit (Life Technologies). Real-time quantitative PCR reactions were performed on the LightCycler 480 system (Roche) with LightCycler 480 SYBR Green I Master. Reactions were run in duplicates, triplicates or quadruplicates, and β-actin (*Actb*) or β(3)-tubulin (*Tubb3*) levels were used as an endogenous control for normalization using the ΔΔCt method24. Real-time PCR primers were designed using the Universal ProbeLibrary (Roche) as exon-spanning whenever possible and answered the following criteria: linear amplification over three orders of magnitude of target concentration, no amplification product in control samples that were not reverse-transcribed (that is, control for contamination with genomic DNA), no amplification product in control samples where no template was added (that is, control for primer dimers), amplification of one singular product as determined by melt-curve analysis and analysis of the product in agarose gel electrophoresis and sequencing of the PCR product. The qPCR primers used in this study are listed in Supplementary Table 6.

For analysis of light-induced gene expression in wild-type mice, the gene expression levels were analysed in four mice (two males and two females) at each time point. The data were calculated as fold change relative to the average of the overnight dark-housed condition and normalized to the average of the maximally induced time point. Data in figures represent the mean and s.e.m. of four mice.

For assessing *Igf1* levels in cortical cultures infected with shRNA-expressing lentiviral constructs, qPCRs were performed in quadruplicates for each condition and fold changes were calculated relative to the non-infected non-stimulated cultures. Data were normalized to the maximally induced condition in each biological replicate, and data in figures represent the mean and s.e.m. of three biological replicates.

**RiboTag-puriﬁcations, RiboTag-qPCR and RiboTag-seq.** Immunoprecipitation and puriﬁcation of ribosome associated RNA was performed essentially as described24, but with minor modiﬁcations: lysis of the samples was performed in the presence 10 mM Ribonucleoside Vanadyl Complex (NEB, Ipswich, MA), and immunoprecipitation was performed with the body (HA-7, 12 μg per immunoprecipitation, Sigma). In brief, the visual cortices were dissected, flash frozen in liquid nitrogen and then kept at −80 °C until further processing. Visual cortices from three individual animals (each sample contained both male and female animals) were pooled for each biological replicate, and three biological replicates were performed. After lysis of the tissues and before immunopuriﬁcation, a small fraction of lysate of each sample (that is, ‘input’) was set aside and total RNA was extracted with TRIzol reagent followed by the RNEasy Micro Kit’s procedure (Qiagen, Valencia, California). After immunopuriﬁcation of the ribosome-associated RNAs, RNA quality was assessed on a 2100 BioAnalyzer (Agilent, Palo Alto, California) and RNA amounts were quantiﬁed using the Qubit 2.0 Fluorometer (Life Technologies). Only samples with RNA numbers above 8.0 were considered for analysis by qPCR and RNA-seq. For all RNA samples of sufficient integrity, 5–10 ng of RNA were SPIA-ampliﬁed with the Ovation RNA Ampliﬁcation System V2 (NuGEN, San Carlos, California), yielding typically 5–8 μg of cdNA per sample.

Quantitative RT–PCR was performed as described above and relative expression levels were determined in every experiment by normalizing the Ct-values to those of beta-Actin (*Actb*) from the 0 h input using the ΔΔCt method24. To determine the fold-enrichment (IP/Input), the actin-normalized expression levels for every time point of every biological replicate were averaged, and the grand averages from the IP and Input were divided to ﬁnd the IP/Input ratio. To calculate fold-induction for each biological replicate, each time point was divided by the maximal value occurring in that biological replicate, such that the maximal value was set to 1 in each biological replicate. The mean and standard error were calculated at each time point from these normalized values. All samples were analysed by qPCR for purity and light-induced gene expression before analysis by high throughput sequencing.

**RNA-seq and analysis.** SPIA-ampliﬁed samples from RiboTag-immunoprecipitated fractions for each of the ﬁve stimulus conditions and each of the ﬁve Cre lines were prepared as described above and processed in triplicate (75 samples total). For preparing sequencing libraries, 2 μg of each ampliﬁed cdNA were fragmented to a length of 200–400 bp using a Covaris S2 sonicator (Acoustic Wave Instruments) using the following parameters: duty cycle: 10%, intensity: 5, cycles per burst: 200, time: 60 s, total time: 5 min. After validating the fragment length of the sonicated cdNA using a 2100 BioAnalyzer (Agilent, Palo Alto, California), 2 μg of the fragmented cdNA were used for sequencing library prepa-ration using the PrepX DNA kit on an Apollo 324 robot (IntegenX). The quality of completed sequencing libraries was assessed using a 2100 BioAnalyzer (Agilent, Palo Alto, California) and the completed libraries were sequenced on an Illumina HiSeq 2000 instrument, following the manufacturer’s standard protocols for single-end 50 bp sequencing with single index reads. Sequencing typically yielded 30–80 million usable non-strand-specific reads per IP sample. Reads were mapped to the mm9 genome using TopHat (v.2.0.13) and Bowtie (2.1.0.9)25. On average, ~70% of mapped IP reads were uniquely mapped to the mm9 genome allowing for 0 mismatches and were therefore assignable to genic features (one RiboTag-seq library (Sts-cre, standard-housing, biological replicate 2) was excluded from analysis due to low mappability). Values from all IP libraries were normalized using Cuffnorm (v.2.2.1), and values from the Cuffnorm output file ‘genes-Count_Table’ (normalized reads) were taken as a proxy for gene expression. P-values were generated for each Cre line for each dark–light conditions using Cuffdiff (v.2.2.1) using the time series (-T) flag based on three biological replicates.

**Identification and classiﬁcation of experience-regulated transcripts.** To identify transcripts regulated by visual experience, for each biological replicate of Cre each line, the fold change in normalized reads was calculated for each gene at every time point (dark-housed/standard-housed, 1 h light/dark-housed, 3 h light/ dark-housed; 7.5 h light/dark-housed). Genes were flagged as experience-regulated in a given Cre line if they met the following conditions in at least one sample: (1) P value < 0.005, (2) mean fold change of twofold or greater, (3) fold changes of 2 or higher in 2 of 3 biological replicates, (4) the mean expression value in at least one sample must be above absolute expression threshold (set at the 40th percentile of all observed values).

To determine in which Cre lines genes were regulated by experience, genes were simply classified according to the above criteria. However, for this analysis we excluded the Gad2-cre line, since P-, St- and Vip-cre all label subsets of the neurons labelled by Gad2-cre. However, we did detect genes regulated solely in Gad2-cre, but no other Cre lines; we reasoned that these genes are probably regulated by experience in a population of 5HT3AR+/VIP+ neurons that are contained in Gad2-cre but none of the other Cre lines.

We classified the set of experience-regulated genes into categories ‘early’, ‘late’, and ‘long-term’ based on the fastest kinetics observed. When genes were found to be elevated and/or suppressed at multiple time points, we assigned them to the categories based on the most rapid observed change. For example, while Fos levels are elevated over dark housing at 1, 3 and 7.5 h of light exposure and suppressed after 7.5 h of dark housing, Fos is classified as ‘early-up’ because it is elevated at 1 h after light exposure.

**Linkage analysis.** All linkage analysis was performed using the ‘single’ method and ‘Cityblock’ metric using Matlab’s linkage function. To determine the branch-order significance of the cladogram resulting from clustering of the 602 experience-regulated genes, we generated 1,000 cladograms from 602 sets of random expressed genes (including experience-regulated genes, with replacement) and asked how often we generated a cladogram with an identical branch order at the level of the Cre lines. Only 11 sets of 1,000 random genes sets generated an identical tree. For the purposes of this analysis, we only compared the branches above the level of the Cre lines.

**Identification of cell-type-specific transcripts.** To identify cell-type-enriched transcripts, an enrichment score was calculated for every transcript in every Cre line for each biological replicate. This enrichment score was calculated by dividing the maximum expression value observed in a given Cre line by the maximum expression value observed across all conditions for all other Cre lines
acetylation was performed as described. Pre-hybridization was done overnight at
in situ after synthesis and validated for labelling with Dioxigenin or Fluorescein. For
Igf1 in vitro probe. Riboprobes for
in vitro − wild-type C57Bl6 mice were dark-housed and light-exposed for 7.5 h as described
Double-fluorescent ISH.

AGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCT). The
epitope (GAACAAAAACTCATCTCAGAAGAGGATCTG), Furin cleavage site
TGTTGACGCGCTCCAATTT; sh2: TACGCCGGTTAGAAATGTA) and the
Igf1.4 (cds) of
between the
was based on
by a human ubiquitin promoter (hUbc). The sequence for
pAAV-hUbc-Flex-F2A-eGFP, whereby the Cre-dependent inserts were driven
yielded plasmids termed pAAV-hUbc-eGFP and eGFP alone were cloned by synthesizing the
in these shRNA constructs is based on
mR-25 (CCCTCTCAACACTTG)17. shRNA-expressing AAV-constructs (pAAV-U6-shRNA-hUbc-Flex-eGFP) were
used by first making the Flex-GFP-Gephyrin
probe was detected by exposing

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For determining serum IGF1 levels, we used the IGF1 Quantikine ELISA kit (R&D
boiling SDS sample buffer and subjected to Western blot analysis essentially as

Cloning of riboprobes, knockdown and expression constructs. Cloning of all
constructs was done using standard cloning techniques, and the integrity of all
cloned constructs was validated by DNA sequencing. Templates for the riboprobes
for Igf1, Gad1, Pvlwb, Slt, and Vip were prepared by PCR amplification of DNA
fragments generated from total RNA isolated from adult C57Bl6 mouse brains (see
Supplementary Table 7 for primer sequences) and cloning of the respective PCR
fragments into the pBlueScript II vector (Agilent Technologies).

Lentiviral shRNA constructs were generated by cloning shRNA stem loop
sequences against Igf1 (Igf1 shRNA 1: GGTGGATGCTTCCTCTGTTG; Igf1 shRNA 2: TGAGGAGCTGGAGGATAGTA) and Luciferase (Luc, control: ACTTTAGCTGAGTACTCGAGTTGAGTTGAGTACGCTGAGTACTTCG) into a modified version of pLentiLox3.726 in which the CMV promoter driving the expression of eGFP was replaced with an
hUbc promoter and in which the loxP sites surrounding the hUbc-eGFP cassette
were removed. The loop sequence used in these shRNA constructs is based on
in which the
μm thick) of the visual cortex were cut on

Primary cultures of cortical neurons were prepared from E16.5 mouse embryos as described10. In brief, 3 × 104 neurons per well were plated in 24-well coated with poly-
l-lysine (20 μg ml \(^{-1}\)) and laminin (3.4 μg ml \(^{-1}\)). Cultures were maintained
in neurobasal medium supplemented with B27 (Invitrogen), 1 mM L-glutamine,
100 U ml \(^{-1}\) penicillin/streptomycin, and one-third of the media in each well was
replaced every other day. For testing of viral shRNA constructs, the cultures were
infected at DIV 3 with concentrated viral stocks for 5 h at an MOI of 6. After
infection, the cultures were washed twice in plain neurobasal medium after which
the conditioned medium was returned to the dish and the cultures were continued
to be maintained as described. At DIV 7, neuronal cultures were treated overnight
with 1 μM TTX and 100 μM AP-5 to silence spontaneous activity before the cultures
were depolarized at DIV 8 with 55 mM extracellular KCl as described31 and lysed
in TRizol after 6 h of stimulation.

Western blotting. Western blots with HEK293T constructs and ELISA for determining serum IGF1 levels. HEK293T cells were used for testing the expression and the biological
activity of the epitope-tagged IGF1.4 constructs. HEK293T cells were cultured in
DMEM (Life Sciences) containing 10% FCS and penicillin/streptomycin. Cells were
transfected using lipofectamine (Life Technologies) and 18 h post transfection,
DMEM (Life Sciences) containing 10% FCS and penicillin/streptomycin. Cells were
transfected using lipofectamine (Life Technologies) and 18 h post transfection,
DMEM containing 0.1% FCS. The conditioned media were applied
immediately for stimulating non-transfected HEK293T that were serum starved

In the sections at room temperature in the dark for 20 min to TSA Plus Cy3 reagent (Perkin Elmer) diluted 1:100 in TSA working solution, after which the sections
were washed three times in TNT buffer. Before the immunodetection
of the second probe, the peroxidases for detecting the first probe were inactivated by
treating the sections for 30 min with 3% H\(_2\)O\(_2\), followed by three washes in PBS.
After an additional blocking step in blocking buffer for 1 h at room temperature, the
anti-fluorescein-POD antibody (Roche) was applied at a concentration of
1:1000 in blocking buffer overnight at 4°C. Following three washes in buffer B1 and
an additional wash in buffer TNT, the probes of the subtype markers were detected by
exposing the sections at room temperature in the dark for 15 min to TSA Plus
Cy3 and Cy5 reagents (Perkin Elmer) diluted 1:100 in TSA working solution, after which the sections
were washed three times in TNT buffer. Finally, the sections were
counterstained with DAPI (4′,6-diamidino-2-phenylindole, Molecular Probes) and
mounted using Fluoromount-G (Southern Biotech). In each experiment, controls
for hybridization specificity were included (sense probe for Igf1) as well as controls
for ensuring the specificity of the immunodetection of the digoxigenin- and
fluorescein-labelled riboprobes.

Fish for Chr, Prok2 and Fbln2 could not be detected reliably, the visual cortices in each
section were imaged on a Zeiss Axio Imager microscope with a 10× objective and
3 × 5 fields-of-view were ‘stitched’ into one compound image; in all cases, image
exposures were kept constant throughout a given experiment for each channel.
Compound images of each visual cortex were then imported to Photoshop, and
additional layers were created for each probe (that is, one layer for the GOI and
one layer for the subtype marker in each compound image). The cells positive
for each probe were then marked with a dot in the new respective layer by two
independent investigators in a blinded manner (one investigator marking GOI-positive cells and the other investigator marking subtype-marker-positive cells).
Finally, the layers containing the dots of the identified positive cells were compiled
into a separate image file together with the DAPI-layer and imported into ImageJ.
In ImageJ, the images were analysed in a blinded manner by defining the visual
cortex and its layers as regions of interest (ROI) based on the DAPI staining and
quantifying the number of cells positive for either one or both markers per ROI.
For each combination of probes (GOI together with each of the subtype markers),
two visual cortices from four animals were analysed (a total of eight visual cortices
for each combination).

Virus production and neuronal cultures. Concentrated lentiviral stocks were
prepared and titered essentially as described10. AAV stocks were prepared at the
University of North Carolina (UNC) Vector Core and at the Children’s Hospital
Boston Vector Core; see also Supplementary Table 8 for further details on AAV
stocks.
Perfusions, immunohistochemistry and morphological analysis of IGF1 cKO visual cortices. Mice were anaesthetized with 10% ketamine and 1% xylazine in PBS by intraperitoneal injection. When fully anaesthetized, the animals were transcardially perfused with ice-cold PBS for 5 minutes followed by 15 minutes of cold 4% PFA in PBS. Brains were dissected and post-fixed for one hour at 4°C in 4% PFA, followed by three washes (each for 30 min) in cold PBS, and cryoprotection overnight at −20°C in 2.7 M sucrose in PBS at 4°C. Brains were placed in Tissue-Tek Cryo-OCT compound (Fisher Scientific), frozen on dry ice and stored at −80°C. Coronal sections (20 μm thick) of the visual cortices were subsequently cut using a Leica CM1950 cryostat and used for subsequent experiments.

For immunolabelling, the slides were blocked for 1 h with PBS containing 5% normal goat serum and 0.1% Triton X-100 (blocking solution). The samples were incubated overnight with different primary antibodies diluted in blocking solution, washed three times with PBS and then incubated for 45 min at room temperature with secondary antibodies and/or Hoescht stain (ThermoFisher Scientific). Slides were mounted in FluoromountG (Southern Biotech) and imaged on a Zeiss Axios Imager microscope. The following antibodies were used: mouse anti-HA (HA-7; Sigma; 1:10000), chicken anti-GFP (GFP-1020, Aves labs; 1:1500), goat anti-mouse IgG (H + L) Alexa Fluor 488 (Highly Cross-Adsorbed, Life Technologies; 1:1000), goat anti-chicken IgY (H + L) Alexa Fluor 488 (Life Technologies; 1:1000).

For analysing the brains of Igf1 Vip-cre WT and cKO mice, brains of three-week-old WT and cKO littermates were placed on the same slide to minimize variation. After cryosectioning, the slides were either counterstained immediately or stored at −20°C before they were counterstained and imaged. Counterstaining was done with DAPI (4′,6-diamidino-2-phenylindole, Molecular Probes) in PBS for 15–30 min at room temperature, after which the sections were washed once in PBS and mounted in FluoromountG (Southern Biotech). For cell counting experiments, coronal visual cortex sections were imaged using a Zeiss Axios Imager microscope with a 10× objective and typically 3 × 5 fields-of-view were 'stitched' into one compound image. In all cases, image exposures were kept constant throughout a given experiment for each channel. Custom ImageJ and MATLAB macros were used to quantify the area of each cortical layer, the number of tdTomato-positive cells per layer, and the size of tdTomato-positive cells. Briefly, regions of interest (ROI) encompassing the visual cortex and its layers were defined based on the DAPI counterstaining. While the width of these ROIs was kept constant throughout the analysis of all sections, the height of the ROIs was adjusted in each image according to the DAPI counterstaining in each section and the areas of each layer in each section were recorded. For analysing the number and soma size of tdTomato-positive cells in each layer, a threshold for each channel was determined based on multiple user-defined negative regions. Channels were thresholded and binarized, and a mask of each channel was created. The number of tdTomato-positive cells was determined by taking the logical AND of the DAPI and tdTomato channel masks and counting the number of components greater than 4 pixels in size in the double overlap of the masks of the two channels in each layer ROI. The soma size was calculated as the area of these double-overlapping components.

For paired pulse experiments, no drugs were used in the ACSF. A stimulating electrode (ISO-Flex, A.M.P.I.) was positioned approximately 100 μm below the cell, and 0.1 ms electrical pulses were given while adjusting the stimulus intensity and electrode position until the first pulse was between 100 and 500 μA. Inter-stimulus interval was varied and 10–30 p.sweep between each sweep. Pulse amplitudes were obtained from average sweeps of at least ten trials. Cells were held at −70 mV to record IPSCs and −70 mV to record EPSCs. For evoked IPSCs, no drugs were used in the ACSF. Simultaneous paired whole-cell recordings were obtained from an eGFP-expressing VIP neuron and a morphologically identified pyramidal neuron located not more than five cell bodies away from the VIP neuron. Both cells were held at 0 mV, and a 5 μs light pulse from a blue LED (Thorlabs) was used to evoke IPSCs. Light intensity and the objective position were varied until the VIP neuron IPSC amplitude was between 200 and 500 pA. Inter-stimulus interval was varied and 10–30 p.sweep between each sweep. Pulse amplitudes were obtained from average sweeps of at least ten trials.
Neurons were reconstructed using NeuronJ (ImageJ), and Sholl analysis was performed using a custom script in MATLAB.

Monocular deprivation (MD) procedure. Eyelids were trimmed and sutured under isoflurane anaesthesia (1–2% in O2) as previously described. The integrity of the suture was checked daily and mice were used only if the eyelids remained intact throughout the duration of the deprivation period. One eye was closed for 4 days starting between P26 to P28. The eyelids were reopened immediately before recording, and the pupil was checked for clarity.

Mouse visual-evoked potential (VEP). VEPs were recorded from anaesthetized mice (50 mg kg−1 Nembutal and 0.12 mg chlorprothixene) using standard techniques described previously. The contra- and the ipsilateral eye of the mouse were presented with horizontal black and white sinusoidal bars that alternated contrast (100%) at 2 Hz. A tungsten electrode was inserted into the binocular visual cortex at 2.8 mm from the midline where the visual receptive field was approximately (100%) at 2 Hz. A tungsten electrode was inserted into the binocular visual cortex of Cre mice (Igf1RCreER; AAV-ChR2-mCherry) as previously described. The integrity of the suture was checked daily and mice were used only if the eyelids remained intact throughout the duration of the deprivation period. One eye was closed for 4 days starting between P26 to P28. The eyelids were reopened immediately before recording, and the pupil was checked for clarity.

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Extended Data Figure 1 | Validation of the sensory stimulation protocol and the RiboTag-based cell-type-specific purification of mRNA.

a, Quantitative real-time PCR (qPCR) for known experience-regulated genes on RNA isolated from the visual cortex of mice that were dark-housed for varying durations (overnight, 3 days, 7 days or 14 days) and then either euthanized in the dark or exposed to light for 1, 3 or 7.5 h, and then euthanized. Data are normalized to the maximal value in each data set and represent the mean and standard error of four biological replicates.

b, qPCR for known experience-regulated genes on RNA isolated from the visual cortex of mice that were dark-housed for 14 days and then either exposed to light for 1, 3 or 7.5 h (dark + light, black) or kept in the dark during these hours (dark + dark, red). All mice of a given time point were dissected in very close temporal proximity. Data are normalized to the maximal value in each data set and represent the mean and standard error of four biological replicates.

c, qPCR for known cell-type-specific marker genes on RNA isolated from RiboTag mice expressing Cre in distinct neuronal subtypes. Data are normalized to the maximal value in each data set and represent the mean and standard error of four biological replicates.

d, qPCR for known early-induced transcription factors on RNA isolated from RiboTag mice expressing Cre in distinct neuronal subtypes. Data are normalized to the maximal value in each data set and represent the mean and standard error of three biological replicates.
Extended Data Figure 2 | Validation of the RiboTag-seq approach.

**a**, Matrix of Spearman correlation coefficients between biological replicates across all samples (scale of correlation coefficients extends from 0.7 to 1, see colour bar) (S, standard housing; 0, dark-housed only; 1/3/7.5, 1/3/7.5 h of light exposure after dark housing, respectively).

**b**, Line plots of RNA-seq data showing the expression values (normalized reads across all exons of a gene) for cell-type-specific marker genes and ubiquitously expressed house-keeping genes in different Cre lines (Emx1-cre, red; Gad2-cre, blue; Pv-cre, brown; Sst-cre, green; Vip-cre, purple) across all time points of the experiment. Data represent the mean and standard error of three biological replicates.
Extended Data Figure 3 | See next page for caption.
Extended Data Figure 3 | Characterization of the experience-induced gene programs in subtypes of cortical neurons. a, Average matrix of Spearman correlation coefficients computed from the expression values of 1000 random sets of 602 genes (including experience-regulated genes, with replacement). b, Matrix of the Spearman correlation coefficients computed from the expression levels of control transcripts that match the expression distribution of experience-regulated genes (n = 602). c, Cladogram resulting from hierarchical clustering of all samples (except samples from Gad2-cre). Cladograms were computed using the mean expression values (that is, normalized reads across all exons of a gene) for all expressed transcripts (n = 14,280). d, Cladogram resulting from hierarchical clustering of the mean expression values of a set of control transcripts that match the expression distribution of experience-regulated genes (n = 602). e, Pie charts showing the subdivision of experience-regulated genes on the basis of kinetics in each set of Cre lines (red, rapidly induced; grey, induced with delayed kinetics; orange, induced only after two weeks of dark housing; green, rapidly suppressed; magenta, suppressed with delayed kinetics; blue, suppressed only after two weeks of dark housing). f, Left, matrix of Spearman correlation coefficients between Cre lines computed using the mean expression values (normalized reads across all exons of a gene) of early-induced genes one hour after light exposure. Right, matrix of Spearman correlation coefficients between Cre lines computed using the mean expression values of late-induced genes 7.5 h after light exposure. For each matrix, the correlations upon permuting the expression values are also shown (colour scale at right, scale begins at zero.)
Extended Data Figure 4 | Characterization of cell-type-specific and experience-induced genes in subtypes of cortical neurons. a, Scatter plots showing the log₁₀ expression values for each expressed gene in a given Cre line (x axis) plotted against the maximum log₁₀ expression values for that gene found in all other Cre lines (y axis). Black line denotes unity, and the red line is the 5.5-fold enrichment threshold set to include Vglut1 as a cell-type-specific gene in Emx1-cre. Data represent the mean values of three biological replicates. b, Scatter plots of all expressed genes, for each Cre line plotting the mean log₂ fold enrichment in that Cre line (x axis) against the mean log₂ of the absolute value of the maximum fold change observed in that Cre line. Data represent the mean values of three biological replicates. Genes that pass both enrichment and induction thresholds in 3 of 3 biological replicates are shown in red. c, Bar graph showing the maximum expression value (in normalized reads) for VIP-neuron-specific experience-regulated genes.
Extended Data Figure 5 | Conditional knockout of Igf1 in VIP neurons does not affect cortical morphology or gross morphology of VIP neurons. a, Example image of cortices from Igf1 wild-type (WT) (Vip-cre/+, LSL-tdTomato/+, Igf1WT/WT) or conditional-knockout (cKO) (Vip-cre/+, LSL-tdTomato/+, Igf1fl/fl) mice. VIP neurons are labelled in white, with DAPI shown in blue (cortical layers are indicated on the left, scale bar, 200μm). b–d, Bar graphs showing the area of each cortical layer (b), number of VIP neurons per image per layer (c), or soma size of VIP neurons (d) in Igf1 wild-type (black) or conditional-knockout (red) mice. Data represent the mean and standard error of three biological replicates.
Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | Igf1 knockdown in VIP neurons affects inhibitory inputs onto VIP neurons but not onto neighbouring neurons. a, AAV shRNA constructs. shRNA cassettes against Igf1 or a control gene (Luc) were cloned downstream of the U6 promoter into an AAV vector that drives Cre-dependent expression of eGFP. b, qPCR validation of the efficacy of Igf1 shRNA constructs. Cultured cortical neurons were infected with lentiviral constructs either expressing no shRNA (vector only), a control shRNA (against Luc) or shRNAs against Igf1. Four days post-infection the cultures were quieted overnight with TTX and AP-5 and then harvested either before or after being depolarized with 55 mM KCl for 6 h; RNA was then isolated and qPCR was performed. Data are normalized to the maximal value in each replicate and represent the mean and standard error of three biological replicates. c, Bar graph showing normalized soma size of P21 visual cortex VIP neurons infected with control shRNA or shRNA targeting Igf1 (shRNA control, n = 103; shRNA Igf1, n = 174; P = 0.41, Mann–Whitney U-test). d, Bar graphs showing the levels of IGF1 in the serum of P20 mice that were injected intracortically with AAVs driving the expression of control shRNA (black) or Igf1 shRNA. Data represent the mean and s.e.m. of the serum IGF1 levels of four mice per group. e–l, Bar graphs and cumulative distribution plots showing mIPSC amplitudes and frequency/inter-event interval upon early widespread knockdown of Igf1 in VIP (e, f), SST (g, h), PV (i, j) and excitatory (k, l) neurons after injection of AAVs into P3 cortices of the respective Cre mice. VIP neurons (identified as eGFP-positive cells in Vip-cre mice): control and Igf1 shRNA, amplitude P = 0.96, frequency P = 0.04. SST neurons (identified as eGFP-positive cells in Sst-cre mice): control and Igf1 shRNA, amplitude P = 0.89, frequency P = 0.55. PV neurons (identified as eGFP-positive cells in Pv-cre mice): control and Igf1 shRNA, amplitude P = 0.084, frequency P = 0.93. Pyramidal neurons (identified by morphology): control and Igf1 shRNA, amplitude P = 0.84, frequency P = 0.15). All P values are derived from Mann–Whitney U-tests; numbers inside bars indicate the number of cells recorded.
Extended Data Figure 7 | See next page for caption.
Extended Data Figure 7 | Effects of IGF1 overexpression in excitatory and SST-positive neurons. a, RiboTag-seq identifies Igf1.4 as the major Igf1 isoform expressed in VIP neurons. Representative tracks of histograms of the RNA-seq reads in each Cre line across the Igf1 genomic locus. Data are from the 7.5 h light exposure RiboTag-seq data sets. b, AAV constructs for the Cre-dependent expression of HA-/Myc-tagged IGF1 (Igf1.4) and eGFP (that is, IGF1-OE, top) or of eGFP alone (that is, control, bottom). F2A, Furin cleavage site followed by the 2A peptide; black and white triangles represent a Cre-dependent Flex-switch. c, Western blot analysis of IGF1-receptor activation status in lysates of serum starved HEK293T cells that were stimulated with conditioned media (CM) containing epitope-tagged isoforms of IGF1. CM was produced by transfecting HEK293T cells with the respective construct and collecting the culture media. IGF1-receptor is detected with antibodies against either activated IGF1 receptor (anti-IGF1R pTyr1136/1138) or total IGF1 receptor (anti-IGF1R total). Molecular weight markers are on the right and the arrow indicates the band of the IGF1 receptor. d, e, Bar graphs and cumulative distribution plots showing mIPSC frequency/inter-event interval (d) and amplitude (e) of mIPSCs recorded from eGFP-positive neurons in P20 Sst-cre mice that were intracortically injected with AAVs driving the expression of control (C) or IGF1-OE (OE) constructs (amplitude, P = 0.16; frequency, P = 0.01; Mann–Whitney U-test; numbers inside bars indicate the number of cells recorded). f, g, Bar graphs and cumulative distribution plots showing mIPSC frequency/inter-event interval (f) and amplitude (g) of mIPSCs recorded from eGFP-positive neurons in P20 Emx1-cre mice that were intracortically injected with AAVs driving the expression of control (black, n = 20) or IGF1-OE AAVs (red, n = 20). Amplitude, P = 0.99; frequency, P = 0.01, Mann–Whitney U-test. h–j, Scatter plots of IGF1-interacting proteins showing the log_{10} normalized mean expression values in Vip-cre neurons versus each of the other Cre lines (Emx1 (h), PV (i), Sst (j)). k, Quantification of EPSC paired-pulse recordings from VIP neurons infected with control shRNA- (black n = 9) or Igf1 shRNA- (red n = 11) expressing AAVs. The ratio of the second EPSC amplitude divided by the first EPSC amplitude is plotted against inter-stimulus interval (P = 0.1, two-way ANOVA).
Extended Data Figure 8 | VIP neuron-derived IGF1 does not disrupt ocular dominance plasticity. a, Widespread infection of VIP neurons by AAV-shRNA-hUbc-Flex-eGFP. High-titre injection of AAVs into the visual cortex of P18–20 Vip-cre/+ mice leads to infection of the majority VIP neurons (green, eGFP; red, anti-VIP; blue, DAPI; arrowheads, infected VIP neurons; scale bars, 150 μm, 20 μm in the inlet). b, c, Average of VEP traces recorded in the visual cortices of mice that were injected with AAVs expressing control shRNA (black/grey) or shRNA against Igf1 (red/pink) shRNA and that were (grey, pink) or were not (black, red) subjected to monocular deprivation in the eye contralateral to the recording site (MD versus NoMD, respectively). d, Monocular deprivation induces a significant reduction in the VEP amplitude in response to low spatial frequency stimulation in mice that had AAVs expressing control shRNA and Igf1 shRNA injected into their visual cortices (control shRNA NoMD, n = 5 mice; control shRNA MD, n = 7 mice; Igf1 shRNA NoMD, n = 5; Igf1 shRNA MD, n = 8. *P < 0.05, Mann–Whitney U-test). e, Mice that had AAVs expressing control shRNA (black) and Igf1 shRNA (red) injected into their visual cortices display normal ocular dominance plasticity as monocular deprivation (MD) induces a shift to the ipsilateral eye in both groups (control shRNA NoMD, n = 5 mice; control shRNA MD, n = 7; Igf1 shRNA NoMD, n = 5; Igf1 shRNA MD, n = 8 mice. **P < 0.0001, one-way ANOVA with Tukey's post hoc test).