Supplementary Methods

Affinity probe detection of ephrin-A/EphA proteins.

Vibratome sections of freshly dissected thalamic tissue were cut horizontally or coronally at 75 μm. These planes of section best reveal the outer-inner (eye-specific) axis of the ferret LGN. Retinas were left in the skull (to allow precise orientation along the nasal-temporal axis) cryosectioned horizontally at 15-20 μm and mounted onto positive-charge slides for AP staining. Adjacent sections were processed in parallel for endogenous AP activity using SEAP (Secreted Endogenous Alkaline Phosphatase). EphA3-AP lacks specificity for individual ephrin-A family members, but has the advantage of labeling all the ephrin-As capable of binding EphA receptors. \( n \)=4-6 LGNs were examined per age at P0, P1, P2, P3, and P4. For retinas, tissue from ferrets age P0, P2, P4, P6, P8, P10 and P15 were examined; \( n \)=3-6 retinas per age.

In situ hybridization

Mouse probes: Amplified PCR fragments were ligated into pCR4-TOPO (Invitrogen, Carlsbad, CA), verified by sequence analysis, and linearised to allow in vitro transcription of antisense and sense cRNA probes using either T7 or T3 phage RNA polymerase (MAXIscript, Ambion, Austin, TX). Plasmid pmephrin-A5-1 contains a 347 bp insert complementary to the published mouse ephrin-A5 sequence (bp 218-560, gi|25955441|) and plasmid pmEphA5-2 encodes 334 bp of the published mouse EphA5 sequence (bp 2209-2542, gi|35505521|). Amplified PCR fragments were ligated into pCR4-TOPO (Invitrogen, Carlsbad, CA), verified by sequence analysis, and linearised to allow in vitro transcription of antisense and sense cRNA probes using either T7 or T3
phage RNA polymerase (MAXIscript, Ambion, Austin, TX). Plasmid pmephrin-A5-1 contains a 347 bp insert complementary to the published mouse ephrin-A5 sequence (bp 218-560, gi|25955441|) and plasmid pmEphA5-2 encodes 334 bp of the published mouse EphA5 sequence (bp 2209-2542, gi|35505521|).

Ferret probes: using the same primers used to generate mouse EphA5, we PCR amplified a fragment of ferret EphA5 from perinatal ferret superior and inferior colliculus and ligated the subsequent fragment into pCR4-TOPO. The resulting template, pfEhA5-1 was sequence verified, linearized and used to generate a 331 bp cRNA probe against ferret EphA5. The partial ferret EphA5 cDNA cloned from perinatal superior colliculus shared 93% homology with the published sequences for Homo sapiens EphA5 (bp 1958-2288, gi|32967316|), and 90% homology with mouse EphA5 (bp 2209-2540, gi|35505521|). The expression pattern with mouse-specific riboprobes matched the ferret mRNA expression patterns.

For isotopic in situ hybridization histochemistry, sense and antisense radiolabelled cRNA probes were generated in the presence of [α-33P]UTP or [α-35S]UTP and hybridized to tissue sections from postnatal ferret as previously described41. Sections were incubated in hybridization solution containing 10^4 cpm/ 1 anti-sense RNA probes overnight at 60°C. The following day, sections were sequentially washed in the following solutions: 4X SSC at 60°C, twice, 30 minutes each; ribonuclease A (0.02mg/ml in 0.01 M Tris HCl buffer, pH 8.0, and 1mM EDTA, 2.9% NaCl) at 45°C, 1 hour; 2X SSC, room temperature, twice, 30 minutes each. Hybridized sections were mounted, the sections were lipid extracted in chloroform and ethanol, coated in autoradiographic emulsion
(NTB-2, Kodak, New Haven, CT)) and exposed for 3 weeks at 4°C. Sense-specific controls showed only background hybridization.

For non-isotopic in situ hybridization histochemistry, digoxigenin (DIG) labeled sense and antisense cRNA probes were generated as for isotopic in situ hybridization with the exception of substituting the Ambion MAXIscript transcription buffer with a DIG transcription buffer (Roche, Indianapolis, IN). Transcribed cRNAs were verified by agarose gel electrophoresis. Sections were blocked in DIG blocking reagent (Roche, Indianapolis, IN) and alkaline phosphate conjugated anti-DIG Fab fragments were used at 1:1000 dilution (Roche, Indianapolis, IN).

4-6 LGNs were examined for ephrin-A5 mRNA per age at P0, P1, P2, P3, P4, P10; horizontal sections, $n=2-3$ LGNs per age; coronal sections, $n=2-3$ LGNs per age. For retinal expression of EphA5 and ephrin-A mRNAs, tissue from ferret retinas age P0, P2, P4, P6, P8, P10 and P15 were examined; $n=3-6$ retinas per age.

**Localization of overexpressed EphA5 protein using EphA5:V5 expression vector**

A Gateway recombinant cloning entry vector was obtained by PCR amplification of full length EphA5 using the following primers; sense, 5’-

GGGGACAAGTTTGTACAAAAAAGCAGGCTATGCGGGGCTCCGGGCCC-3’,

antisense, 5’-

GGGGACCACTTTGTACAAAAAAGCAGGCTATGCGGGGCTCCGGGCCC-3’,

followed by recombination with the donor vector pDONR 221 (Invitrogen). The resulting entry clone was then recombined with the destination vector pcDNA-DEST40 (Invitrogen) to generate the EphA5-V5 fusion tagged vector.
Whole retina labeling of eye-specific projections.

Cholera toxin Beta subunit (CTβ) was injected into the vitreal chamber using a 33gauge needle inserted at the corneo-scleral junction. CTβ conjugated to Alexa dye 488 (green label) was used for the right eye, and CTβ conjugated to Alexa dye 594 (red label) was used for the left eye (5 l; 0.5% in sterile physiological saline; Molecular Probes: Eugene OR; CTβ has no biological activity). 24 hours later, ferrets were over-dosed with sodium pentobarbitol then transcardially perfused with 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose, sectioned horizontally at 40 m, mounted and coverslipped.

Imaging and quantification of eye-specific projections to the LGN

Images were digitally acquired (Zeiss AxioCam) imported to Photoshop (Adobe), cropped to exclude the optic tract and then set to a threshold 30% above background (background was designated as a non-retinorecipient portion of the tissue slice 1mm lateral to the midline of the thalamus). The 30% value is based on previous studies in ferret\textsuperscript{12,15,20} and evaluation of signal:noise in tissue from different age animals. Long and short axis measures were determined by the length of the maximum extent along which label was present. Aspect ratios were calculated by dividing the length of the long axis of the ipsilateral eye input to the LGN by the short axis of the ipsilateral eye input to the LGN. To determine the extent of ipsilateral eye terminations along the anterior-posterior axis of the LGN, the long axis of the ispi-eye label was divided by the long axis of the LGN in the same tissue section. 4-6 sections through the middle 200-300 m portion of the LGN were analyzed for each animal. For preparation of photomicrographs, images were
imported to Photoshop (Adobe) for cropping, resizing and alignment. In some cases, artifact was removed from outside the region of interest.

**Labeling and quantification of individual retinogeniculate axons.**

Ferrets were over-dosed with sodium pentobarbital and transcardially perfused with 4% paraformaldehyde. The brain was postfixed for 48 hours and then sectioned at 100 μm in the horizontal plane on a vibratome. Images were obtained on a Zeiss Axioplan microscope equipped with digital camera, then converted to grayscale and the luminance inverted to aid visualization. In control animals, layers A and A1 were visualized by the pattern of autofluorescence. The perturbation in eye-specific patterning caused by EphA overexpression made analysis of normal borders of A and A1 impossible in those ferrets. Cellular layers are not present in the ferret LGN at the ages examined in our study. However, autofluorescence allowed visualization of the optic tract and inner limit of the LGN in all cases.

**Recording of spontaneous retinal activity**

An individual retinal wave defines a domain where ganglion cells fire in a coordinated fashion. These domains typically remain refractory for periods of 1-2 mins after a wave passes through that region. After this refractory period, subsequent waves may enter the domain such that over time, the entire retina is tiled by this correlated activity. As a result, cell pairs within this critical distance will be correlated and cell pairs that are well-separated will be less correlated because they participate in separate wave events. To quantify this, we calculated the cross-correlation functions for the cells isolated in these
recordings and summarized them with a Correlation Index (CI). The CI measures how much more likely a pair of cells is to fire together within a particular time window than they would by chance. For example, a CI of 20 means that the pair of cells are 20 times more likely to fire together than by chance; a CI of 1 means that they are just as likely to fire together as a pair of independent cells. A CI less than 1 means that they tend not fire together.

The correlation index is given by

\[ N_{ab}(-w,+w) * T / \left( N_a(0,T) * N_b(0,T) * 2*w \right) \]

Where \( N_{ab}(-w,+w) \) is the number of spike pairs from cells \( a \) and \( b \) for which cell \( b \) fires within +/-w seconds of cell \( a \), \( T \) is the duration of the recording in seconds, \( N_a(0,T) \) and \( N_b(0,T) \) are the total number of spikes from cell \( a \) and \( b \) during the recording, and \( 2*w \) is the width of the correlation window. \( N_{ab} \) was computed using \( w=0.1s \) and the cross-correlation function was binned at .05s. The particular values of the correlation index depend on the choice of the correlation window \( w \). A value of .1s was chosen based on what has been commonly used by other researchers as a reasonable time-scale for activity-dependent modification of synaptic strength.