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# Disruption of NMDA receptor function prevents normal experience-dependent homeostatic synaptic plasticity in mouse primary visual cortex

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Abbreviated title: NMDARs in homeostatic synaptic plasticity of V1

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**Abstract**

Homeostatic regulation of synaptic strength allows for maintenance of neural activity within a dynamic range for proper circuit function. There are largely two distinct modes of synaptic plasticity that allow for homeostatic adaptation of cortical circuits: synaptic scaling and sliding threshold (BCM theory). Previous findings suggest that the induction of synaptic scaling is not prevented by blocking NMDA receptors (NMDARs) while sliding threshold model posits that the synaptic modification threshold of LTP and LTD readjusts with activity hence, the outcome of synaptic plasticity is NMDAR-dependent. While synaptic scaling and sliding threshold have been considered two distinct mechanisms, there are indications from recent studies that these two modes of homeostatic plasticity may interact or that they may operate under two distinct activity regimes. Here we report using both sexes of mouse that acute genetic knockout of the obligatory subunit of NMDAR or acute pharmacological block of NMDAR prevents experience-dependent homeostatic regulation of AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs) in layer 2/3 of visual cortex. This was not due to gross changes in postsynaptic neuronal activity with inhibiting NMDAR function as determine by c-Fos expression and 2 photon $Ca^{2+}$ imaging in awake mice. Our results suggest that experience-dependent homeostatic regulation of intact cortical circuits is mediated by NMDAR-dependent plasticity mechanisms, which supports a sliding threshold model of homeostatic adaptation.

63 **Significance statement**

64 Prolonged changes in sensory experience lead to homeostatic adaptation of excitatory synaptic
65 strength in sensory cortices. Both sliding threshold and synaptic scaling models can account for
66 the observed homeostatic synaptic plasticity. Here we report that visual experience-dependent
67 homeostatic plasticity of excitatory synapses observed in superficial layers of visual cortex is
68 dependent on NMDA receptor function. In particular, both strengthening of synapses induced by
69 visual deprivation and the subsequent weakening by reinstatement of visual experience were
70 prevented in the absence of functional NMDA receptors. Our results suggest that sensory
71 experience-dependent homeostatic adaptation depends on NMDA receptors, which supports the
72 sliding threshold model of plasticity and input-specific homeostatic control observed *in vivo*.
73

## Introduction

Neuronal circuits constantly undergo changes through development, experience, and learning that allow for adaptation to different environments or internal states. Correlation based synaptic plasticity mechanisms such as long-term potentiation (LTP) and long-term depression (LTD) are largely responsible for storing such information. However, LTP and LTD have innate positive feedback loop that requires additional homeostatic mechanisms to allow stability of neural circuits undergoing plasticity. Several models of homeostatic plasticity can achieve this function, including synaptic scaling (Turrigiano, 2008) and the sliding threshold model (Cooper and Bear, 2012).

According to synaptic scaling, prolonged reduction in neuronal activity leads to an upscaling of the strength of excitatory synapses, while a period of enhanced activity results in a downscaling (Turrigiano et al., 1998). Synaptic scaling was initially proposed to occur globally across the majority of synapses in a multiplicative manner to preserve relative differences in synaptic weight (Turrigiano et al., 1998). Initial demonstrations of synaptic scaling were done by observing changes in the amplitude of miniature excitatory postsynaptic currents (mEPSCs) in cultured neurons upon pharmacological manipulation of neural activity, and its induction is largely independent of NMDA receptor (NMDAR) activation (O'Brien et al., 1998; Turrigiano et al., 1998). Similar homeostatic changes in mEPSCs, which has been interpreted as synaptic scaling, can be induced in pyramidal neurons of rodent primary visual cortex (V1) by dark exposure (DE) (Goel et al., 2006; Goel and Lee, 2007; He et al., 2012), intraocular TTX injection (Desai et al., 2002), enucleation (He et al., 2012), and retinal lesions (Keck et al., 2013). Similar to what is observed in cultured neurons, synaptic scaling in V1 is largely mediated by the insertion or removal of AMPA receptors (AMPARs) (Goel et al., 2006; Goel et al., 2011).

Sliding threshold model states that prolonged periods of altered activity result in the modification of the threshold for LTP and LTD induction. The synaptic modification threshold shifts bidirectionally depending on the history of neuronal activity: an extended period of low activity slides the threshold to favor LTP, while high activity shifts it to favor LTD (Abraham and Bear, 1996; Cooper and Bear, 2012). Sliding threshold has been demonstrated in rodent V1, in which dark-rearing (DR) or DE leads to a lower threshold for LTP induction (Kirkwood et al., 1996; Philpot et al., 2003; Guo et al., 2012). Synaptic modification threshold "slides" by changes in either the induction mechanisms of LTP/LTD, such as alterations in NMDAR function (Quinlan et al., 1999; Philpot et al., 2003) and inhibition (Steele and Mauk, 1999), or the expression mechanisms of LTP/LTD, such as changes in AMPAR phosphorylation (Huang et al., 2012).

Although seemingly different, both synaptic scaling and sliding threshold allow homeostatic adaptation of synapses to maintain neuronal activity within a physiologically relevant, yet stable dynamic range. Previous investigations have shown that both changes in mEPSC amplitude and changes in the modification threshold happen *in vivo* in V1. They have, however, failed to address how these two processes interact, if at all, and to which extent. Recent evidence suggests that different levels of activity change may trigger synaptic scaling versus sliding threshold mode of homeostatic plasticity in V1 (Bridi et al., 2018). Based on the reported differences in the requirement of NMDAR activation for synaptic scaling and sliding threshold models, we examined the role of NMDARs on visual experience-dependent changes in mEPSCs of L2/3 neurons of mouse V1, which have been interpreted as synaptic scaling (Desai et al., 2002; Goel et al., 2006; Goel and Lee, 2007; He et al., 2012; Keck et al., 2013). We reasoned that if the observed scaling of mEPSCs with visual experience is a consequence of LTP/LTD due

to the sliding threshold, then these changes would depend on NMDAR activation. Using cell-type specific knockout mice or an antagonist of NMDARs, we found evidence supporting a key role of NMDARs in mediating experience-dependent homeostatic synaptic plasticity in V1.

## Materials and Methods

### *Visual experience manipulation*

All animal handling and manipulations were approved by the Institutional Animal Care and Use Committee (IACUC) at Johns Hopkins University and followed the guidelines established by the National Institutes of Health (NIH). Male and female $NR1^{flox}$ mice (https://www.jax.org/strain/005246; RRID: IMSR_JAX:005246) were raised under a 12 hours light/dark cycle until postnatal day 25-35 (P25-P35). At this point a group of mice was placed in 24-hour dark conditions for 2 days (2 days dark exposure, DE). Animals in the dark were cared for by using infrared vision goggles. A group of DE mice were taken out of the dark and re-exposed to light for 2 hours (2 hours light-exposed, LE). Age matched control animals were continuously raised in the normal 12 hours light/dark cycle (Ctl).

### *Targeted viral transfection*

Male and female $NR1^{flox}$ mice between P23-P27 were bilaterally injected with an adeno-associated viral vector expressing Cre-GFP under the control of CaMKII promoter (AAV9.CaMKII.HI.eGFP-Cre.WPRE.SV40; Penn Vector Core, University of Pennsylvania, Cat#: AV-9-PV2521) in V1. Layer 2/3 of V1 was targeted by using the following stereotaxic coordinates relative to Bregma: posterior -3.6mm, lateral 1.5mm, and depth -0.3mm. Mice recovered on a heated pad until movement, eating and drinking behaviors were evident. Animals

were returned to the mouse colony after recovery and remained under 12 hours light/dark conditions until experimental use. Viral expression and knockout of NR1 gene was confirmed experimentally 6-7 days after transfection as determined by significantly reduced NMDAR currents (Fig. 1). Manipulation of visual experience therefore commenced 1 week (6-7d) after viral injections. Control mice underwent the same procedure, but instead were injected with a GFP-expressing adeno-associated virus (AAV9.CaMKII0.4.eGFP.WPRE.rBG; Penn Vector Core, University of Pennsylvania, Cat#: AV-9_PV1917).

***In vivo CPP application***

For control normal-reared and DE groups, NMDAR antagonist D-4-[(2*E*)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid (d-CPP; Tocris, Minneapolis, MN; Cat#: 1265) was delivered intraventricularly for 2 days via Alzet osmotic minipumps (DURECT Corporation, Cupertino, CA; Cat#: 1007D) coupled to a brain infusion cannula (Alzet, DURECT Corportation, Cupertin, CA; Cat# 8851). Control groups were infused with saline instead of d-CPP. In brief, all osmotic minipumps (0.5 µL/hr) were backfilled with either d-CPP (10 µM) or saline solution and primed by incubating in saline solution at 37˚C for at least 5 hours before implantation. Implantation surgery was done under constant administration of 1.5-2% isoflurane/oxygen mix under aseptic conditions. Anesthesia level and vital signs were monitored during the surgery. Craniotomy was performed for cannula insertion by drilling a small hole in the skull at stereotaxic coordinates -0.22 mm posterior, 1 mm lateral from Bregma to target the lateral cerebral ventricle using a dental drill and a sterilized 0.5 mm drill-bit. The neck was aseptically cleaned, a small cut was made at the base and blunt forceps were used to separate the fascia, then an osmotic mini-pump was inserted subcutaneously. The cannula was guided to the

drilled hole on the skull and secured in place with dental cement (TEETS denture material; Patterson dental, MN; Cat#: 223-3773). Following the surgery, mice were recovered on a 30˚C heat pad, and returned to the home cage where drinking water was supplemented with 0.07 mg/mL carprofen (Sigma-Aldrich 33975; CAS#: 53716-49-7) (Ingrao et al., 2013). For DE group, mice were allowed to recover for at least 12 hours before placed inside a darkroom for 2 days. For LE groups, d-CPP (10 mg/kg) or saline were delivered intraperitoneally (200 µl volume) in the dark room 10 min before light exposure.

***Primary visual cortex slice preparation***

Mice between P25-P35 were deeply anesthetized with isoflurane gas in a chamber placed in a chemical fume hood. Anesthesia was delivered to dark exposed animals in a light-tight chamber. After confirming the absence of pinch or righting reflex, mice were decapitated and the brain was immediately placed in ice-cold dissection buffer containing the following (in mM): 212.7 sucrose, 10 dextrose, 3 $MgCl_2$, 1 $CaCl_2$, 2.6 KCl, 1.23 $NaH_2PO_4 \cdot H_2O$, and 26 $NaHCO_3$, which was bubbled with 95% $O_2$/5% $CO_2$ gas. Blocks containing V1 were rapidly isolated and sectioned coronally into 300 µm thick slices, while submerged in ice-cold dissection buffer, using a vibratory tissue slicer (PELCO easiSlicer, Ted Pella; product#: 11000). Slices were transferred to a submersion holding chamber filled with artificial cerebrospinal fluid containing (in mM): 124 NaCl, 5 KCl, 1.25 $NaH_2PO_4 \cdot H_2O$, 26 $NaHCO_3$, 10 dextrose, 2.5 $CaCl_2$, and 1.5 $MgCl_2$, bubbled with 95% $O_2$/5% $CO_2$. The slices recovered for 1 hour at room temperature before electrophysiological recordings started.

***Electrophysiological recordings***

Slices were transferred to a submersion-type recording chamber and perfused with oxygenated ACSF (bubbled 95% $O_2$/5% $CO_2$ at 32 ± 2°C) at a rate of 2mL/min. The chamber was mounted on a fixed stage under an upright microscope (E600 FN; Nikon, Tokyo, Japan) with oblique infrared illumination. Pyramidal neurons in L2/3 of V1 were visually identified and patched using a glass pipette with a tip resistance between 3 and 5 MΩ, which was filled with internal solution containing (in mM): 120 CsOH, 120 Gluconic acid, 10 phosphocreatine, 0.5 GTP, 4 ATP, 8 KCl, 1 EGTA,10 HEPES and 5 QX-314. An Axon patch-clamp amplifier (Multiclamp 700B, Molecular Devices) was used for voltage-clamp recordings and data was acquired through Igor Pro software (WaveMetrics, http://www.wavemetrics.com/products/igorpro/igorpro.htm; RRID:SCR_000325). Only data from cells with input resistance (Ri) > 150 MΩ and series resistance (Rs) < 25 MΩ were analyzed.

*NMDAR/AMPAR ratio* Glutamatergic currents were recorded in response to electric stimulation delivered through a bipolar glass electrode placed in V1 L4 or L2/3. Recordings were done in the presence of 20 µM bicuculline methiodide (Enzo Life Sciences, product #: BML-EA149-0050) in the ACSF. The stimulation intensity was adjusted so that a single-peak response was produced with an onset latency of 2-3ms. The AMPA receptor component was taken as the average peak amplitude of responses recorded at Vh=-70 mV. The NMDA receptor component was taken as the average amplitude of responses recorded at Vh=+40 mV 70 ms after onset. Responses were recorded every 10 s and a minimum of 10 responses were averaged for each component.

*miniature EPSCs* AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs) were isolated by recording with 1 µM tetrodotoxin citrate (TTX; Abcam; product#: ab120055), 20 µM bicuculline methiodide, and 100 µM DL-2-amino-5 phosphonopentanoic acid (DL-APV; Sigma-Aldrich A5282; CAS#: 76326-31-3) in the ACSF. Events were recorded at

212 Vh=-80 mV for a minimum of 4 minutes initiated 1-2 minutes after cell break-in. The recorded
213 data were digitized at 2 kHz by a data acquisition board (National Instruments), acquired with
214 Igor Pro software and analyzed using the MiniAnalysis program (Synaptosoft,
215 http://www.synaptosoft.com/MiniAnalysis/; RRID:SCR_002184). The detection threshold for
216 mEPSCs was set to 3 times the root mean square (RMS) noise and events with a rise time > 3 ms
217 were excluded from analysis. Events within bursts (more than 2 events, inter-event-interval < 10
218 ms) were excluded from the measurement of amplitudes. The average of total isolated events
219 (200-220) was used to calculate the decay time constant for each neuron. Cells were discarded if
220 Ri or Rs changed more than 15% during the duration of the recording.
221
222 ***Biocytin processing***
223 Slices used for electrophysiological recordings were immediately fixed in 10% formalin
224 (Sigma-Aldrich, HT5014; MDL: MFCD00003274) solution overnight at 4°C. Slices were rinsed
225 0.01M phosphate buffered saline (PBS) at room temperature and permeabilized in 2% Triton X-
226 100 (Fisher) in PBS for 1 h. Slices were then incubated in 1:2000 solution of avidin-Texas Red
227 conjugate (Life Technologies; A820) in 1% Triton X (in PBS) overnight. After incubation, slices
228 were washed in PBS, mounted on glass slides, and coverslipped with Prolong Gold Anti-fade
229 (Fisher; product #: P36930) mounting medium. Images were taken using a Zeiss LSM 510
230 META confocal microscope.
231
232 ***Immunohistochemistry***
233 $NR1^{flox}$ mice were deeply anesthetized with isoflurane vapors in a closed chamber placed
234 in a fume hood. 2dDE animals were anesthetized in a light-tight chamber. Animals were

perfused transcardially with PBS followed by 10% formalin solution. The brains were then extracted and kept in 10% formalin overnight. V1 was isolated and sectioned coronally in 40 µm thick slices. Free floating slices containing V1 were incubated with 1% sodium borohydride (Fisher; Cat#: S-678-10) for 15 minutes at room temperature and then washed with PBS. The same slices were blocked for 2 hours in a solution containing 3% goat serum (Sigma-Aldrich; product #: 9023) and 0.3% Triton-X in PBS or mixture of 10% normal goat serum (Jackson Immunoresearch; product code#: 005-000-121), 5% normal donkey serum (Jackson Immunoresearch; product code#: 017-000-121) and 0.5% Triton-X in PBS. Cortical slices were then incubated with antibodies against c-Fos and Neuronal nuclei protein (NeuN) in the blocking buffer overnight. Slices were rinsed and then incubated for 2 hours with fluorescently labeled secondary antibodies. Slices were then washed with PBS, incubated with DAPI and mounted on glass slides with Prolong Gold Anti-fade medium. The antibody concentrations were as follows: 1:20,000 rabbit anti-cFos (Calbiochem, Cat#PC38; RRID:AB_2106755) or 1:500 rabbit anti-c-Fos (Cell Signaling Technology, Cat#2250S; RRID:AB_10692514), 1:200 mouse anti-NeuN (Millipore MAB377; RRID: AB_2298772), 1:200 donkey anti-mouse Alexa Fluor 488 (Thermo Fisher; RRID: AB_141607), 1:200 goat anti-rabbit Alexa Fluor 633 (Thermo Fisher; RRID: AB_2535731), goat anti-mouse Alexa Fluor 555 (Thermo Fisher; RRID: AB_2535844). Slices were imaged using either Zeiss LSM 700 or 800 confocal microscope with a step size of 0.5 µm. All images were analyzed using Volocity software.

***Two Photon $Ca^{2+}$ imaging in awake head fixed mice***

Male and female Emx1-Cre x Ai96 mice (https://www.jax.org/strain/005628 RRID: MGI:2684615, https:/www.jax.org/strain/024106) underwent head plate and cranial window

implantation surgery at p23-p25. The head plate and cranial windows, consisting of one 5 mm and two 3 mm round coverslips, were based on an existing design (Goldey et al., 2014). After 3-4 days of recovery, the mice were habituated to head-fixation in a body tube for 4-5 days. Habituated mice readily accepted sucrose and did not react aversively to visual stimuli. 2-photon calcium imaging was performed at P33-P35, using a custom-built microscope based on a Janelia Farm design (https://wiki.janelia.org/wiki/display/shareddesigns/MIMMS). GCaMP6s expressing neurons were imaged through a 16x 0.8 NA Nikon using a Chameleon Ultra 2 laser (Coherent) at 940 nm. Due to relatively weak fluorescence of the Ai96 line, imaging depth was limited to 150-220 μm with laser power set to 75-100 mW. Images were acquired at 30 Hz using Scanimage 2018 (Pologruto et al., 2003) and analyzed using custom scripts written in Matlab (Mathworks).

*Visual stimuli and data acquisition* During the imaging session, mouse was head-fixed in the body tube with visual stimuli displayed on a monitor centered 25 cm from the contralateral eye (60 Hz, mean luminance 30 cd/m$^2$). The stimuli were moving sinusoidal gratings at 8 orientations (45° increments), with spatial frequency 0.05 cycles/degree, temporal frequency 3 cycles/second. The gratings were shown for 3 s followed by 6 s interstimulus interval and were organized into blocks of 10 stimuli, with each block containing all the gratings and two additional blank stimuli in random order. After 12 repetitions of each stimulus (lasting 18 minutes), the mouse was taken out of the body tube and given an i.p. injection of either saline or 10 mg/kg CPP (Tocris, Cat#: 01773). A second round of imaging was then done 30-60 minutes after the injection in the same region. Depth was manually adjusted to match pattern of cell bodies from first round of imaging.

*Data processing and analysis* After cross-correlation based frame alignment, ROIs were manually drawn around visible cell bodies and cell body masks were calculated in semi-automated manner using correlation with a seed pixel. Neuropil signal was estimated from pixels within ROIs that were at least 2 pixels away from the mask boundary. Fluorescent traces for both mask signal and neuropil were filtered by 0.5 second running average to reduce noise. Fluorescent baseline $F_0$ was calculated as the running $10^{th}$ percentile over 1800 frames (~1 minute) of the mask signal. Fluorescence signal from cell bodies was then calculated as $\Delta F/F_0 = ((F_{mask}-0.7*F_{neuropil})- F_0)/ F_0$. Visual responses were calculated as the mean $\Delta F/F_0$ over the 3 s duration of the stimulus minus mean $\Delta F/F_0$ over 1 s preceding the visual stimulus. Spontaneous activity was calculated as the mean $\Delta F/F_0$ over 7 s window following the onset of blank stimuli. To determine whether the neuron was visually responsive, the difference between visual response and blank stimulus response was compared to distribution of such differences for scrambled data (1000 re-samplings). The neuron was considered visually responsive if the difference was higher than at least 950 (95%) of differences from the scrambled distribution. Note that this excludes neurons that are suppressed by visual stimuli or show off-response to visual stimuli. A similar procedure was used to determine whether the neuron had significant orientation selectivity. The orientation selectivity index $OSI = (R_{pref}-R_{ortho})/(R_{pref}+R_{ortho})$ was calculated and then compared to distribution of OSI calculated from scrambled data. If the OSI was higher than 95% of OSIs from the scrambled distribution, the neuron was considered significantly orientation selective.

***Experimental Design and Statistical Analysis***

Data are presented as mean ± SEM. All statistical analyses were done using Prism 7.0 (GraphPad Software; RRID: SCR_002798). One-factor analysis of variance (ANOVA) was used to compare multiple groups followed by a Tukey's multiple comparison *post hoc* test. Unpaired Student's t-tests were used for two group comparisons. The Kolmogrov-Smirnov (K-S) test was used to compare cumulative probabilities. A p-value $< 0.05$ was used as a measure of significance in t-tests, ANOVAs, and Tukey's or Bonferroni *post hoc* analyses. For K-S tests, p-values $< 0.01$ were used as a measure of significance. Asterisk (*) is used in both text and figures to denote statistical significance.

## Results

### *Neuron-specific NMDAR KO*

To test whether NMDAR is necessary for experience-dependent homeostatic synaptic plasticity in V1, we aimed to specifically knockout (KO) NMDARs in principal neurons. This was achieved by targeted injection of an adeno-associated viral construct expressing Cre-GFP under the control of the CaMKII promoter (AAV9.CamKII.Cre-eGFP; Cre-GFP condition) into V1 L2/3 of $NR1^{flox}$ transgenic mice (Tsien et al., 1996). In this scheme, the expression of Cre recombinase leads to excision of the *Grin1* gene, which encodes the obligatory NMDAR NR1 (GluN1) subunit. To control for effects only due to viral transfection, a second group of $NR1^{flox}$ transgenics were injected with a GFP-expressing viral construct (AAV9.CaMKII.GFP; GFP-only condition). We verified that viral transfection efficiency was similar for both constructs by quantifying the percentage of GFP-positive cells relative to the total number of neurons in a given tissue section of V1 L2/3 (Fig. 1A). To determine whether Cre-GFP condition leads to effective knockout of NMDARs, we used whole-cell voltage clamp to measure

NMDAR/AMPAR ratios after viral injections. We corroborated the specificity of the functional NMDAR knockout by also measuring NMDAR/AMPAR ratios from non-GFP expressing neurons that were neighbors to the knockout cells (neighbors) (Fig. 1B, C). We found a significant decrease in NMDAR currents for NMDAR knockout cells (NMDAR KO; Cre-GFP) 7 days after viral injection (Fig. 1C). These results confirm both the specificity and the effectiveness of the virally mediated NMDAR KO used in this study.

***NMDAR KO abolishes experience-dependent homeostatic changes in synaptic strength***

Next we examined whether NMDARs play a role in homeostatic synaptic plasticity by measuring changes in the strength of excitatory synapses on V1 L2/3 pyramidal neurons following manipulations to visual experience in the presence or absence of NMDARs. Homeostatic synaptic plasticity has been characterized both *in vitro* and *in vivo* as an increase in synaptic strength after prolonged periods of decreased neuronal activity and a decrease in synaptic strength after periods of increased activity (O'Brien et al., 1998; Turrigiano et al., 1998; Desai et al., 2002; Goel and Lee, 2007). Previous studies have established that 2 days of visual deprivation in the form of dark exposure (DE) increases the strength of excitatory synaptic transmission, as measured by mEPSC amplitude, which is rapidly reversed by reinstating visual experience for a short period (light exposure, LE) (Goel and Lee, 2007; Gao et al., 2010).

In GFP-only control neurons, the average mEPSC amplitude was significantly increased after 2-days of DE and returned to normal-reared control (Ctl) values after 2-hours of LE (Fig. 2A). Changes in the average mEPSC amplitude was also evident in the distribution of mEPSC amplitudes plotted in cumulative probability graphs (Fig. 2A), where the distribution of mEPSC amplitudes of control and DE group were significantly different while those between control and

LE did not show statistical significance. Alterations in visual experience had no significant effect on the average frequency of mEPSCs of GFP-only condition neurons (Fig. 2A). These results are consistent with previous studies showing that homeostatic synaptic plasticity in V1 L2/3 mainly manifests as postsynaptic change in AMPA receptors (Goel et al., 2006; Goel et al., 2011; He et al., 2012).

In contrast, mEPSCs recorded from NMDAR KO neurons (Cre-GFP condition) lacked regulation by changes in visual experience. There was no significant change in either the average amplitude or frequency of mEPSCs across control, DE or LE groups (Fig. 2B). Moreover, we did not observe any significant difference in mEPSC amplitude distribution across the 3 groups, as shown by overlapping cumulative probability graphs (Fig. 2B). There was no significant difference in basal mEPSC amplitude between normal-reared GFP-only controls and NMDAR KO neurons (Student's t-test: $t=1.416$, $p=0.1713$). However, we noted a significant increase in baseline frequency of mEPSC in control normal-reared NMDAR KO neurons when compared to normal-reared GFP-only neurons (GFP-only: $2.5 \pm 0.15$ Hz, $n=9$; NR1 KO: $4.9 \pm 0.57$ Hz, $n=14$; unpaired Student's t-test: $t=3.258$, $^{*}p=0.0038$). This is similar to an observation made in a previous study measuring mEPSCs from NMDAR KO neurons in CA1 (Adesnik et al., 2008), which implicated NMDARs in regulating the number of functional synapses. Unexpectedly, data from neighbor neurons, which did not express Cre-GFP and have intact NMDAR current (Fig. 1C), also failed to modulate the average mEPSC amplitude with changes in visual experience (Fig. 2C). While there was no significant change in the average mEPSC amplitude across groups, there was a statistically significant increase in the distribution of mEPSC amplitudes of LE group as seen in the cumulative probability graph (Fig. 2C). In addition, unlike the NMDAR KO neurons or GFP-only condition, these neighbor neurons showed significant increase in mEPSC

frequency after LE relative to control conditions (Fig. 2C). At this point, we cannot explain the phenotype of neighbor neurons except that NMDAR KO may not simply have a cell autonomous regulation of homeostatic plasticity in V1 L2/3 neurons. In any case, our results indicate that knocking out NMDARs prevents experience-dependent homeostatic synaptic plasticity and therefore support a necessary role of NMDARs in this process.

***NMDAR function is required for experience-dependent homeostatic synaptic plasticity***

Our data so far suggest that NMDAR KO prevents experience-dependent homeostatic synaptic plasticity. One caveat of our results from virally mediated NMDAR KO experiments is that NMDAR KO cells lacked upscaling by DE, hence we could not confirm whether NMDAR is required also for downscaling of mEPSCs when DE mice are re-exposed to light. In order to test this, a more acute manipulation of NMDAR function is needed hence we used pharmacology to acutely inhibit NMDAR function just during the LE. We blocked NMDAR function pharmacologically by administration of D-4-[(2*E*)-3-Phosphono-2-propenyl]-2-piperazinecarboxylic acid (d-CPP), which is a selective and competitive antagonist of NMDAR (Lehmann et al., 1987). Mice pertaining to the LE group were placed in the dark room for two days without any drug to allow normal scaling up process by DE, and then received d-CPP via an intraperitoneal injection (i.p., 10 mg/kg) 10 minutes before light re-exposure. To control for i.p. injection, a group of mice received the same volume of saline injection instead of d-CPP before light re-exposure. This design allows for specific testing of the role of NMDAR in LE-induced downscaling, which was not possible to determine in NMDAR KO condition where DE-induced upscaling was absent. In order to determine the effect of LE, we also had a group of control normal-reared and DE mice receiving saline. Because DE induced upscaling requires 2 days,

saline was administered into the lateral ventricle using an osmotic mini-pump coupled to a cannula to allow 2 days of saline infusion during DE. Control normal-reared also received 2 days of saline via osmotic mini-pump to control for any effect of osmotic mini-pump surgery. We found that d-CPP injection right before LE prevented the normal downscaling of average mEPSC amplitude (Fig. 3A), which was also evident when comparing the cumulative probability of mEPSCs recorded from saline LE versus d-CPP LE groups (Fig. 3B). There was no significant difference in average mEPSC frequency across groups (Fig. 3C). These results suggest that NMDAR function is also required for downscaling mEPSCs in LE condition.

We also attempted to see whether blocking NMDAR function with d-CPP blocks scaling up of mEPSCs with DE as observed in NMDAR KO neurons. This was to determine whether the failure to scale up mEPSCs in NMDAR KO neurons is due to missing the NMDAR protein itself, which is known to have a structural role in organizing downstream signaling molecules at synapses via its intracellular domain (Sprengel et al., 1998; Kohr et al., 2003), or absence of NMDAR function. For the purpose of this experiment d-CPP was perfused for 2 days into the lateral ventricle through an osmotic minipump coupled to a cannula for Ctl normal-reared or DE animals. Unexpectedly, we found that 2 days of d-CPP infusion in control normal-reared mice significantly increased the amplitude of mEPSCs when compared to mice receiving the same duration of saline (Fig. 3D). This presents difficulty in interpreting our results from d-CPP treated DE mice (average mEPSC amplitude = 11.52 ± 0.45 pA, n=13). Despite the lack of conclusive data from d-CPP treated DE group, our result from LE group suggests that functional NMDARs are required for rapid downscaling mEPSCs with visual experience. Taken together with our NMDAR KO data, these results support the previous conclusion that functional NMDARs are required to undergo proper experience-dependent synaptic scaling.

***NMDAR disruption does not alter overall postsynaptic activity***

A potential concern regarding the lack of homeostatic adaptation observed with NMDAR function block is that it may reflect an inability of the network to modulate activity levels with visual experience. Changes in postsynaptic activity levels are thought to drive homeostatic synaptic plasticity (Ibata et al., 2008; Goold and Nicoll, 2010)(but see (Fong et al., 2015)). Therefore, we investigated whether disrupting NMDAR function altered the overall activity of V1 L2/3 neurons. To do this, we used the expression of the immediate early gene c-Fos as a proxy for neuronal activation under different conditions (Hoffman et al., 1993; Joo et al., 2016).

We first examined how global block of NMDAR function by d-CPP may have altered V1 L2/3 neurons, hence compared c-Fos expression in V1 L2/3 neurons under Ctl, DE and LE conditions in saline and d-CPP infused mice (Fig. 4A-C). As in our mEPSC recording studies (Fig. 3), Ctl and DE group received saline or d-CPP via osmotic pump for 2 days, while LE group was placed in a darkroom for 2 days to allow normal DE-induced up-scaling and only received saline or d-CPP via i.p. injection 10 min before light re-exposure. V1 slices were co-stained for cFos and NeuN (neuronal marker), and the fraction of c-Fos positive neurons in L2/3 were quantified by dividing the number of c-Fos positive cells by the number of NeuN positive cells. We found that the fraction of c-Fos positive neurons decreased with DE and returned to control levels with LE in saline infused mice, which is consistent with a reduction in activity levels with visual deprivation. We observed the same pattern of regulation in c-Fos positive neuronal fraction in mice infused with d-CPP, which suggests that a global block of NMDAR function in the whole brain does not grossly alter the level of neuronal activation leading to c-Fos expression in V1 L2/3.

To test if selective KO of NMDAR in V1 neurons also preserves neuronal activity across visual manipulations, we compared the fraction of c-Fos positive neurons following viral KO of NMDARs by expressing Cre-GFP or control-GFP in $NR1^{flox}$ mice (Fig. 4D-F). Here we only quantified the fraction of c-Fos positive cells among neurons that were co-labeled with GFP and NeuN, hence the quantification corresponds to activity of GFP or Cre-GFP transfected neurons. In V1 sections from control GFP expressing mice, we did not observe a clear down regulation of c-Fos expression in control GFP expressing neurons with DE, but there was significant increase in c-Fos expression with LE. In V1 sections from Cre-GFP expressing mice (NMDAR KO), we found that DE decreased c-Fos expression which returned to normal levels with LE similar to what we saw in saline infused animals. Despite the caveat that we cannot explain the lack of a significant decrease in c-Fos expression with DE in control GFP expressing neurons, our data nonetheless suggest that KO of NMDAR in neurons do not grossly alter their activity levels across different visual manipulations. Collectively, our results suggest that the disruption in homeostatic regulation of excitatory synapses in the absence of NMDAR function is not likely due to major alterations in the overall activity of V1 L2/3 neurons.

Assessing neural activity with immediate early gene expression, such as c-Fos, has innate limitation in that they can only monitor whether the neural activity is beyond the threshold for immediate early gene induction, but cannot determine fine scale changes in neural activity. In order to more directly measure the effect of NMDAR blockade on V1 L2/3 neuronal activity, we performed *in vivo* calcium imaging from awake mice before and after CPP injection (Fig. 5A). Individual neurons sometimes displayed differences in response to moving sinusoidal gratings pre and post CPP injection (Fig. 5B). However, the distribution of these differences was not significantly different from that for control mice receiving saline for spontaneous activity

464 measured during blank screen presentation (Fig. 5C left panel) or activity measured during
465 presentation of visual stimuli (Fig. 5C middle panel). This also held true if we restricted analysis
466 only to neurons that were significantly visually activated (Fig. 5C right panel). NMDA spikes
467 have previously been implicated in stimulus selectivity of layer 4 neurons in the barrel cortex
468 (Lavzin et al., 2012), so we considered the possibility that CPP might affect orientation tuning of
469 the neurons (Fig. 5D). The response at preferred orientation did not change significantly in mice
470 receiving saline versus CPP (Fig. 5D left panel). Likewise, the changes in orientation selectivity
471 index were not significant (Fig 5D right panel), although there was a trend that CPP may have
472 affected OSI in a small subpopulation of L2/3 neurons. Overall, our results indicate that blocking
473 NMDARs with CPP is unlikely to grossly affect responses of V1 L2/3 neurons in a way that
474 could account for the observed effects on plasticity.
475
476 **Discussion**
477 The mechanisms of homeostatic adaptation to changes in sensory experience has been
478 largely explained by two distinct models: sliding threshold and synaptic scaling. While these two
479 models are considered different, there is emerging body of recent work suggesting that these two
480 modes of homeostatic plasticity may share similarities and may interact with each other to
481 regulate synaptic strength (see (Fox and Stryker, 2017; Keck et al., 2017) for recent discussions).
482 Furthermore, there is evidence that sliding threshold or synaptic scaling mode of homeostatic
483 plasticity may be employed depending on the regime of activity changes *in vivo* (Bridi et al.,
484 2018). Based on the consensus of the field that synaptic scaling is largely independent of
485 NMDAR activation, here we tested the role of NMDARs in homeostatic synaptic plasticity
486 induced *in vivo* by changes in visual experience. By disrupting NMDAR function through cell

specific knock-out or pharmacology, we showed that the principal neurons within L2/3 of V1 require functional NMDARs to undergo proper visual experience-dependent homeostatic adaptation of excitatory synaptic strength. Disruption of normal experience-dependent homeostatic plasticity by removing NMDAR function was not due to gross changes in the activity of V1 L2/3 neurons. Our results support a role for NMDAR-dependent mechanisms in homeostatic synaptic plasticity induced *in vivo*.

We observed that NMDAR function is required for proper scaling of mEPSCs in V1 L2/3 neurons following a few days of DE or brief re-exposure to light (LE). While previous studies have shown that there is co-regulation of NMDAR together with AMPAR under inactivity conditions (Watt et al., 2000) and changes in NMDAR function after visual deprivation (Quinlan et al., 1999; Philpot et al., 2003; Guo et al., 2012), homeostatic synaptic scaling is largely thought of as occurring independent of NMDAR activity (Turrigiano et al., 1998; Turrigiano, 2008) and is absent in several genetic models with preserved LTP/LTD (Stellwagen and Malenka, 2006; Hu et al., 2010). It has previously been proposed that a switch in NMDAR function slides the synaptic modification threshold for LTP/LTD induction after DE (Quinlan et al., 1999; Philpot et al., 2003; Guo et al., 2012). Our results suggest a possibility that scaling up of mEPSCs could be a consequence of sliding down of synaptic modification threshold. Lowered synaptic modification threshold by DE would promote LTP across a large population of synapses, and hence manifest as global scaling up of excitatory synapses. This would imply that the amount of activity in V1 under DE condition is sufficient to act on the lowered threshold to induce NMDAR-dependent LTP. This contradicts *in vitro* studies done in cultured neurons, where prolonged blockade of action potentials was able to scale up excitatory synapses. Our data suggest that this may not be the case *in vivo*, where we surmise there may be sufficient activity in

the deprived cortex that can activate NMDARs to potentiate synaptic strength across a large number of synapses following a reduction in LTP threshold. Indeed, we recently reported that homeostatic upscaling of mEPSCs in V1 L2/3 neurons with DE is dependent on spontaneous activity and upregulation of GluN2B (Bridi et al., 2018). GluN2B containing NMDARs have a longer current duration (Monyer et al., 1994), which may enable better integration of lower frequency activity as occurs during spontaneous firing. Our current data add to this by showing that NMDAR function in V1 L2/3 neurons is necessary for upscaling with DE using cell-type specific genetic KO, and also demonstrate that NMDAR function is necessary for scaling down of mEPSCs with LE.

In addition to disrupting normal visual experience-dependent regulation of mEPSCs, we unexpectedly observed that blocking NMDAR function with d-CPP for 2 days significantly scales up mEPSCs under control normal-reared conditions. This suggests that there may be on-going activation of NMDARs by normal visual experience that actively reduces mEPSCs. This is reminiscent of what we observed in Arc KOs, which also show larger basal mEPSCs in V1 L2/3 neurons under normal-reared conditions and lack visual experience-dependent regulation of mEPSCs (Gao et al., 2010). While d-CPP scaled up mEPSCs under basal conditions, we did not observe an increase in mEPSC amplitude in the NMDAR KO neurons (Fig. 2). It seems unlikely that the absence of basal "upscaling" in NMDAR KO was simply due to the incomplete removal of pre-existing NMDARs during the 6-7day Cre expression because the NMDAR current is mostly absent at that time point (Fig. 1). It is possible that the difference may have resulted from d-CPP having a more global effect while NMDAR KO was done in a cell-type specific manner in V1 L2/3. We cannot rule out an alternative possibility that complete blockade of NMDAR triggers mEPSCs to potentiate, which would be consistent with some of the *in vitro* studies

showing NMDAR blockade facilitates scaling up of synapses (Sutton et al., 2006; Aoto et al., 2008) by blocking spontaneous $Ca^{2+}$ transients mediated by NMDARs (Reese and Kavalali, 2015). Despite such caveats, the fact that both methods detect failures in experience-dependent homeostatic regulation suggests that it is the function of NMDARs that is critical for proper regulation of AMPAR-mEPSCs by changes in visual experience.

Despite initial studies showing induction of synaptic scaling as being largely independent of NMDARs, there is some evidence from previous studies *in vitro* demonstrating interaction between NMDAR function and synaptic scaling. For example, blocking NMDARs has been shown to accelerate synaptic upscaling in cultured neurons (Sutton et al., 2006) and decreasing NMDAR calcium permeability has been shown to downscale AMPAR currents (Pawlak et al., 2005). Other potential mechanism relating NMDAR activation with scaling involve "unsilencing" of synapses after activity blockade *in vitro* that promotes further LTP induction (Arendt et al., 2013). These findings suggest that NMDAR activity can also have profound influence on synaptic scaling mechanisms in addition to sliding the threshold for LTP/LTD.

Synaptic scaling has been largely considered a cell-autonomous process that is triggered by readout of postsynaptic spikes, or more precisely postsynaptic depolarization (Ibata et al., 2008; Goold and Nicoll, 2010). For example, optogenetic activation of postsynaptic neurons was shown to be sufficient to drive down-scaling of excitatory synapses (Goold and Nicoll, 2010), and blocking somatic spikes was sufficient to scale up synapses (Ibata et al., 2008). However, a recent study suggested that synaptic scaling is not dependent on postsynaptic spike rate *per se*, but due to changes in glutamatergic inputs (Fong et al., 2015). This suggests that the level of activation of glutamate receptors could ultimately be the condition monitored by neurons in order to trigger homeostatic adaptation. Our data is consistent with the latter, where

556 glutamatergic transmission level may be detected by the activation of NMDARs to induce
557 changes in AMPAR-mediated mEPSCs.

558 Our data provide *in vivo* evidence for a homeostatic mechanism that requires NMDAR
559 activation in order to cope with changes in visual experience. These results suggest either a role
560 for NMDAR function in *in vivo* synaptic scaling or that homeostatic scaling of synapses is a
561 manifestation of Hebbian forms of plasticity triggered by lowered or increased synaptic
562 modification threshold according to changes in visual experience. If it is the latter, a major
563 implication is that *in vivo* homeostasis could be implemented in an input specific manner.
564 Indeed, DE has been shown to increase the synaptic strength of lateral intracortical inputs to V1
565 L2/3 neurons without affecting those originating from L4 (Petrus et al., 2015), which could be
566 the basis for non-multiplicative synaptic scaling observed in adult V1 (Goel and Lee, 2007). A
567 recent study reported that homeostatic scaling of dendritic spines in V1 following monocular
568 enucleation occurs in a dendritic branch specific manner (Barnes et al., 2017), which is
569 consistent with input specific regulation. Input-specific homeostatic synaptic plasticity has also
570 been observed in the hippocampus where inactivity scales up excitatory synaptic transmission in
571 feedforward synapses while decreasing mEPSC frequency in recurrent synapses within CA3
572 (Kim and Tsien, 2008). Collectively, these observations suggest that input-specific homeostatic
573 adaptation is likely a general phenomenon across different brain circuits that receive input from
574 several sources. Synapse-specific homeostatic plasticity has been observed in reduced
575 preparations where activity was selectively manipulated at individual synapses via genetic
576 methods of silencing specific synapses (Hou et al., 2008; Lee et al., 2010; Beique et al., 2011).
577 One such study demonstrated that NMDAR subunit composition can be selectively altered at
578 individual synapses (Lee et al., 2010), which allows for synapse-specific adjustment of sliding

579 threshold. Such input-specific homeostatic control will allow cortical neurons that participate in
580 multiple functional circuits to adapt effectively to changes in select inputs to provide stability
581 without compromising the function of other synapses. Our results suggest that one way to
582 achieve such input-specific homeostatic control is via NMDAR-dependent plasticity
583 mechanisms.
584

585 **References**


586 Abraham WC, Bear MF (1996) Metaplasticity: the plasticity of synaptic plasticity. *Trends*
587 *Neurosci* 19:126-130.
588 Adesnik H, Li G, During MJ, Pleasure SJ, Nicoll RA (2008) NMDA receptors inhibit synapse
589 unsilencing during brain development. *Proc Natl Acad Sci U S A* 105:5597-5602.
590 Aoto J, Nam CI, Poon MM, Ting P, Chen L (2008) Synaptic signaling by all-trans retinoic acid in
591 homeostatic synaptic plasticity. *Neuron* 60:308-320.
592 Arendt KL, Sarti F, Chen L (2013) Chronic inactivation of a neural circuit enhances LTP by
593 inducing silent synapse formation. *J Neurosci* 33:2087-2096.
594 Barnes SJ, Franzoni E, Jacobsen RI, Erdelyi F, Szabo G, Clopath C, Keller GB, Keck T (2017)
595 Deprivation-Induced Homeostatic Spine Scaling In Vivo Is Localized to Dendritic
596 Branches that Have Undergone Recent Spine Loss. *Neuron* 96:871-882 e875.
597 Beique JC, Na Y, Kuhl D, Worley PF, Huganir RL (2011) Arc-dependent synapse-specific
598 homeostatic plasticity. *Proc Natl Acad Sci U S A* 108:816-821.
599 Bridi MCD, de Pasquale R, Lantz CL, Gu Y, Borrell A, Choi SY, He K, Tran T, Hong SZ, Dykman A,
600 Lee HK, Quinlan EM, Kirkwood A (2018) Two distinct mechanisms for experience-
601 dependent homeostasis. *Nat Neurosci* 21:843-850.
602 Cooper LN, Bear MF (2012) The BCM theory of synapse modification at 30: interaction of theory
603 with experiment. *Nat Rev Neurosci* 13:798-810.
604 Desai NS, Cudmore RH, Nelson SB, Turrigiano GG (2002) Critical periods for experience-
605 dependent synaptic scaling in visual cortex. *Nat Neurosci* 5:783-789.
606 Fong MF, Newman JP, Potter SM, Wenner P (2015) Upward synaptic scaling is dependent on
607 neurotransmission rather than spiking. *Nat Commun* 6:6339.
608 Fox K, Stryker M (2017) Integrating Hebbian and homeostatic plasticity: introduction. *Philos*
609 *Trans R Soc Lond B Biol Sci* 372.
610 Gao M, Sossa K, Song L, Errington L, Cummings L, Hwang H, Kuhl D, Worley P, Lee HK (2010) A
611 specific requirement of Arc/Arg3.1 for visual experience-induced homeostatic synaptic
612 plasticity in mouse primary visual cortex. *J Neurosci* 30:7168-7178.
613 Goel A, Lee HK (2007) Persistence of experience-induced homeostatic synaptic plasticity
614 through adulthood in superficial layers of mouse visual cortex. *J Neurosci* 27:6692-6700.
615 Goel A, Jiang B, Xu LW, Song L, Kirkwood A, Lee HK (2006) Cross-modal regulation of synaptic
616 AMPA receptors in primary sensory cortices by visual experience. *Nat Neurosci* 9:1001-
617 1003.
618 Goel A, Xu LW, Snyder KP, Song L, Goenaga-Vazquez Y, Megill A, Takamiya K, Huganir RL, Lee HK
619 (2011) Phosphorylation of AMPA receptors is required for sensory deprivation-induced
620 homeostatic synaptic plasticity. *PLoS One* 6:e18264.
621 Goldey GJ, Roumis DK, Glickfeld LL, Kerlin AM, Reid RC, Bonin V, Schafer DP, Andermann ML
622 (2014) Removable cranial windows for long-term imaging in awake mice. *Nat Protoc*
623 9:2515-2538.
624 Goold CP, Nicoll RA (2010) Single-cell optogenetic excitation drives homeostatic synaptic
625 depression. *Neuron* 68:512-528.

626 Guo Y, Huang S, de Pasquale R, McGehrin K, Lee HK, Zhao K, Kirkwood A (2012) Dark exposure
627 extends the integration window for spike-timing-dependent plasticity. *J Neurosci*
628 32:15027-15035.
629 He K, Petrus E, Gammon N, Lee HK (2012) Distinct sensory requirements for unimodal and
630 cross-modal homeostatic synaptic plasticity. *J Neurosci* 32:8469-8474.
631 Hoffman GE, Smith MS, Verbalis JG (1993) c-Fos and related immediate early gene products as
632 markers of activity in neuroendocrine systems. *Front Neuroendocrinol* 14:173-213.
633 Hou Q, Zhang D, Jarzylo L, Huganir RL, Man HY (2008) Homeostatic regulation of AMPA receptor
634 expression at single hippocampal synapses. *Proc Natl Acad Sci U S A* 105:775-780.
635 Hu JH, Park JM, Park S, Xiao B, Dehoff MH, Kim S, Hayashi T, Schwarz MK, Huganir RL, Seeburg
636 PH, Linden DJ, Worley PF (2010) Homeostatic scaling requires group I mGluR activation
637 mediated by Homer1a. *Neuron* 68:1128-1142.
638 Huang S, Trevino M, He K, Ardiles A, Pasquale R, Guo Y, Palacios A, Huganir R, Kirkwood A (2012)
639 Pull-push neuromodulation of LTP and LTD enables bidirectional experience-induced
640 synaptic scaling in visual cortex. *Neuron* 73:497-510.
641 Ibata K, Sun Q, Turrigiano GG (2008) Rapid synaptic scaling induced by changes in postsynaptic
642 firing. *Neuron* 57:819-826.
643 Ingrao JC, Johnson R, Tor E, Gu Y, Litman M, Turner PV (2013) Aqueous stability and oral
644 pharmacokinetics of meloxicam and carprofen in male C57BL/6 mice. *J Am Assoc Lab*
645 *Anim Sci* 52:553-559.
646 Joo JY, Schaukowitch K, Farbiak L, Kilaru G, Kim TK (2016) Corrigendum: Stimulus-specific
647 combinatorial functionality of neuronal c-fos enhancers. *Nat Neurosci* 19:642.
648 Keck T, Keller GB, Jacobsen RI, Eysel UT, Bonhoeffer T, Hubener M (2013) Synaptic scaling and
649 homeostatic plasticity in the mouse visual cortex in vivo. *Neuron* 80:327-334.
650 Keck T, Toyoizumi T, Chen L, Doiron B, Feldman DE, Fox K, Gerstner W, Haydon PG, Hubener M,
651 Lee HK, Lisman JE, Rose T, Sengpiel F, Stellwagen D, Stryker MP, Turrigiano GG, van
652 Rossum MC (2017) Integrating Hebbian and homeostatic plasticity: the current state of
653 the field and future research directions. *Philos Trans R Soc Lond B Biol Sci* 372.
654 Kim J, Tsien RW (2008) Synapse-specific adaptations to inactivity in hippocampal circuits
655 achieve homeostatic gain control while dampening network reverberation. *Neuron*
656 58:925-937.
657 Kirkwood A, Rioult MC, Bear MF (1996) Experience-dependent modification of synaptic
658 plasticity in visual cortex. *Nature* 381:526-528.
659 Kohr G, Jensen V, Koester HJ, Mihaljevic AL, Utvik JK, Kvello A, Ottersen OP, Seeburg PH,
660 Sprengel R, Hvalby O (2003) Intracellular domains of NMDA receptor subtypes are
661 determinants for long-term potentiation induction. *J Neurosci* 23:10791-10799.
662 Lavzin M, Rapoport S, Polsky A, Garion L, Schiller J (2012) Nonlinear dendritic processing
663 determines angular tuning of barrel cortex neurons in vivo. *Nature* 490:397-401.
664 Lee MC, Yasuda R, Ehlers MD (2010) Metaplasticity at single glutamatergic synapses. *Neuron*
665 66:859-870.
666 Lehmann J, Schneider J, McPherson S, Murphy DE, Bernard P, Tsai C, Bennett DA, Pastor G,
667 Steel DJ, Boehm C, et al. (1987) CPP, a selective N-methyl-D-aspartate (NMDA)-type
668 receptor antagonist: characterization in vitro and in vivo. *J Pharmacol Exp Ther* 240:737-
669 746.

Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12:529-540.

O'Brien RJ, Kamboj S, Ehlers MD, Rosen KR, Fischbach GD, Huganir RL (1998) Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* 21:1067-1078.

Pawlak V, Schupp BJ, Single FN, Seeburg PH, Kohr G (2005) Impaired synaptic scaling in mouse hippocampal neurones expressing NMDA receptors with reduced calcium permeability. *J Physiol* 562:771-783.

Petrus E, Rodriguez G, Patterson R, Connor B, Kanold PO, Lee HK (2015) Vision Loss Shifts the Balance of Feedforward and Intracortical Circuits in Opposite Directions in Mouse Primary Auditory and Visual Cortices. *J Neurosci* 35:8790-8801.

Philpot BD, Espinosa JS, Bear MF (2003) Evidence for altered NMDA receptor function as a basis for metaplasticity in visual cortex. *J Neurosci* 23:5583-5588.

Pologruto TA, Sabatini BL, Svoboda K (2003) ScanImage: flexible software for operating laser scanning microscopes. *Biomed Eng Online* 2:13.

Quinlan EM, Olstein DH, Bear MF (1999) Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proc Natl Acad Sci U S A* 96:12876-12880.

Reese AL, Kavalali ET (2015) Spontaneous neurotransmission signals through store-driven Ca(2+) transients to maintain synaptic homeostasis. *Elife* 4.

Sprengel R, Suchanek B, Amico C, Brusa R, Burnashev N, Rozov A, Hvalby O, Jensen V, Paulsen O, Andersen P, Kim JJ, Thompson RF, Sun W, Webster LC, Grant SG, Eilers J, Konnerth A, Li J, McNamara JO, Seeburg PH (1998) Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. *Cell* 92:279-289.

Steele PM, Mauk MD (1999) Inhibitory control of LTP and LTD: stability of synapse strength. *J Neurophysiol* 81:1559-1566.

Stellwagen D, Malenka RC (2006) Synaptic scaling mediated by glial TNF-alpha. *Nature* 440:1054-1059.

Sutton MA, Ito HT, Cressy P, Kempf C, Woo JC, Schuman EM (2006) Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* 125:785-799.

Tsien JZ, Huerta PT, Tonegawa S (1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell* 87:1327-1338.

Turrigiano GG (2008) The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* 135:422-435.

Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391:892-896.

Watt AJ, van Rossum MC, MacLeod KM, Nelson SB, Turrigiano GG (2000) Activity coregulates quantal AMPA and NMDA currents at neocortical synapses. *Neuron* 26:659-670.

712 **Figure Legends**

713 **Figure 1. Neuron-specific NMDAR knockout**

714 **(A)** GFP and Cre-GFP expressing viruses result in similar transfection efficiency for V1 neurons
715 (mean percentage of transfected neurons: GFP-only = 67.7 ± 4.6%, Cre-GFP = 60.8 ± 3.9%;
716 unpaired t-test: t=1.634, p=0.1079; number of slices quantified reported, 6 mice per condition).
717 **(B)** Confocal image of biocytin filled NMDAR KO (green arrow, expressing Cre-GFP in the
718 nucleus) and neighbor (orange arrow) neurons in V1 L2/3.
719 **(C)** Left: Comparison of average NMDAR/AMPAR ratio for each condition (mean
720 NMDAR/AMPAR ratio for GFP-only 0.25 ± 0.04, NR1 KO 0.05 ± 0.007, neighbor 0.22 ± 0.07;
721 one-way ANOVA: F(2,13)=5.502, *p=0.0186; Tukey's multiple comparison *post hoc*: GFP vs
722 NR1 KO *p=0.02, GFP vs neighbor p=0.93, neighbor vs NR1 KO p=0.05; number of cells
723 reported). Right: Example traces of NMDAR (measured at +40 mV holding potential) and
724 AMPAR (measured at -70 mV holding potential) mediated currents in GFP-only, NMDAR KO
725 and neighbor neurons. Traces were normalized to match the amplitude of AMPAR current.
726

727 **Figure 2. NMDAR knockout neurons lack homeostatic regulation of mEPSCs**
728 Top row: Average mEPSC traces from normal-reared (Ctl), 2 days DE, and 2 hours LE. Second
729 row: Bar graph comparison of average mEPSC amplitude from Ctl, DE and LE groups. Average
730 mEPSC amplitude for each cell is plotted in gray circle. Third row: Cumulative probability
731 graphs of mEPSC amplitudes from Ctl (gray solid line), DE (black solid line, and LE (black
732 dashed line). Fourth row: Example mEPSC recording traces from Ctl (top), DE (middle) and LE
733 (bottom). Bottom row: Comparison of average mEPSC frequency across Ctl, DE and LE groups.
734 Average mEPSC frequency for each cell is plotted in gray circle.

735 **(A)** Results from neurons transfected with GFP-only virus. There was a significant increase in
736 average mEPSC amplitude with DE which is reversed by LE (mean amplitude: Ctl = 11.38 ±
737 0.51 pA, DE = 13.29 ± 0.55 pA, LE = 10.59 ± 0.49 pA; one-way ANOVA: F(2,26)=7.213,
738 *p=0.0032; Tukey's multiple comparison *post-hoc*: Ctl vs DE *p=0.040, Ctl vs LE: p=0.574, DE
739 vs LE *p=0.003). DE significantly shifted the cumulative probability curve of mEPSC
740 amplitudes to the right with DE (Kolmogorov-Smirnov test: *p<0.0001), which then returned to
741 Ctl distribution with LE (Kolmogorov-Smirnov test: p=0.0138). There was no significant change
742 in mEPSCs frequency across groups (mean frequency: Ctl = 2.43 ± 0.14 Hz, DE = 2.92 ± 0.28
743 Hz, LE = 3.30 ± 0.53 Hz; one-way ANOVA: F(2,26)=1.196, p=0.3192).
744 **(B)** Results from Cre-GFP transfected neurons. NMDAR KO neurons failed to undergo
745 significant changes in average mEPSC amplitude (Ctl = 12.85 ± 0.76 pA, DE = 12.55 ± 0.95 pA,
746 LE = 11.96 ± 0.86 pA; one-way ANOVA: F(2,35)=0.2863, p=0.7528). Despite no difference in
747 average mEPSC amplitude, cumulative probability curves of mEPSC amplitudes from Cre-GFP
748 expressing NMDAR KO neurons show a slight shift towards higher amplitudes in Ctl group
749 relative to DE (Kolmogorov-Smirnov test: p=0.0005) and LE (Kolmogorov-Smirnov test:
750 p=0.0005), but there was no significant difference between DE and LE (Kolmogorov-Smirnov
751 test: p= 0.1441). There was no significant change in the average mEPSC frequency across groups
752 (Ctl = 4.92 ± 0.57 Hz, DE = 4.49 ± 0.48 Hz, LE = 4.93 ± 0.58 Hz; one-way ANOVA:
753 F(2,35)=0.1947, p=0.8240).
754 **(C)** Results from neighbor neurons that did not express Cre-GFP. There was no significant
755 change in the average mEPSCs amplitude across groups (Ctl = 11.71 ± 0.88 pA, DE = 11.47 ±
756 0.36 pA, LE = 13.86 ± 0.97 pA; one-way ANOVA: F(2,25)=2.465, p=0.1054). While there was
757 no significant difference in cumulative probability of mEPSC amplitudes between Ctl and DE

values (Kolmogorov-Smirnov test: p=0.0168), there was a significant shift towards larger values for LE when compared to DE (Kolmogorov-Smirnov test: *p< 0.0001) or Ctl (Kolmogorov-Smirnov test: *p< 0.0001). There was a significant change in the average mEPSC frequency after LE relative to control conditions (Ctl = 3.28 ± 0.55 Hz, DE = 5.15 ± 0.62 Hz, LE = 6.57 ± 0.71 Hz; one-way ANOVA *p=0.0034, F(2,25)=7.182; Tukey's multiple comparison *post hoc*: Ctl vs DE p=0.091, Ctl vs LE *p=0.003, DE vs LE: p=0.291).

**Figure 3. Lack of visual experience-dependent scaling down of mEPSCs with NMDAR blockade**

**(A)** Comparison of average mEPSC amplitude changes in saline treated groups and LE group that was treated with d-CPP to block NMDAR activity. Left: Average mEPSC traces from saline treated normal-reared (Ctl), 2 days DE, and 2 hours LE compared to d-CPP treated LE group. Right: Bar graph comparison of average mEPSC amplitude from saline treated Ctl, DE and LE groups compared to d-CPP treated LE group (Saline Ctl = 10.55 ± 0.42 pA, Saline DE = 12.14 ± 0.35 pA, Saline LE = 10.69 ± 0.55 pA, CPP LE = 12.35 ± 0.73 pA; ANOVA: F(3,35) = 3.139, *p = 0.0375; Bonferroni's Multiple Comparison test: Saline LE vs CPP LE *p < 0.05). Average mEPSC amplitude for each cell is plotted as gray circle.

**(B)** Cumulative probability of mEPSC amplitudes from saline treated LE group (black dashed line) and d-CPP treated LE group (black solid line) show statistically significant difference (Kolmogorov-Smirnov test: *p < 0.0001)

**(C)** There was no significant difference in average mEPSC frequency across groups (Saline Ctl = 4.62 ± 0.85 Hz, Saline DE = 5.41 ± 0.64 Hz, Saline LE = 4.05 ± 0.61 Hz, CPP LE = 5.62 ± 0.82

Hz; ANOVA: F(3,35) = 0.9418, p = 0.4309). Average mEPSC frequency for each cell is plotted as gray circle.

**(D)** Treatment of d-CPP for 2 days in control normal-reared mice significantly increased the average mEPSC amplitude compared to saline treated controls. Left: Average mEPSC traces. Middle: Comparison of average mEPSC amplitude (saline Ctl: 10.55 ± 0.42 pA, n=9; d-CPP Ctl: 13.19 ± 0.52 pA, n= 10; unpaired Student's t-test t(17)=3.88, *p=0.0012). Right: Comparison of average mEPSC frequency (saline Ctl: 4.62 ± 0.85 Hz, n=9; d-CPP Ctl: 5.59 ± 0.73 Hz, n=12; unpaired Student's t-test t(19)=0.85, p=0.4031).

**Figure 4. Comparison of neuronal activity measured with c-Fos expression**

**(A-C)** Comparison of c-Fos expression in V1 L2/3 neurons from Ctl, DE, and LE mice treated with saline or d-CPP. Representative confocal images of V1 L2/3 from Ctl, DE, and LE mice receiving saline **(A)** or d-CPP **(B)** infusion. Sections were stained with NeuN (green) and c-Fos (magenta). Scale bars: 36 µm. **(C)** Comparison of quantified fraction of c-Fos positive neurons, which were calculated as (number of c-Fos positive neurons)/(number of NeuN positive neurons). For saline group, DE significantly decreased the fraction of c-Fos positive neurons, which increased to Ctl values with LE (Ctl = 0.62 ± 0.03, DE = 0.14 ± 0.02, LE = 0.63 ± 0.04; ANOVA: F(2,33) = 103.24, *p < 0.0001; Tukey's multiple comparison *post hoc*: Ctl vs DE *p < 0.0001, Ctl vs LE p = 0.9931, DE vs LE *p < 0.0001). For d-CPP group, DE significantly decreased the fraction of c-Fos positive neurons, which increased to Ctl values with LE (Ctl = 0.54 ± 0.32, DE = 0.13 ± 0.02, LE = 0.58 ± 0.02; ANOVA: F(2,32) = 93.5424, *p < 0.0001; Tukey's multiple comparison *post hoc*: Ctl vs DE *p < 0.0001, Ctl vs LE p=0.4234, DE vs LE *p < 0.0001).

**(D-F)** Comparison of c-Fos expression in GFP transfected V1 L2/3 neurons from Ctl, DE or LE mice. Representative confocal images of V1 L2/3 from NR1-flox mice that received viral transfection of GFP-only **(D)** or Cre-GFP (NMDAR KO) **(E)**. Sections were stained with NeuN (blue) and c-Fos (red). GFP expression from either GFP-only or Cre-GFP is shown in green. Scale bars: 36 μm. **(F)** Comparison of the fraction of c-Fos positive GFP neurons, which were calculated as (number of c-Fos and GFP positive neurons)/(number of total GFP positive neurons). For GFP-only group, there was a significant increase in the fraction of c-Fos positive GFP neurons following LE compared to DE (Ctl = 0.35 ± 0.04, DE = 0.25± 0.04, LE = 0.54 ± 0.09; ANOVA: $F(2,15)=6.35$, *p=0.0100; Tukey's multiple comparison test: Ctl vs DE p=0.5654, Ctl vs LE p=0.1503, DE vs LE **p=0.0077). In NMDAR KO (Cre-GFP) group, there was a significant decrease in the fraction of c-Fos positive Cre-GFP neurons in DE, which increased back to Ctl levels with LE (Ctl = 0.49 ± 0.03, DE = 0.31± 0.06, LE = 0.56 ± 0.08; ANOVA: $F(2,16)=5.52$, *p=0.0149; Tukey's multiple comparison test: Ctl vs DE *p=0.04, Ctl vs LE =0.6629, DE vs LE *p=0.0212).

**Figure 5. Blocking NMDARs with d-CPP injection does not acutely affect visually evoked responses in V1**

**(A)** Example of imaged region in an Emx1-Ai96 mouse before (left) and after (right) intraperitoneal CPP injection. Both images are maximum projections of GCaMP6s fluorescence. **(B)** Visually evoked responses from ROIs shown in (A) before (gray) and after (red) CPP injection. The dashed line denotes onset of the visual stimulus and its height corresponds to 0.5 $\Delta F/F_0$. Each trace is 11 seconds long.

**(C)** Distribution of visual response change after application of saline (blue) versus CPP (red). (Left panel) Comparison of changes in spontaneous activity of all neurons before and after saline or CPP injection. Spontaneous activity was measured during a 7 s window when a blank screen was presented. N=107 neurons across 7 mice for saline and N=81 neurons across 5 mice for CPP. Kolmogorov-Smirnov test, P=0.448. (Middle panel) Response change for all activated neurons, regardless of whether the level of activation is significant. The same neurons as in the left panel. Kolmogorov-Smirnov test, P=0.087. (Right panel) Response change only for significantly visually activated neurons. N=36 neurons across 7 mice for saline and N=36 neurons across 6 mice for CPP. Kolmogorov-Smirnov test, P=0.46.

**(D)** (Left panel) Change of response at preferred orientation after application of saline (blue) versus CPP (red). Unpaired t-test, P=0.24. (Right panel) Change of orientation selectivity after application of saline (blue) versus CPP (red). Unpaired t-test, P=0.069. Only neurons with significant orientation tuning were included in this analysis (N=30 neurons for saline conditions and N=28 neurons for CPP condition).