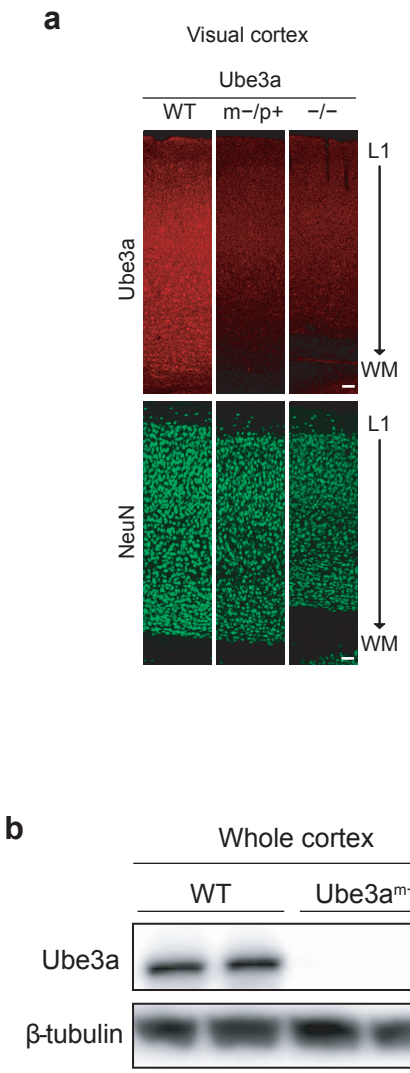
Ube3a is required for experience-dependent maturation of the neocortex

Koji Yashiro, Thorfinn T. Riday, Kathryn H. Condon, Adam C. Roberts, Danilo R. Bernardo, Rohit Prakash, Richard J. Weinberg, Michael D. Ehlers, and Benjamin D. Philpot

SUPPLEMENTARY FIGURES, TABLES, & METHODS

Nature Neuroscience: doi:10.1038/nn.2327



Supplementary Figure 1: Maternal expression of Ube3a in the cortex. **a**, Immunohistochemical analysis of Ube3a expression in the visual cortex from young (P24) WT, Ube3a$^{m-/p+}$, and Ube3a $^{m-/p-}$ mice. Whereas strong Ube3a immunoreactivity was observed in all cortical layers except for layer 1 (L1) and white matter (WM) in WT mice, it was very weak in both Ube3a$^{m-/p+}$ and Ube3a $^{m-/p-}$ mice. NeuN antibody stains neuronal cell bodies. Scale bars, 100 µm. **b**, Immunoblot analysis of Ube3a expression in whole cortical lysates from WT and Ube3a$^{m-/p+}$ mice at P25-27. Each lane contains cortical lysate from a different mouse. β-tubulin was used as an expression control.

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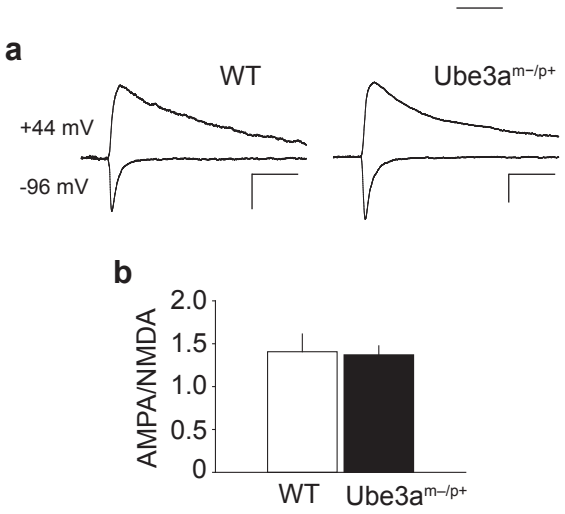

Supplementary Figure 2: The ratio of AMPA receptor- to NMDA receptor-mediated currents is normal in Ube3a$^{m-/p+}$ mice. a, Example traces: EPSCs were evoked by stimulating layer 4 and recording in layer 2/3 pyramidal neurons while cells were voltage-clamped at either −96 mV or +44 mV. AMPA receptor-mediated currents were measured at the EPSC peak, while NMDA receptor-mediated currents were recorded at +44 mV and measured 50 msec after the stimulus artifact. Scale bars: WT 50 msec, 50 pA; Ube3a$^{m-/p+}$, 50 msec, 100 pA. **b,** Average AMPA/NMDA current ratios for WT (n = 16 cells) and Ube3a$^{m-/p+}$ mice (n = 11 cells), showing no significant difference (WT, 1.4 ± 0.2; Ube3a$^{m-/p+}$, 1.4 ± 0.1; p = 0.89).

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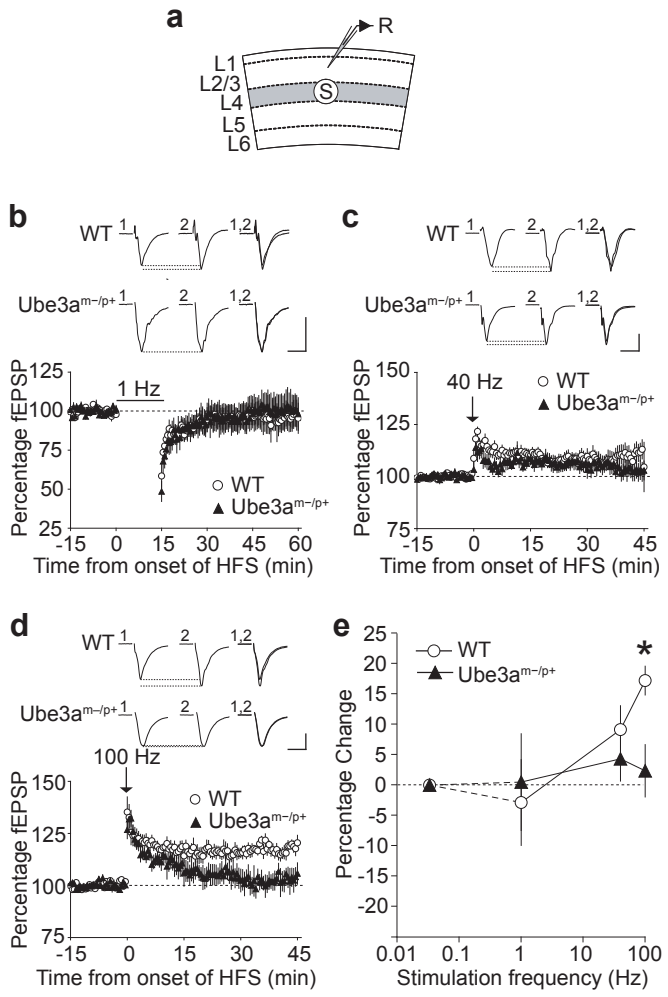
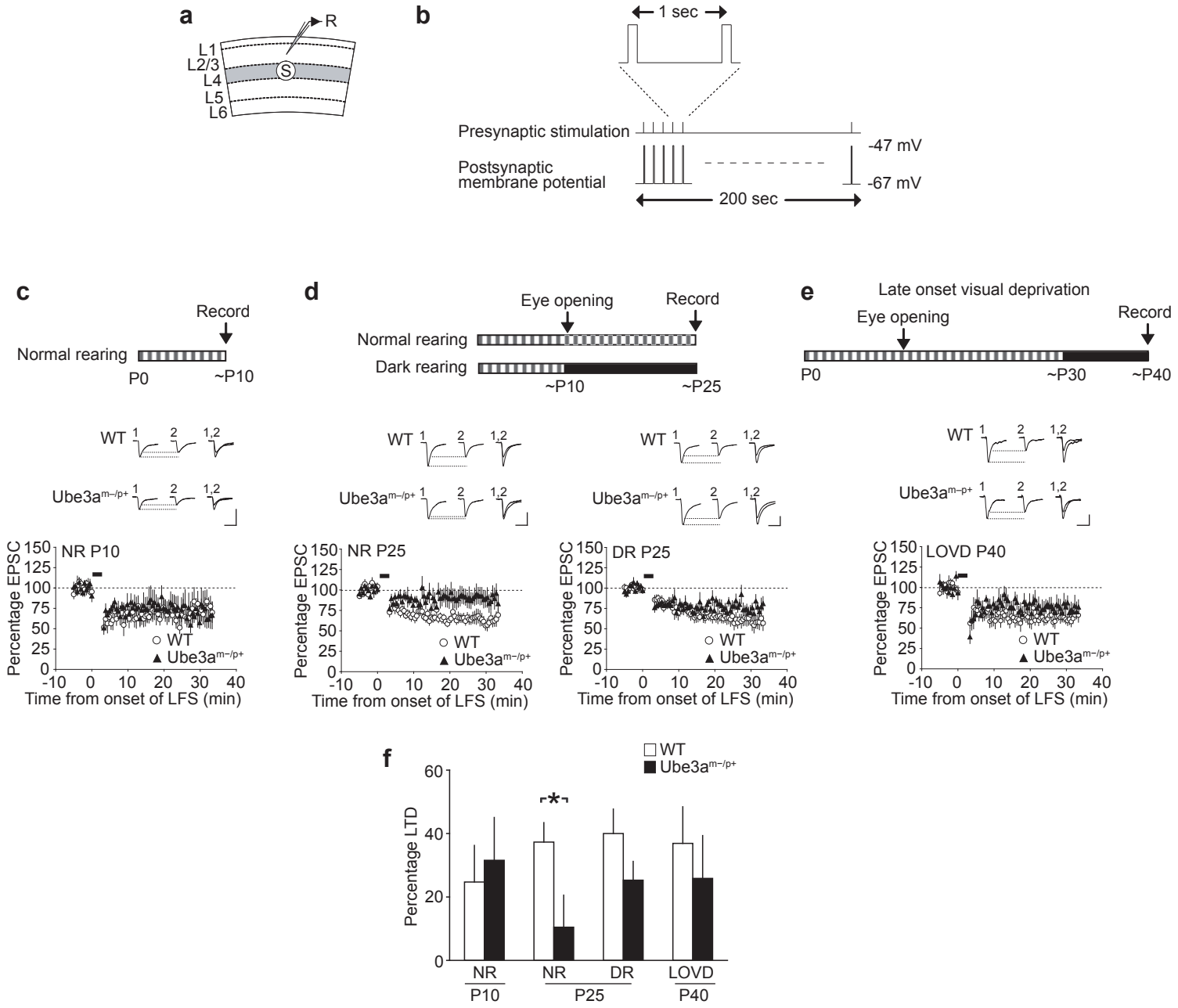


Supplementary Figure 3: The absence of Ube3a causes deficits in bidirectional synaptic plasticity in the visual cortex of <u>adult</u> mice. **a**, Schematic of the recording configuration. **b**, Synaptic responses of WT (open circle) and Ube3a$^{m-/p+}$ (closed triangle) mice were measured before and after application of a conditioning stimulation to the layer 4 (L4) to L2/3 pathway of the visual cortex. LTD was induced with 1 Hz stimulation for 15 min in visual cortex slices from adult mice. Top traces are representative averaged traces of 15 min baseline (1), 30-45 min period after LTD induction, (2) and their overlays (1, 2). Scale bars: 5 ms, 1 mV. Bottom graph describes average change in field EPSP (Percentage fEPSP) upon delivery of a 1 Hz stimulus (indicated by the bar) (WT, 97.1 ± 7.1%; Ube3a$^{m-/p+}$, 100.5 ± 8.0%; n = 9, 9; p = 0.75). LTD was not induced in either genotype. **c**, Same as b, except that plasticity-inducing stimulation consisted of three 40 Hz trains (indicated by an arrow). Whereas this stimulation induced a weak LTP in WT mice, it failed to do so in Ube3a$^{m-/p+}$ mice (WT 109.2 ± 4.0%; Ube3a$^{m-/p+}$, 104.4 ± 3.7 %; n = 10, 8 slices; p = 0.39)**.** **d**, Same as c, except that the LTP-inducing stimulation consisted of two 100 Hz trains (indicated by an arrow). Whereas this stimulation induced strong LTP in WT mice, it did not alter fEPSP amplitudes in Ube3a$^{m-/p+}$ mice (WT, 117.3 ± 2.4 %; Ube3a$^{m-/p+}$, 102.4 ± 4.3%; n = 6, 7 slices; p < 0.02)**.** **e**, Frequency-response functions derived from visual cortex of WT and Ube3a$^{m-/p+}$ mice. Data points represent percent changes in fEPSP 30-45 min after the delivery of conditioning stimulations. The data points for 0.033 Hz are inferred from baseline stimulation delivered once every 30 sec, which induced no obvious synaptic modifications.

Nature Neuroscience: doi:10.1038/nn.2327



Supplementary Figure 4: Experience-dependent loss of LTD at excitatory synapses onto layer 2/3 pyramidal neurons in AS mice. **a**, Whole-cell recording configuration: EPSCs evoked at layer 4 (L4) were recorded in L2/3 pyramidal neurons. **b**, LTD induction protocol. **c**, Baseline synaptic responses of WT (open circle) and Ube3a$^{m-/p+}$ (closed triangle) mice were measured before and after application of conditioning stimuli in normally reared (NR) mice at ~P10. A top schematic describes rearing condition. Traces in the middle are representative averaged traces of 5 min baseline (1), 25-30 min period after LTD induction (2), and their overlays (1, 2). Scale bars: 20 ms, 100 pA. Bottom graph describes average change in EPSC (Percentage EPSC) upon delivery of the conditioning stimulus (indicated by the bar). The pairing protocol induced LTD in both WT and Ube3a$^{m-/p+}$ mice (Percentage EPSC: WT, 75.3 ± 11.5, n = 8 cells; Ube3a $^{m-/p+}$, 68.4 ± 13.5, n = 8 cells; p = 0.71). **d**, Same as c, except that LTD was measured in young (~P25) mice reared normally (left) or in a complete darkness (right). In normally reared mice (NR), whereas the pairing protocol induced LTD in WT mice, it did not alter EPSC amplitudes in Ube3a$^{m-/p+}$ mice (Percentage EPSC: WT, 62.3 ± 6.1, n = 11 cells; Ube3a$^{m /p+}$, 90.1 ± 10.1, n = 9 cells; $p < 0.03$). In dark-reared (DR) mice, LTD was equally induced in both WT and Ube3a$^{m-/p+}$ mice (Percentage EPSC: WT, 62.7 ± 9.1, n = 7 cells; Ube3a$^{m /p+}$, 74.7 ± 5.9 , n = 7 cells; p = 0.30). **e**, Same as c, except that LTD was measured in ~P40 mice, provided with late-onset visual deprivation (LOVD). LTD was equally induced in both WT and Ube3a$^{m-/p+}$ mice (Percentage EPSC: WT, 63.1 ± 5.4, n = 9 cells; Ube3a$^{m /p+}$, 25.6 ± 9.5, n = 6 cells; p = 0.30). **f**, Visual experience causes developmental loss of LTD in Ube3a$^{m- /p+}$ mice. Data represent means ± SEM of percentage LTD measured 25-30 min after the delivery of conditioning stimuli. Whereas LTD was comparable between WT and Ube3a$^{m-/p+}$ mice at P10, it was reduced in young (~P25) Ube3a$^{m-/p+}$ mice when they were reared normally. This developmental loss was prevented by DR and LOVD.

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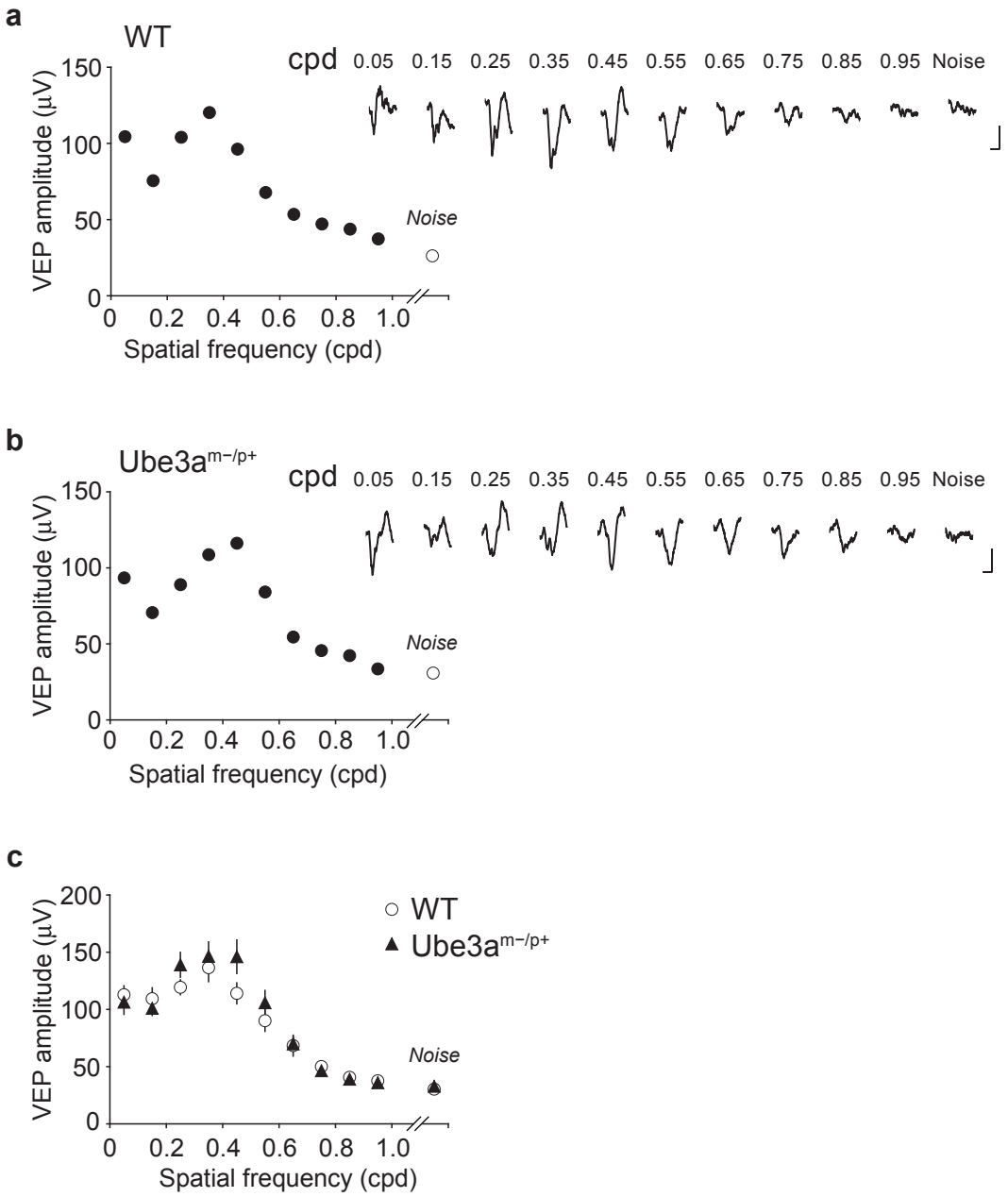

Supplementary Figure 5: Normal visual acuity in AS mice. **a**, Examples of VEP recordings in WT mice. VEPs were evoked by presenting reversing gratings of different spatial frequencies. Closed circles represent amplitudes of VEPs in response to the gratings at maximal contrast. The open circle indicates the amplitude of noise measured at 0% contrast. VEP amplitudes decrease with increasing spatial frequency of stimulus indicated as cycles per degree (cpd). Scale bars: 50 μV, 50 msec. **b**, Same as a, except that recordings were conducted in Ube3a$^{m-/p+}$ mice**.** **c**, Averaged VEP amplitudes as a function of spatial frequency in WT (n = 15 mice) and Ube3a$^{m-/p+}$ mice (n = 13 mice). No statistically significant differences were found between WT and Ube3a$^{m-/p+}$ mice ($p > 0.05$, two-way repeated measures ANOVA).

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Supplementary Table 1. Passive membrane properties of layer 2/3 pyramidal neurons in which mEPSC's are recorded

	Infant (P8 – 11)			Young (P21 – 28)			Adult (P98 – 101)		
	WT	Ube3A$^{m-/p+}$	p value	WT	Ube3A$^{m-/p+}$	p value	WT	Ube3A$^{m-/p+}$	p value
Neuron number	11	11		11	12		12	12	
V_m (mV)	-55.5 ± 3.5	-56.8 ± 3.1	0.78	-73.0 ± 1.5	-70.0 ± 1.9	0.24	-75.0 ± 1.4	-72.6 ± 1.7	0.29
Ri (MΩ)	511.2 ± 51.6	487.9 ± 47.5	0.74	114.3 ± 10.1	163.4 ± 13.6	0.01	122.0 ± 1.4	140.7 ± 13.5	0.30
C_m (pF)	66.4 ± 4.1	68.9 ± 2.8	0.61	147.8 ± 5.5	115.2 ± 5.5	0.0004	151.7 ± 8.7	121.6 ± 9.6	0.03
Tau (ms)	1.0 ± 0.1	1.0 ± 1.8	0.32	2.8 ± 0.2	2.3 ± 0.1	0.06	3.1 ± 0.1	2.7 ± 0.2	0.10

	Young WT (P21 – 28)			Young Ube3A$^{m-/p+}$ (P21 – 28)			DR WT vs DR m-/p+
	NR	DR	p value	NR	DR	p value	p value
Neuron number	11	12		12	14		
V_m (mV)	-73.0 ± 1.5	-71.9 ± 1.9	0.48	-70.0 ± 1.9	-73.6 ± 1.4	0.14	0.49
Ri (MΩ)	114.3 ± 10.1	144.7 ± 11.7	0.06	163.4 ± 13.6	156.7 ± 13.3	0.73	0.51
C_m (pF)	147.8 ± 5.5	129.6 ± 8.0	0.08	115.2 ± 5.5	117.1 ± 5.4	0.81	0.26
Tau (ms)	2.8 ± 0.2	2.4 ± 0.2	0.20	2.3 ± 0.1	2.1 ± 0.1	0.43	0.26

Resting membrane potential (V_m), input resistance (Ri), Membrane capacitance (Cm), and Membrane time constant (Tau) were measured using Mutliclamp (Axon instruments) while cells are voltage-clamped at -70 mV. Significant differences were found in membrane capacitance between the two genotypes of normally reared young and adult mice, and in input resistance of normally reared young mice. These differences may indicate smaller cell size of Ube3A$^{m-/p+}$ mice.

Nature Neuroscience: doi:10.1038/nn.2327

Supplementary Table 2: Raw values for data presented in manuscript figures 1-6. For statistical analyses, unpaired student t-tests were used unless noted.

Fig 1d: Average mEPSC amplitude.

	P10	P25	P100
WT	16.6 ± 1.2 pA (11 cells)	11.8 ± 0.3 pA (11 cells)	10.1 ± 0.3 pA (12 cells)
Ube3a$^{m-/p+}$	18.0 ± 0.9 pA (11 cells)	11.0 ± 0.4 pA (12 cells)	11.1 ± 0.5 pA (12 cells)
p value	0.36	0.15	0.11

Fig 1e: Average mEPSC frequency.

	P10	P25	P100
WT	0.8 ± 0.2 Hz (11 cells)	10.7 ± 0.8 Hz (11 cells)	10.9 ± 1.3 Hz (12 cells)
Ube3a$^{m-/p+}$	0.7 ± 0.1 Hz (11 cells)	6.8 ± 0.9 Hz (12 cells)	7.5 ± 0.7 Hz (12 cells)
p value	0.52	< 0.003	< 0.03

Fig. 2c: Average mEPSC amplitude.

	WT	Ube3a$^{m-/p+}$
DR	11.5 ± 0.5 pA (12 cells)	11.7 ± 0.3 pA (14 cells)
NR	11.8 ± 0.3 pA (11 cells)	11.0 pA ± 0.4 pA (12 cells)
p value	0.68	0.16

Fig. 2d: Average mEPSC frequency.

	WT	Ube3a$^{m-/p+}$
DR	7.0 ± 0.5 Hz (12 cells)	6.0 ± 0.6 Hz (14 cells)
NR	10.7 ± 0.8 Hz (11 cells)	6.8 ± 0.9 Hz (12 cells)
p value	< 0.005	0.46

Fig 2f: Average density of dendritic spines.

	DR	NR
WT	0.53 ± 0.07 spines/µm (25 cells)	0.77 ± 0.04 spines/µm (33 cells)
Ube3a$^{m-/p+}$	0.41 ± 0.05 spines/µm (25 cells)	0.57 ± 0.03 spines/µm (32 cells)
p value	0.15	< 0.0003

Fig 3b: Average % baseline fEPSP response after 1 Hz stimulation.

	1 Hz stimulation
WT	84.3 ± 3.5 % (13 slices)
Ube3a$^{m-/p+}$	97.9 ± 4.9 % (7 slices)
p value	< 0.04

Fig 3c: Average % baseline fEPSP response after 40 Hz stimulation.

	40 Hz stimulation
WT	114.8 ± 3.3 % (12 slices)
Ube3a$^{m-/p+}$	104.2 ± 2.0 % (10 slices)
p value	< 0.02

Fig 3d: Average % baseline fEPSP response after 100 Hz stimulation.

	100 Hz stimulation
WT	110.4 ± 3.3 % (12 slices)
Ube3a$^{m-/p+}$	112.7 ± 2.2 % (10 slices)
p value	0.59

Fig 4c: Average % baseline fEPSP response after 40 Hz stimulation in dark-reared mice.

	40 Hz stimulation
WT	113.2 ± 2.4 % (18 slices)
Ube3a$^{m-/p+}$	113.6 ± 2.4 % (18 slices)
p value	0.91

Nature Neuroscience: doi:10.1038/nn.2327

Supplementary Table 2 (con't): Raw values for data presented in manuscript figures 1-6.

Fig 4d: Average % baseline fEPSP response after 1 Hz stimulation in dark-reared mice.

	1 Hz stimulation
WT	86.5 ± 4.3 % (16 slices)
Ube3a$^{m-/p+}$	89.7 ± 2.9 % (17 slices)
p value	0.69

Fig 4f: Average % baseline fEPSP response after 1 Hz stimulation in mice given 1 day of light exposure.

	1 Hz stimulation
WT	86.6 ± 3.8 % (19 slices)
Ube3a$^{m-/p+}$	95.0 ± 4.7 % (15 slices)
p value	0.08

Fig 4g: Average % baseline fEPSP response after 1 Hz stimulation in mice given 4 days of light exposure.

	1 Hz stimulation
WT	86.1 ± 4.8% (9 slices)
Ube3a$^{m-/p+}$	107.0 ± 3.4% (6 slices)
p value	< 0.01

Fig 5c,d: Average % baseline fEPSP response after 1 Hz stimulation.

	1 Hz stimulation
NR WT	98.0 ± 4.7 % (10 slices)
LOVD WT	86.4 ± 3.0 % (10 slices)
LOVD Ube3a$^{m-/p+}$	88.2 ± 3.3 % (11 slices)
p value (one-way ANOVA followed by Tukey)	< 0.05 (NR WT vs. LOVD WT or LOVD Ube3a$^{m-/p+}$)

Fig 6b: C/I ratio in WT mice.

	Deprived (13 mice)	Non-deprived (14 mice)
Day 0	2.0 ± 0.1	2.0 ± 0.2
Day 3	1.4 ± 0.1	2.5 ± 0.3
p value (paired t-test)	< 0.01	0.14

Fig 6c: C/I ratio in Ube3a$^{m-/p+}$ mice.

	Deprived (11 mice)	Non-deprived (9 mice)
Day 0	2.1 ± 0.1	2.1 ± 0.2
Day 3	2.3 ± 0.3	2.0 ± 0.1
p value (paired t-test)	0.52	0.53

Fig 6d: VEP amplitude in WT mice.

	Contralateral	Ipsilateral
Control (14 mice)	163.0 ± 17.6 μV	75.0 ± 8.7 μV
MD (13 mice)	109.1 ± 9.9 μV	85.7 ± 9.7 μV
p value	< 0.05	0.42

Fig 6e: VEP amplitude in Ube3a$^{m-/p+}$ mice.

	Contralateral	Ipsilateral
Control (9 mice)	154.1 ± 16.4 μV	81.2 ± 7.2 μV
MD (11 mice)	146.6 ± 20.5 μV	71.7 ± 11.1 μV
p value	0.77	0.47

Nature Neuroscience: doi:10.1038/nn.2327

Supplementary Methods

Whole-cell LTD induction

Recording conditions were the same as for the mEPSC recordings, except that ACSF was not supplemented with tetrodotoxin, APV, and picrotoxin, and the internal solution contained (in mM): 107 CsOH, 107 Gluconic acid, 20 (K)HEPES, 0.2 (K)EGTA, 3.7 NaCl, 5 QX-314, 4 (Mg)ATP, 0.3 (Na)GTP, 10 Na-phosphocreatine and 0.01% w/v Alexa 488, adjusted to pH 7.2, and with sucrose to 290 mOsm. An empirically determined liquid-junction potential (approximately -14 mV) was corrected. EPSCs were elicited with a two-conductor cluster electrode (FHC) placed in layer 4. Test stimuli (200 μsec) were delivered at 0.033 Hz. The test holding potential was −67 mV (reversal potential for Cl^-). Low frequency stimulus long-term depression (LFS-LTD) was induced by pairing 1 Hz presynaptic stimulation with a brief (100 msec) postsynaptic step depolarization from −67 to −47 mV for each of 200 pulses. Each presynaptic stimulation occurred midway (50 msec) into the step depolarization. Experiments were discarded if input resistance was < 200 MΩ (younger mice) or < 100 MΩ (~P40), if series resistance was > 25 MΩ, or if series resistance changed by >30% during the recording.

Ratio of AMPA receptor to NMDA receptor-mediated currents

Recording conditions were the same as for the LTD induction experiments except ACSF contained 2 mM $MgCl_2$, 1 μM glycine, and 50 μM picrotoxin, and internal solution contained (in mM): 102 CsOH, 102 Gluconic acid, 5 TEA-chloride, 3.7 NaCl, 20 (K)HEPES, 0.3 (Na)GTP, 4 (Mg)ATP, 0.2 (K)EGTA, 10 BAPTA, 5 *N*-(2,6-dimethylphenyl carboylmethyl) triethylammonium bromide (QX-314) chloride (Alomone Labs), and 0.01% w/v Alexa 488,

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adjusted to pH 7.2 and to osmolarity ~300 mmol/kg. An empirically determined liquid-junction potential (approximately −16 mV) was corrected. EPSCs were elicited with a two-conductor cluster electrode (FHC) placed in layer 4. Test stimuli (50 μsec) were delivered at 0.066 Hz and evoked EPSCs were recorded at -96 mV and +44 mV. Peaks of an evoked EPSC at -96 mV were considered AMPA receptor-mediated. NMDA receptor-mediated currents were recorded at +44 mV and measured 50 ms after a stimulation artifact, a time when AMPA receptor-mediated currents are largely absent. Experiments were discarded if input resistance was < 200 MΩ or series resistance was > 25 MΩ measured at -96 mV.

Visual acuity

Measurements were conducted in awake mice at P34. Electrodes were implanted at P22 and animals were habituated to the restraint apparatus 24 h prior to the first recording session. Electrical signals were amplified, band pass filtered (0.3-100 Hz), digitized, and averaged (160 events in two blocks) in synchrony with the stimulus contrast reversal. Peak-to-trough amplitude was measured for transient VEPs in response to abrupt contrast reversal (1 Hz). Visual stimuli consisted of full-field horizontal sine wave gratings [0.05-0.95 cycles per degree (cpd) presented in randomized order] at maximal contrast. Responses to 0% contrast (gray screen) were also recorded as a measure of activity not evoked by patterned visual stimuli. Animals were positioned 20 cm away from a 21" CRT (23 cd/m^2 mean luminescence) centered on the midline.

Nature Neuroscience: doi:10.1038/nn.2327