

Supporting Information

Sato and Stryker 10.1073/pnas.1001281107

SI Methods

Optical Imaging of Retinotopic Maps. Retinotopic maps in VC were imaged in adult mice using full-screen bar stimulus under urethane anesthesia as described previously (1). Magnification factor and map scatter were determined as described previously (1). Response area was measured by counting the number of pixels exhibiting a response magnitude >30% of the maximum. Map position denotes the distances from the reference point on the map at which the 0° isoelevation contour crosses the 0° isoazimuth contour to the midline and lambda sutures. In each animal, two pairs of azimuth and elevation maps were imaged, and the parameters of those maps were averaged.

Immunofluorescence Microscopy. The following primary antibodies were used: mouse monoclonal anti-E6AP/Ube3a (clone E6AP-330, 1:1,000; Sigma-Aldrich), rabbit polyclonal anti-E6AP/Ube3a (1:500; Bethyl), mouse monoclonal anti-CaMKII (clone 6G9, 1:1,000; Millipore), mouse monoclonal anti-GAD67 (clone 1G10.2, 1:1,000; Millipore), and mouse monoclonal anti-parvalbumin (clone PARV-19, 1:2,000; Sigma-Aldrich). The Sigma-Aldrich and Bethyl antibodies against Ube3a gave similar staining. The secondary antibodies used were Alexa Fluor 488-labeled goat anti-rabbit IgG and Alexa Fluor 594-labeled goat anti-mouse IgG antibodies (1:500; Invitrogen).

Determination of Cell Density and Cortical Thickness. For cell density analysis, images of DAPI staining were captured with the minimum aperture size to keep the thickness of optical cross-sections minimal. The number of DAPI-stained nuclei (mainly neuronal, but also including those of other cell types) in lower layer 2/3 and layer 5 of the binocular VC designated by DiI tracks was counted in a 100 × 100-μm field using Image J software (National Institutes of Health). Cell density was determined at two different anteroposterior levels, each of which contained two fields for counting. Those four values were averaged to obtain the representative density. Cortical thickness was measured at four different levels in anterior binocular VC using Leica LAS AF software, and the values were averaged to represent each animal.

Labeling of Retinogeniculate Axons at dLGN. Mice under isoflurane anesthesia received intravitreal injections of Alexa Fluor 488- and Alexa Fluor 594-conjugated cholera toxin B subunits (2.5 μL of 2 mg/mL in PBS; Invitrogen) into the left and right eyes, respectively, using a Hamilton syringe with a 33-G needle. The needle was gently pulled out after being held in place for about 1 min. Mice were perfused at 48 h after injection. Brains were dissected, postfixed, and cut coronally into 100-μm-thick serial sections with a vibratome. After examination of the whole series, images of the dLGN at the largest cross-section were captured with

a Leica confocal microscope. MIPs were obtained from a stack of ~20 images collected at 5 μm for the 10× objective and at 1 μm for the 40× objective.

Immunoblotting. Protein samples prepared from VC (5 or 10 μg of protein/lane) were resolved by SDS/PAGE gel (Ready Gel; Bio-Rad), transferred onto polyvinylidene difluoride membranes [Immuno-Blot (Bio-Rad) or Immobilon-FL (Millipore)], and subjected to immunoblot detection as described previously (2). The following primary antibodies were used: mouse monoclonal anti-E6AP/Ube3a (clone 13, 1:2,000; BD Transduction), rabbit polyclonal anti-E6AP/Ube3a (1:3,000; Bethyl), mouse monoclonal anti-CaMKII (clone 6G9, 1:1,000–4,000; Millipore), rabbit polyclonal anti-active CaMKII (phosphorylated CaMKII at Thr²⁸⁶, 1:1,000–4,000; Promega), rabbit polyclonal anti-GAD67/67 (1:2,000; Millipore), rabbit polyclonal anti-NR2A (1:2,000; Upstate), mouse monoclonal anti-NR2B (clone BWJHL, 1:2,000; Upstate), mouse monoclonal anti-monoubiquitinated and anti-polyubiquitinated proteins (clone FK2, 1:20,000; Biomol), rabbit polyclonal anti-zif268 (otherwise called Egr-1, 1:1,000–2,000; Santa Cruz Biotechnology), and mouse monoclonal anti-β-tubulin III (clone TuJ1, 1:20,000; Millipore). We confirmed in experiments on m-/p+ mice that the BD Transduction and Bethyl antibodies both specifically recognize Ube3a protein. Secondary antibodies used were HRP-conjugated donkey anti-rabbit IgG or anti-mouse IgG antibodies (1:20,000; Jackson ImmunoResearch Laboratories) or IRDye 800CW goat anti-rabbit IgG or IRDye 680 goat anti-mouse IgG antibodies (1:20,000; LI-COR). Signals on the membranes were detected on film with ECL Plus reagents (GE Healthcare) (experiments shown in Fig. S7) or scanned directly with the Odyssey Infrared Imaging System (LI-COR) (Figs. 1 and 4 and Fig. S3). The optical density of bands was measured with Image J or Odyssey 1.2 software (LI-COR).

Behavioral Testing of Visual Acuity Development. Visual acuity of unrestrained, freely moving mice was quantified with a virtual optomotor system (OptoMotry; CerebralMechanics) as described previously (2, 3). To obtain developmental profiles of visual acuity after eye opening, two littermates of m+/p+ and m-/p+ mice at the late second postnatal week were checked every day for eye opening, and the behavioral tests were performed every other day from the day of eye opening (P15) to the beginning of the CP for monocular deprivation (P25). All pups in the litters opened their eyes on the same day regardless of their genotype. During the 12-min session, the highest spatial frequency that induced discernible tracking behavior was measured independently for each eye by changing the direction of the rotating gratings, and an average of those values for the two eyes was determined as the representative threshold.

1. Cang J, et al. (2005) Ephrin-As guide the formation of functional maps in the visual cortex. *Neuron* 48:577–589.
2. Sato M, Stryker MP (2008) Distinctive features of adult ocular dominance plasticity. *J Neurosci* 28:10278–10286.

3. Prusky GT, Alam NM, Beekman S, Douglas RM (2004) Rapid quantification of adult and developing mouse spatial vision using a virtual optomotor system. *Invest Ophthalmol Vis Sci* 45:4611–4616.

