Supplemental Data

Ephrin-As Guide the Formation of Functional Maps in the Visual Cortex

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Supplemental Experimental Procedures

Physiological Preparation and Functional Imaging of Retinotopic Maps

To image mouse cortical retinotopy maps, we followed the method developed by Kalatsky and Stryker (2003). Adult mice were anesthetized with an intraperitoneal injection of urethane (1.0 g/kg in 10% saline solution) supplemented by chlorprothixene (0.2 mg/mouse i.m.). In addition, lidocaine (2% xylocaine jelly) was applied locally to all incisions. Atropine (5 mg/kg mouse) and dexamethasone (0.2 mg/mouse) were injected subcutaneously. The animals were placed in a stereotaxic apparatus, their temperature was maintained at 37.5°C, and electrocardiograph leads were attached to monitor the heart rate continuously throughout the experiment. A tracheotomy was performed in some experiments. A craniotomy was made over the visual area of the left hemisphere; the dura mater was left intact. Low-melting point agarose (3% in saline) and a glass coverslip were placed over the exposed area. All experimental procedures were approved by the UCSF Committee on Animal Research.

Optical images of the cortical intrinsic signal were obtained using a Dalsa 1M30 CCD camera (Dalsa, Waterloo, Canada) controlled by custom software. Using different tandem lens configurations (Nikon, Inc., Melville, NY), "medium-resolution" (85 × 50 mm lenses, 7.2 × 7.2 mm image area) and "high-resolution" (135 × 50 mm lenses, 4.6 × 4.6 mm image area) images were acquired. The surface vascular pattern or intrinsic signal images were visualized with illumination wavelengths set by a green (546 ± 10 nm) or red (610 ± 10 nm) interference filter, respectively. After acquisition of a surface image, the camera was focused 400–600 μm below the pial surface, an additional red filter was interposed between the brain and the CCD camera, and intrinsic signal images were acquired. Frames were acquired by 1M30 camera at the rate of 30 fps and were stored as 512 × 512 pixel images after binning the 1024 × 1024 camera pixels by

2 × 2 pixels spatially and by four frames temporally, reducing the sampling rate for the stored images to 7.5 Hz.

A high refresh rate monitor (Nokia Multigraph 445X, 1024 × 768, 120 Hz) was used to display visual stimuli. Drifting thin bars (2° wide) were generated by a Matrox G450 board (Matrox Graphics, Inc., Quebec, Canada), controlled by custom software, and displayed on the stimulus monitor. The spatial frequency of the drifting bar was 1 cycle/80 degrees, and the temporal frequency was 1 cycle/8 s or 1 cycle/6 s. To stimulate both ipsilateral and contralateral eyes (e.g., see Figure 5), the monitor was placed in front of the mouse, 25 cm away, with its midline aligned to the animal. In order to obtain detailed retinotopic maps (e.g., Figure 2), we stimulated a wide area of the visual field of the right eye (contralateral to the hemisphere being imaged) by placing the monitor to the right with its left edge approximately aligned to the animal.

Reference

Kalatsky, V. A., and Stryker, M. P. (2003). New paradigm for optical imaging: temporally encoded maps of intrinsic signal. Neuron *38*, 529–545.

Supplemental Figures

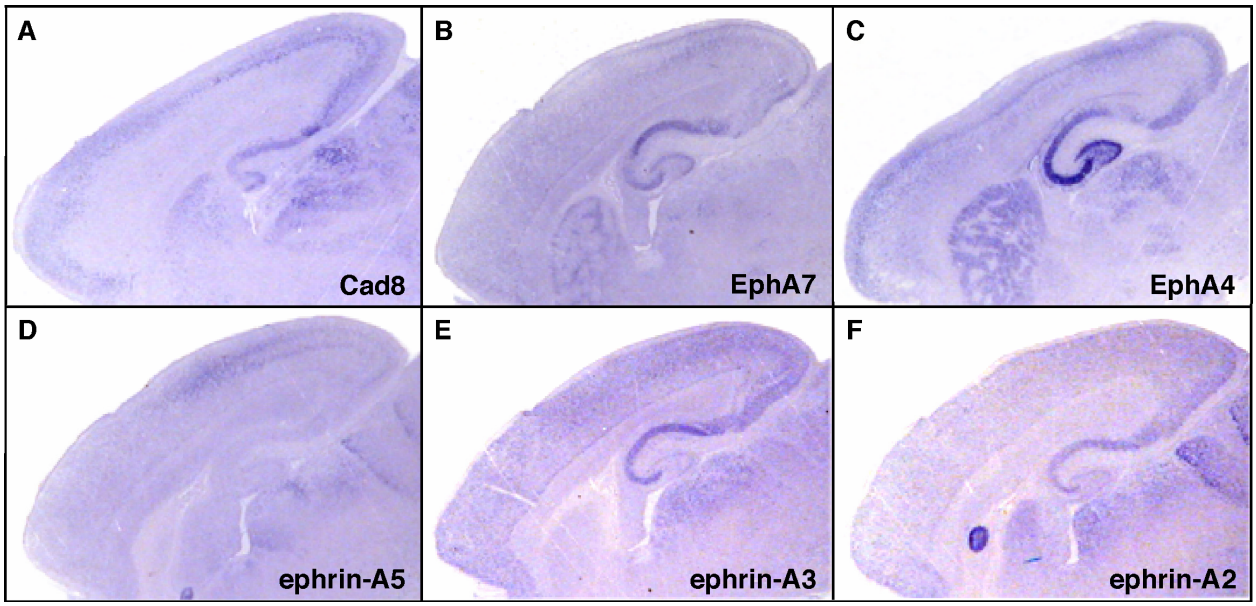

Figure S1. Complementary Expression of EphA Receptors and Ephrin-A Ligands along the Rostral-Caudal Axis of the Developing Cortex

Parasaggital sections from mouse P4 brain were treated with hybridization probes for cadherin 8 (Cad8) (A), EphA7 (B), EphA4 (C), ephrin-A5 (D), ephrin-A3 (E), and ephrin-A2 (F). Rostral is on the left and dorsal is on the top of each panel.

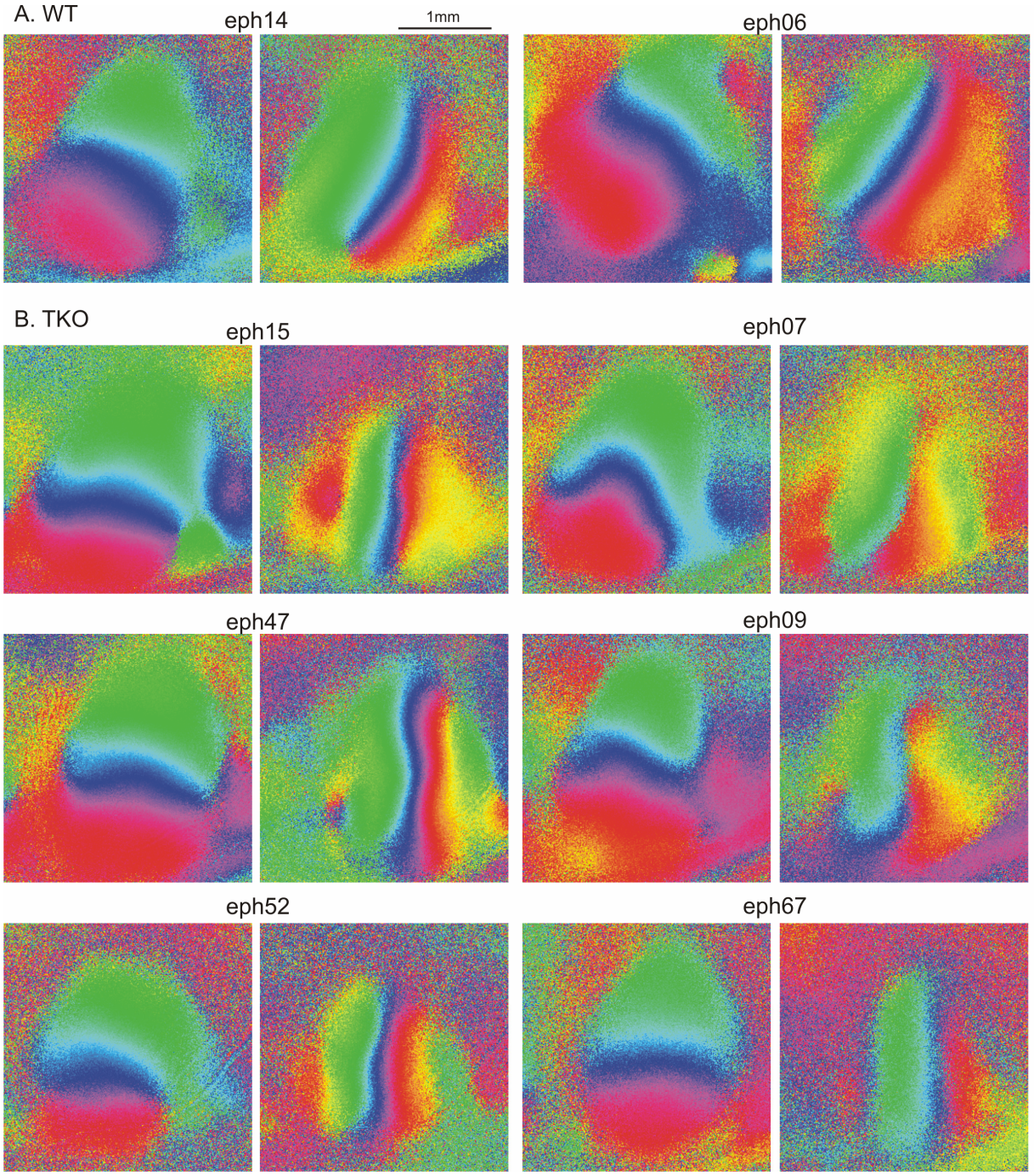

Figure S2. Examples of Cortical Retinotopic Maps in WT and ephrin-A TKO Mice

(A and B) Cortical retinotopic maps of two WT (A) and six TKOs (B). For each animal, both elevation (left panels) and azimuth (right panels) maps are shown according to the same color scale as in Figure 2. The text "eph##" is the ID of the animal from which the maps were obtained.

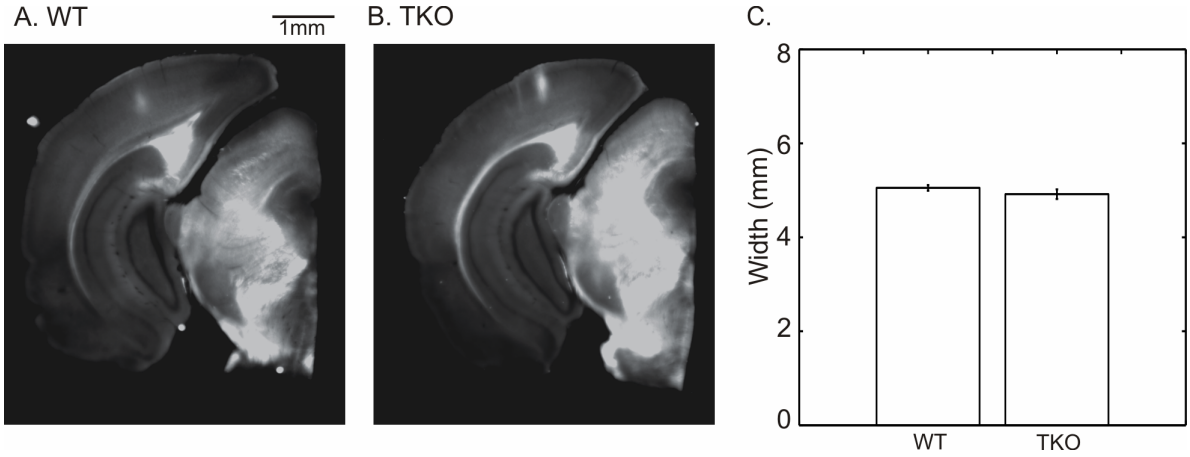

Figure S3. (A and B) Coronal sections of WT (A) and ephrin-A TKO (B) brains at the level of visual cortex. (C) Brain sizes of WT and ephrin-A TKO mice are similar (WT: 5.05 ± 0.06 mm, n = 5; TKO: 4.92 ± 0.10 mm, n = 5; p = 0.29).