Synapse elimination and learning rules co-regulated by MHC class I H2-D<sup>b</sup>

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The formation of precise connections between retina and lateral geniculate nucleus (LGN) involves the activity-dependent elimination of some synapses, with strengthening and retention of others. Here we show that the major histocompatibility complex (MHC) class I molecule H2-D<sup>b</sup> is necessary and sufficient for synapse elimination in the retinogeniculate system. In mice lacking both H2-K<sup>b</sup> and H2-D<sup>b</sup> (K<sup>b</sup>D<sup>b−/−</sup>), despite intact retinal activity and basal synaptic transmission, the developmentally regulated decrease in functional convergence of retinal ganglion cell synaptic inputs to LGN neurons fails and eye-specific layers do not form. Neuronal expression of just H2-D<sup>b</sup> in K<sup>b</sup>D<sup>b−/−</sup> mice rescues both synapse elimination and eye-specific segregation despite a compromised immune system. When patterns of stimulation mimicking endogenous retinal waves are used to probe synaptic learning rules at retinogeniculate synapses, long-term potentiation (LTP) is intact but long-term depression (LTD) is impaired in K<sup>b</sup>D<sup>b−/−</sup> mice. This change is due to an increase in Ca<sup>2+</sup>-permeable AMPA (a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors. Restoring H2-D<sup>b</sup> to K<sup>b</sup>D<sup>b−/−</sup> neurons renders AMPA receptors Ca<sup>2+</sup> impermeable and rescues LTD. These observations reveal an MHC-class-I-mediated link between developmental synapse pruning and balanced synaptic learning rules enabling both LTD and LTP, and demonstrate a direct requirement for H2-D<sup>b</sup> in functional and structural synapse pruning in CNS neurons.

Early in development, before photoreceptors function, retinal ganglion cells (RGCs) spontaneously generate correlated bursts of action potentials called 'retinal waves'<sup>4–5</sup>. Postsynaptic LGN neurons in turn are driven to fire in similar patterns<sup>6,5</sup>, and this endogenous activity is even relayed further into the visual cortex<sup>6</sup>. Although there is consensus that retinal waves and correlated activity are needed for synapse remodelling and segregation of RGC axons into eye-specific layers<sup>7,8</sup>, little is known at the synaptic or molecular level about how natural patterns of activity are read out to drive elimination and structural remodelling before sensory experience. It is assumed that synaptic learning rules are present at retinogeniculate synapses, and that implementation of these rules ultimately leads either to synapse stabilization or elimination. Efforts to discover molecular mechanisms of developmental synapse elimination have implicated several unexpected candidates, all with links to the immune system, including neuronal pentraxins, Complement C1q and MHC class I family members<sup>9–11</sup>. However, it is not known whether any of these molecules regulate plasticity rules at developing synapses. Moreover, because germline knockout mice were examined in each of these examples, it is not known whether neuronal versus immune function is required for synapse elimination in vivo. Here we examine these questions and also test whether genetically restoring H2-D<sup>b</sup> expression selectively to CNS neurons in vivo can rescue synapse elimination in mice that nevertheless lack an intact immune system.

Defective synapse elimination in LGN of K<sup>b</sup>D<sup>b−/−</sup> mutant mice

MHC class I genes H2-D<sup>b</sup> and H2-K<sup>b</sup>, members of a polymorphic family of over 50, are expressed in LGN neurons<sup>10</sup> and were discovered in an unbiased screen in vivo for genes regulated by retinal waves: blocking this endogenous neural activity not only prevents RGC axonal remodelling<sup>1</sup>, but also downregulates expression of MHC1 messenger RNA<sup>12</sup>. Previous studies have suggested that MHC1 molecules regulate synapse number in cultured neurons<sup>13</sup> and are needed for anatomical segregation of RGC axons into LGN layers in vivo<sup>14–16</sup>. To examine if H2-K<sup>b</sup> and H2-D<sup>b</sup> are involved in functional synapse elimination, whole-cell recordings were made from individual neurons in wild-type or K<sup>b</sup>D<sup>b−/−</sup> LGN slices (Fig. 1a)<sup>15,16</sup>. Adult mouse LGN neurons normally receive strong monosynaptic inputs from 1–3 RGC axons, but in development, many weak synaptic inputs are present. Most are eliminated between postnatal day 5 (P5) and P12 before eye opening, while the few remaining inputs strengthen, resulting in adult-like synaptic innervation by P24–P30 (ref. 15). By gradually increasing optic tract stimulation intensity, individual RGC axons with progressively higher firing thresholds can be recruited<sup>15</sup>, generating a stepwise series of excitatory postsynaptic currents (EPSCs) recorded in each LGN neuron. For example, at P21 in wild type, only two steps are present (Fig. 1b), indicating that just two RGC axons provide input to this LGN neuron, as expected. In contrast, in K<sup>b</sup>D<sup>b−/−</sup> LGN neurons, there are many EPSC steps (Fig. 1c), a pattern similar to that in much younger wild-type mice before synapse elimination<sup>15–16</sup>.

To obtain more quantitative information, minimal stimulation was used to estimate single fibre strength (SF-AMPA)<sup>17</sup> (Methods and Extended Data Fig. 1a, b). On average, the amplitude of SF-AMPA in K<sup>b</sup>D<sup>b−/−</sup> neurons is almost half that of wild type, and the cumulative probability distribution of EPSC amplitudes recorded from K<sup>b</sup>D<sup>b−/−</sup> LGN neurons is also consistent with the presence of smaller sized EPSCs (Fig. 1d; note onset latency of SF-AMPA is similar in both genotypes (Extended Data Fig. 1c)). In contrast, maximal synaptic input (Max-AMPA) is not different between wild type and K<sup>b</sup>D<sup>b−/−</sup> (Extended Data Fig. 1d). Fibre fraction, an index of how much each input contributes to total synaptic response<sup>13</sup> (Methods), is half as large in K<sup>b</sup>D<sup>b−/−</sup> than wild type (Fig. 1e), consistent with the idea that the number of RGC synapses in K<sup>b</sup>D<sup>b−/−</sup> LGN neurons is greater than in wild type. An alternative possibility—that differences can arise from altered probability of
neuronal H2-Db rescues elimination and segregation

Neuronal H2-D\(^b\) rescues elimination and segregation

H2-D\(^b\) and H2-K\(^b\) are also critical for immune function and CD8 T-cell development. Both MHCI molecules are expressed in LGN during the period of retinogeniculate synaptic refinement, with H2-D\(^b\) expressed at a higher level than H2-K\(^b\). To separate a contribution of the immune system, and to examine if neuronal expression is sufficient for synapse elimination, H2-D\(^b\) expression was restored exclusively to neurons by crossing H2-D\(^b\) mice to NSE\(^b\) mice in which H2-D\(^b\) expression is regulated under the neuron-specific enolase (NSE) promoter. 'Rescued' offspring littersmates have H2-D\(^b\) expression restored to CNS neurons while the rest of the body remains K\(^b\) (K\(^b\) mice).

Impaired LTD with natural activity patterns

Synapse elimination is thought to involve cellular processes leading to synaptic weakening such as LTD, conversely LTP-like mechanisms.
Figure 2 | H2-D\textsuperscript{b} expression in neurons rescues synapse elimination and eye-specific segregation in K\textsuperscript{D\textsuperscript{b}/–} LGN.  

- **Rescue of synapse elimination at P20–P24.** a, b, EPSC amplitudes versus optic tract stimulus intensity. Insets: example traces. c, Cumulative probability histogram of SF-AMPA. Inset: mean ± s.e.m. for control: D\textsuperscript{b}–/–, n = 19/17/N = 5. Rescue: D\textsuperscript{b}–/– (K\textsuperscript{D\textsuperscript{b}/–}; NSED\textsuperscript{–}/–, n = 17/N = 7), **P < 0.01. d, Fibre fraction is also rescued in K\textsuperscript{D\textsuperscript{b}/–}; NSED\textsuperscript{–}/– (n = 16/N = 7) compared to K\textsuperscript{D\textsuperscript{b}/–}; NSED\textsuperscript{–}/– (n = 18/N = 5), *P < 0.05, Mann–Whitney U-test for c. d. Horizontal grey bars delineate Fig. 1e data (mean ± s.e.m.). e, f, Rescue of eye-specific segregation in K\textsuperscript{D\textsuperscript{b}/–}; NSED\textsuperscript{+}/– at P34. e, Top: coronal sections of dLGN showing pattern of retinogeniculate projections from the ipsilateral (green) and contralateral (red) eyes. Bottom: region of ipsi-contra pixel (white) overlap between the two channels at 60% intensity threshold (T60%). f, Percentage of dLGN area occupied by ipsi-contra overlap. mean ± s.e.m. for K\textsuperscript{D\textsuperscript{b}/–}; NSED\textsuperscript{–}/– (N = 3) and K\textsuperscript{D\textsuperscript{b}/–}; NSED\textsuperscript{+}/– (N = 4) (T60%) (*P < 0.05, two way ANOVA) (see Extended Data Fig. 5). Horizontal grey bar indicates wild-type value at 160% (from ref. 14). n = cells/N = animals.

are postulated for synaptic strengthening and stabilization\textsuperscript{34,37}. In addition, spike-triggered-dependent mechanisms are crucial in Xenopus tectum for visually driven tuning of receptive fields\textsuperscript{13}. In mammalian LGN, LTP\textsuperscript{25} or LTD\textsuperscript{26} can be induced at retinogeniculate synapses using 100 Hz optic tract stimulation, which is far from the endogenous bursting patterns generated by retinal waves (Fig. 1f, g)\textsuperscript{14}. However, realistic patterns of optic tract stimulation mimicking waves, paired with postsynaptic depolarization of LGN neurons, have also been used; results revealed a synaptic learning rule that generates LTP when pre- and postsynaptic activity are coincident\textsuperscript{31,32}, but LTD when presynaptic optic tract activity precedes postsynaptic LGN depolarization within a broad window corresponding to the 60–90 s duty cycle of retinal waves (Fig. 3a–c and Extended Data Fig. 2b)\textsuperscript{31}. Moreover, using these timing patterns in conjunction with optogenetic stimulation of retina is sufficient either to drive or prevent segregation of RGC axons depending on the pattern\textsuperscript{33}. To determine whether synaptic learning rules based on natural activity patterns are altered at K\textsuperscript{D\textsuperscript{b}/–}–retinogeniculate synapses, perforated patch recordings were made in LGN slices from wild type versus K\textsuperscript{D\textsuperscript{b}/–}/– at P8–P13, the relevant period when extensive synapse elimination and eye-specific segregation are actually occurring. First, paired-pulse stimulation was used to examine release probability; the same amount of synaptic depression was observed in wild-type and K\textsuperscript{D\textsuperscript{b}/–}/– mice, indicating similar probabilities (Extended Data Fig. 6). Next, synchronous activity patterns were used, in which 10 Hz optic tract stimulation was paired with LGN depolarization (Fig. 3a, b; 0 ms latency), generating 10–20 Hz bursts of action potentials in LGN neurons mimicking retinal waves\textsuperscript{1,5}. In wild type, synchronous stimulation induced LTP (Fig. 3d, f; 117 ± 8% over baseline; P < 0.001). In K\textsuperscript{D\textsuperscript{b}/–}/– mice, the same protocol elicited LTD indistinguishable from wild type (Fig. 3e, f).

In contrast, induction using asynchronous activity patterns reveals a defect in LTD. In wild type, when optic tract stimulation precedes LGN neuron depolarization by 1.1 s (Fig. 3a, c; 1,100 ms latency), LTD results (Fig. 3g, i; ±12% decrease from baseline; P < 0.001). In contrast, in K\textsuperscript{D\textsuperscript{b}/–}/–, the same induction protocol failed to induce synaptic depression; if anything, a slight but significant potentiation was seen (Fig. 3h, i; 5% increase from baseline; P < 0.005). Thus, whereas LTD using asynchronous pre- and postsynaptic activity patterns is a robust feature of wild-type retinogeniculate synapses during the period of synapse elimination and eye-specific layer formation, it seems to be absent in K\textsuperscript{D\textsuperscript{b}/–}/– mice. This impairment is consistent with the failure of synapse elimination and axonal remodelling in K\textsuperscript{D\textsuperscript{b}/–}/– mice.

**Ca\textsuperscript{2+}-permeable AMPA receptors at K\textsuperscript{D\textsuperscript{b}/–/–} synapses**

Impaired LTD in K\textsuperscript{D\textsuperscript{b}/–}/– mice could be due to altered regulation of NMDA (N-methyl-D-aspartate)-receptor-mediated synaptic responses, as LTD and LTP are known to be dependent on NMDA receptors at a variety of synapses\textsuperscript{26}. Surprisingly, the NMDA/AMPA ratio was not different between genotypes (Extended Data Fig. 7a, b). However, the kinetics of I\textsubscript{AMPA} recorded in K\textsuperscript{D\textsuperscript{b}/–}/– LGN neurons are markedly prolonged compared to wild type (Fig. 4a–d). The slowed decay in K\textsuperscript{D\textsuperscript{b}/–}/– EPSCs is unlikely to be due to different peak I\textsubscript{AMPA} amplitudes (P > 0.1; Fig. 4d), but could occur if there were greater Ca\textsuperscript{2+} influx through AMPA receptors.

Ca\textsuperscript{2+}-permeable AMPA (CP-AMPA) receptors are blocked selectively by bath-applying the specific antagonist NASPM, a synthetic homologue of joro spider toxin\textsuperscript{34}. Indeed in K\textsuperscript{D\textsuperscript{b}/–}/– LGN neurons, 100 μM NASPM blocked 40% of the current recorded at −70 mV but only 20% in wild type (Fig. 4e and Extended Data Fig. 7c), confirming a two-fold increase in CP-AMPA receptor-mediated currents in K\textsuperscript{D\textsuperscript{b}/–}/– neurons. Another diagnostic feature of CP-AMPA receptors is rectification in the current–voltage (I–V) relationship when spermine is present in the internal recording solution\textsuperscript{34,36}. In wild type, the I–V relationship is linear. However in K\textsuperscript{D\textsuperscript{b}/–}/– LGN neurons, rectification is very prominent (Fig. 4f, g and Extended Data Fig. 7d) but can be linearized close to wild-type levels by bath application of NASPM (Fig. 4f, g), indicating that the prominent I–V rectification in K\textsuperscript{D\textsuperscript{b}/–}/– arises from an increase in CP-AMPA receptors.

Differences in composition of GluR subunits are known to modulate AMPA receptor Ca\textsuperscript{2+} permeability, and tetramers containing GluR2 confer Ca\textsuperscript{2+} impermeability\textsuperscript{35}. Indeed, the ratio of GluR1 to GluR2, the most prevalent subunits\textsuperscript{35,37,38}, is slightly increased in developing thalamic neurons (Fig. 4h, i; 35% increase in K\textsuperscript{D\textsuperscript{b}/–}/–; P = 0.07; Extended Data Fig. 7e). Elevated levels of GluR1 subunits suggest that AMPA receptors in K\textsuperscript{D\textsuperscript{b}/–}/– are more likely to be composed of GluR1 homomers,
Figure 3 | Impaired LTD but intact LTP at retinogeniculate synapses in $K^D^{b/-}$ induced with natural activity patterns. a, Diagram illustrating basis for timing-dependent plasticity at developing retinogeniculate synapses. Spontaneous retinal waves propagate from ‘a, b’ towards ‘c, d;’ neighbouring RGCs fire synchronously but asynchronously with respect to RGCs located elsewhere. Waves drive action potentials in postsynaptic LGN neurons with RGCs fire synchronously but asynchronously with respect to RGCs located elsewhere. Waves drive action potentials in postsynaptic LGN neurons with RGCs fire synchronously but asynchronously with respect to RGCs located elsewhere. Waves drive action potentials in postsynaptic LGN neurons with. 

b, c, Top: conditioning protocol for LTP (0 ms latency; b) or LTD (1,100 ms latency; c). Bottom: example membrane potential changes recorded in LGN neurons during conditioning protocol. OT, optic tract. 

d–f, Intact LTP yielding increased Ca$^{2+}$ permeability. Together, results point to an increase in CP-AMPA receptors in $K^D^{b/-}$ mice. Similar increases in CP-AMPA receptors at other synapses are known to shift synaptic learning rules away from LTD and towards LTP$^{39,40}$. If so, the deficit in LTD observed when spermine is present in the internal recording solution, and bath application of NASPM has little additional effect ($P > 0.5$ at $+40$ mV), similar to wild type (Fig. 5e and Extended Data Fig. 9b). Because the Ca$^{2+}$ permeability of AMPA receptors is close to wild-type levels in $K^D^{b/-}$;NSED$^{b+}$ mice, it is possible that LTD is also rescued. Indeed, the I–V relationship is linearized in $K^D^{b/-}$;NSED$^{b+}$ LGN neurons when spermine is present in the internal recording solution, and bath application of NASPM has little additional effect (Fig. 5e and Extended Data Fig. 9b). Because the Ca$^{2+}$ permeability of AMPA receptors is close to wild-type levels in $K^D^{b/-}$;NSED$^{b+}$ LGN neurons, it is possible that LTD is also rescued. Indeed, the same asynchronous activity pattern that failed to induce LTD in $K^D^{b/-}$ (Fig. 3) induces robust LTD (15%; $P < 0.001$) in $K^D^{b/-}$;NSED$^{b+}$ neurons, similar to wild type (Fig. 5f, g). Together, these observations suggest that restoring expression of H2-D$^{b}$ in neurons is sufficient to rescue LTD at retinogeniculate synapses via decreasing the Ca$^{2+}$ permeability of AMPA receptors.

Neuronal H2-D$^{b}$ rescues synaptic function and LTD If H2-D$^{b}$ affects synapse elimination by regulating properties of AMPA receptors, then retinogeniculate EPSCs should be rescued to wild type in the LGN of $K^D^{b/-}$;NSED$^{b+}$ mice. Indeed, the kinetics of $I_{AMPA}$ in $K^D^{b/-}$;NSED$^{b+}$ LGN neurons compared to $K^D^{b/-}$;NSED$^{b-}$ LGN neurons (Fig. 5a–c and Extended Data Fig. 9a), indicating a decrease in Ca$^{2+}$-permeable AMPA receptors. Accordingly, NASPM-dependent inhibition of $I_{AMPA}$ is only 20% in $K^D^{b/-}$;NSED$^{b+}$, significantly reduced from the 40% inhibition observed in littermate $K^D^{b/-}$ mice (Fig. 5d and Extended Data Fig. 9a). Moreover, the I–V relationship is linearized in $K^D^{b/-}$;NSED$^{b+}$ LGN neurons when spermine is present in the internal recording solution, and bath application of NASPM has little additional effect ($P > 0.5$ at $+40$ mV), similar to wild type (Fig. 5e and Extended Data Fig. 9b). Because the Ca$^{2+}$ permeability of AMPA receptors is close to wild-type levels in $K^D^{b/-}$;NSED$^{b+}$ mice, it is possible that LTD is also rescued. Indeed, the same asynchronous activity pattern that failed to induce LTD in $K^D^{b/-}$ (Fig. 3) induces robust LTD (15%; $P < 0.001$) in $K^D^{b/-}$;NSED$^{b+}$ neurons, similar to wild type (Fig. 5f, g). Together, these observations suggest that restoring expression of H2-D$^{b}$ in neurons is sufficient to rescue LTD at retinogeniculate synapses via decreasing the Ca$^{2+}$ permeability of AMPA receptors.

Discussion

A major finding of this study is that the link between activity-dependent synapse pruning during development, and regulation of LTD and CP-AMPA
receptors, requires neuronal MHCI function. It is notable that synaptic elimination fails despite the fact that retinal waves and retinogeniculate basal synaptic transmission are intact. The persistence of multiple innervation in KdD−/− LGN neurons is highly reminiscent of the immature reading out endogenous activity patterns into a lasting structural frame-

Figure 4 | Increased Ca2+-permeable AMPA receptors at retinogeniculate synapses in KdD−/− LGN. a–d, Prolonged decay kinetics of IAMPA in KdD−/− mice. a, Average IAMPA (5–10 EPSCs) for wild-type versus KdD−/− LGN neurons. IAMPA half-width (ms) (b), IAMPA decay time (ms) (c) and peak amplitude (nA) (d) for wild type versus KdD−/− (WT, n = 16/N = 4; KdD−/−, n = 22/N = 5), e, Increased per cent inhibition of peak IAMPA by NASPM (100 μM) in KdD−/− (n = 13/N = 4) versus wild type (n = 9/N = 3) (**P < 0.01; NS, not significant; Mann–Whitney U-test for b, c). f, IAMPA I–V curves (normalized to −40 mV). g, Rectification index for wild type (n = 14/N = 3), KdD−/− (n = 9/N = 3), KdD−/− +20 μM NASPM (n = 16/N = 4) or KdD−/− +100 μM NASPM (n = 6/N = 2) (**P < 0.001 for wild type versus KdD−/−; P > 0.05 for wild type versus KdD−/− + NASPM (20 or 100 μM), Mann–Whitney U-test). Ages studied: P8–P13. Also, see Extended Data Fig. 7. n = cells/N = animals.

Figure 5 | Neuronal expression of H2-Dk restores Ca2+-impermeable AMPA receptors and rescues LTD. a–c, IAMPA half-width (ms) (a), IAMPA decay time (ms) (b), and peak amplitude (nA) (c) for KdD−/−;NSED−+ (n = 9/N = 2) and KdD−/−;NSED− (n = 11/N = 4). d, Reduced per cent inhibition of peak IAMPA by NASPM (100 μM) in KdD−/−;NSED−+ (n = 10/N = 3) compared to KdD−/−;NSED−+ (n = 8/N = 2); *P < 0.05, ***P < 0.001, ***P < 0.001, Mann–Whitney U-test for a–d. e, Rescue of IAMPA linear I–V relationship in KdD−/−;NSED−+ LGN. Rectification index at +40 mV for KdD−/−;NSED−+ (n = 11/N = 3) and KdD−/−;NSED− (n = 13/N = 5) shows significant difference (**P < 0.005); KdD−/−;NSED−+ (+NASPM) (n = 7/N = 3) is not significantly different to KdD−/−;NSED−+ (P > 0.05), Mann–Whitney U-test. See also Extended Data Fig. 9. mean ± s.e.m. f, g, LTD rescued in KdD−/−;NSED−+ LGN neurons.

The rescue experiments performed here indicate that a single MHCI molecule—H2-Dk, when expressed in neurons—is sufficient for functional synaptic elimination and anatomical eye-specific segregation in the LGN. By crossing KdD−/− mice to NSED+ transgenic mice, expression of H2-Dk alone was restored to neurons but not elsewhere in the body, rescuing LTD, functional synaptic elimination, Ca2+-impermeable AMPA receptors and structural remodelling at retinogeniculate synapses. Notably, these brain phenotypes are rescued even though the immune system is still impaired in KdD−/−;NSED−+ mice. Until this experiment, it was not known if any one MHCI molecule is sufficient either in vitro or in vivo, nor has it been possible to separate the general effects of immune compromise from the absence of H2-Dk and/or H2-Kb in neurons. Together, our observations argue for a key role for H2-Dk in reading out endogenous activity patterns into a lasting structural framework. In the human genome, as in mice, the MHCI (HLA) locus is large and highly polymorphic. Recent genome-wide association studies have consistently linked specific single nucleotide polymorphisms in MHCI to schizophrenia42–44. Our observations offer possible mechanistic insight: alterations in expression levels of specific MHCI molecules at neuronal synapses could trigger changes in activity-dependent plasticity and synaptic pruning during critical periods of human development, generating lasting alterations in circuits and behaviour.
METHODS SUMMARY

All experimental protocols were approved by Stanford University Animal Care and Use Committees. K\(^{+/—}\) mice were provided by H. Ploeg\(^{16}\) and NSEFP\(^{+/—}\) mice by M. B. A. Oldstone. Crosses of these two lines generated K\(^{+/—}\) NSEFP\(^{+/—}\) mice plus littermate controls. Electrophysiological recordings were made from LGN neurons by cutting parasagittal brain slices containing dorsal lateral geniculate nucleus (dLGN) and optic tract; synaptic transmission and degree of innervation was assessed as previously described\(^{11}\). For plasticity experiments at retinogeniculate synapses, perforated patch-clamp technique and induction protocols with natural activity patterns were used\(^{12}\). Multi-electrode array recordings of retinal waves and anatomical labeling of retinogeniculate projections to determine status of eye-specific segregation were carried out according to ref. 18 and ref. 14. Pharmacological investigation of CP-AMPA receptors was carried out according to ref. 34. All experiments were conducted and analysed blind to genotype except in Fig. 4 (genotype was obvious to experimenter because of phenotype). Sample sizes were chosen for each experiment to reach statistical significance (P value equal to or less than 0.05; details given in Methods and figure legends).

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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All experimental protocols were approved by Stanford University Animal Care and Use Committees. K\(^{+}\)/-/- mice were provided by H. Ploegh \(^{16}\) and NSE\(^{-}\) mice by M. R. A. Oldstone\(^{24}\). These mice were maintained on C57BL/6 backgrounds.

Crossed composition in mM: 125 CsCl, 6.4 MgCl\(_2\), 0.45 CaCl\(_2\), 2 mM HEPES, 4 Mg-ATP, 10 phosphocreatine, 0.3 Na-GTP, pH 7.3 with KOH. Note that antimonial patch recordings were necessary to track macropatch components required for plasticity\(^{20,24,45}\). Amylphorin B stock solution (40 mg ml\(^{-1}\) in DMSO) was prepared fresh every day, from which 100–200 \(\mu\)g ml\(^{-1}\) internal solution was prepared hourly and kept on ice. The tip of the patch elec-

trode was filled with internal solution without amylphorin B before back-filling with amylphorin B containing internal solution. Following the formation of GQ seal, gradual drop of access resistance was monitored until stabilizing at 20–200 MΩ. Trials with sudden changes in Rs (clearly discernible due to rupture of perforation or clogging of patch pipette by amylphorin B precipitation) were discarded. EPSC changes with stable Rs within \(\pm 20\) MΩ were used for analysis except when gradual changes in Rs were correlated with changes in EPSCs size. After obtaining a stable baseline for 10–20 min, the configuration was switched to current-clamp mode and plasticity induction protocols were applied by pairing optic tract stimulation with postsynaptic current injection for 1 s to generate 10–20 Hz action potentials either synchronously or with 1,100 ms latency (Fig. 3a–c). \(V_m\) (resting membrane potential measured at \(t = 0\) configuration in perforated patch clamp mode) was not different between wild type (\(-58.5 \pm 1.2\) mV; \(N = 16\) cells from 16 animals), \(K^{+}\)EPSC (\(-59.6 \pm 1.3\) mV; \(N = 10\)), \(KbDb\)-EPSC (\(-60.5 \pm 1.9\) mV; \(N = 13\)) or \(K^{+}\)EPSC\(^{-}\) \(NSEDb\) (\(-62.2 \pm 2.5\) mV; \(N = 7\) \(p = 0.1, 1\)-test). The patch protocol was repeated 10 times over a 40 s interval. Data were acquired with resting membrane potentials (measured at \(t = 0\) greater than \(-53\) mV. For the NASPM LTD rescue experiments (Extended Data Fig. 8), 20\(\mu\)M NASPM was added to the bath, and the asynchronous pairing protocol (10 times over a 40 s interval) was repeated twice, separated by 20 min. \(N\) one cell per each experiment.

Percentage change is mean \(\pm\) s.e.m. of EPSC amplitude averaged over 30 min after conditioning period as compared to baseline per cell.

For optic tract stimulation, concentric bipolar (CBABD70; FHC Inc.) or low-resistance glass-electrode filled with ACSF were used for stimulating electrodes without noticeable differences. Stimulus strength was regulated using an ISO-Flex stimulus isolator (A.M.F.I.) and applied at 0.025–0.033 Hz. For plasticity experi-

ments, stimulus strength was set to achieve stable EPSCs (10–120 pA) activated by a single or a few fibres, and stimuli were applied at 0.025 Hz. To measure spino-

dependent \(I-V\) rectification, stimulation intensity was adjusted to evoke \(I_{AMP}\) currents in the 100–300 pA range, due to the presence of \(Ca^{2+}\)-permeable AMPA receptor-mediated synaptic responses in \(K^{+}\)/-/- mice.

**Evaluation of the number of synaptic inputs.** The number of connected fibres was estimated using two approaches modified from ref. 15. First, synaptic responses were recorded while varying optic tract stimulus intensity from minimal to maximal. Stimulation in increments of 1.0–2.5 \(\mu\)A was used between 0 \(\mu\)A and 100 \(\mu\)A, and larger increments (10–20 \(\mu\)A) were used at stimulation intensities greater than 100 \(\mu\)A. The stimulus-response profile provides an estimate of the number of RGC inputs to the cell. We also estimated the number of inputs to each LGN neuron based on the single-fibre AMPA current elicited at \(-70\) mV by minimal stimulation as a per cent of the total current elicited by maximal stimulation for the same cell (Fibre fraction). For minimal stimulation, the method is based on the assumption that minimal stimulation elicits synaptic responses from only a single fibre in the optic tract (SF-AMPA). After a synaptic response was observed, the intensity of electrical stimulation was lowered to obtain a failure rate higher than 50% (20–50 repeats). Synaptic responses with an onset latency of \(<5\) ms within a 1-ms window were regarded as direct, monosynaptic responses. The mean peak amplitude of successful synapses were taken as the threshold of the single fibre synaptic response (SF-AMAP). Onset latency of SF-AMPA by minimal stimulation (estimated as time to reach 10% of peak \(I_{AMP}\)) was not different between wild type, \(K^{+}\)/-/-, \(KbDb\)-EPSC\(^{-}\) \(NSEDb\) or \(KbDb\)-EPSC\(^{-}\) \(NSEDb\) (Extended Data Fig. 1c).

For maximal stimulation, electrical stimulation was increased until the peak synaptic currents reached steady-state level (1–600 \(\mu\)A range). Fibre fraction was calculated as a ratio of single-fibre synaptic strength (SF-AMPA) to maxi-

mal synaptic strength (Max-AMPA) from the same cell (FF = SF/Max-AMPA per cell, %). The number of cells recorded per animal was limited to 4 for SF-AMPAs and Max-AMPA measurements.

**Kinetons of EPSCs** were measured as both the width at half-maximal EPSC amplitude (half-width), and decay time within 80–20% range of peak EPSCs using Clampfit.

**Multi-electrode array recordings of retinal waves.** Retinas were isolated from wild-
type and \(K^{+}\)/-/- mice and pieces of retina were placed retinal-ganglion-cell-side 

**Multi-electrode array recordings of retinal waves.** Retinas were isolated from wild-
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**Kinetons of EPSCs** were measured as both the width at half-maximal EPSC amplitude (half-width), and decay time within 80–20% range of peak EPSCs using Clampfit.
down on a 60-electrode multi-electrode array arranged in an 8 × 8 grid (excluding the four corners) with 10 μm diameter electrodes at 100 μm spacing (Multi Channel Systems). The retinas were held in place with a weighted piece of dialysis membrane and superfused continuously with artificial cerebral spinal fluid (ACSF) containing (in mM): 119.0 NaCl, 26.2 NaHCO3, 11 glucose, 2.5 KCl, 1.0 K2HPO4, 2.5 CaCl2, and 1.3 MgCl2. ACSF was oxygenated with 95% O2 and 5% CO2 and maintained at 32 °C, pH 7.4. Each preparation was allowed to equilibrate for 20 min before data acquisition. Spontaneous firing patterns were then recorded for 60 min. Voltage traces on each electrode were sampled at 20 kHz and filtered between 120 and 2,000 Hz. Events that crossed a spike threshold were sorted online to identify single units using Plexon Offline Sorter software. Single units were identified by combining principal component analysis together with a valley-seeking algorithm and were inspected manually. The mean firing rate of all units throughout the recording was calculated and units with a mean firing rate less than 10% of overall mean firing rate were excluded from further analysis. Spike-sorted data were analysed in MATLAB (Mathworks). To identify bursts, a modified Poisson Surprise algorithm was used as described previously. Mean burst values for each retina were grouped according to age (P5–P8 and P10–P12) and genotype (wild type and KΔD−/−). Differences between means of different genotypes within each age group were evaluated for statistical significance using a t-test. To determine spatial properties of waves, a pair-wise correlation index, CI, was computed as a function of distance between two cells for all spikes in the recording, using a method described previously.4 The correlation index gives a measure of the likelihood relative to chance that a pair of neurons fire together within a 100-ms time window. The distance between cells was approximated as the distance between the electrodes on which the activity of the cells was recorded. The cell pairs were grouped according to their intercellular distance, and medians were computed over all cell pairs in a given distance group for each individual retina. The median correlation indices were then averaged across retinas, for each age and genotype, and plotted as a function of intercellular distance.

**Anterograde labelling of retinal ganglion axons and multiple threshold analysis.** All methods were performed according to previously described studies.3,4,6,8,9,17 P5–34 mice were anaesthetized with isoflurane. Cholera toxin B (CTB) subunit (1–2 μl) conjugated to AF594 was injected in the left eye (1 mg ml−1 dissolved in 0.2% DMSO, Invitrogen). After 24 h, animals were perfused and fixed by transcardial perfusion of 0.1 M phosphate buffered saline (PBS), then ice-cold 4% paraformaldehyde (PFA) in 0.1 M PBS. Brains were postfixed in 4% PFA overnight. 100-μm thick sections were cut on a freezing microtome. Sections were mounted with Prolong Antifade Gold (Invitrogen), and after coverslipping were imaged on a Zeiss Leica PS2 confocal microscope (Leica Microsystems), dLGN sections taken at the middle of the rostral-caudal extent of dLGN where the area of the ipsilateral eye projection is greatest were imaged in red and green channels. Images were acquired and analysed such that the peak intensity values were below maximum, and multiple threshold analysis was used to vary red and green channels at intensity thresholds of 20%, 40%, 60%, 80% and 100% of maximum. To measure the amount of overlap between inputs from both eyes, pixels overlapping in both red and green channels were identified using Image J (NIH) and the Colocalization plug-in tool. Colocalization indices were then averaged across retinas, for each age and genotype, and plotted as a function of intercellular distance.

**GluR1/2 expression levels.** Thalami were isolated from P22 wild-type or KΔD−/− mice, and synaptosome-enriched fractions prepared as described.4,11,12 Freshly isolated individual thalami were homogenized in homogenization buffer (10 mM HEPES pH 7.5, 0.5 mM EGTA, 33% sucrose, 4 mM Pefabloc SC PLUS (Roche), and 0.2 mM phenylmethanesulphonyl fluoride (PMSF)), and centrifuged (10 min at 2,000g). Supernatants were passed through three layers of 100 μm pore nylon membranes, and then through 5 μm nitrocellulose filters. Fractions were centrifuged (10,000g for 10 min), and pellets re-suspended in homogenization buffer. Protein amounts were determined by BioRad Protein Assay (BioRad) (about 200 μg synaptosomal protein was obtained per thalamus), and SDS–PAGE sample buffer (Novex) was added to a 1× concentration. After heating (85 °C for 4 min), equivalent amounts of protein from each sample (20 μg per sample) were electroblotted to PVDF membranes. Western blots were performed with antibodies to GluR1 (Millipore, AB1504), GluR2 (Abcam, AB133477), N-tubulin (Abcam, AB18207), or GAPDH (Abcam, AB9485). For quantification, gels were scanned and intensities of bands were quantified using the Image J program. After quantification, intensity values for GluR1 and GluR2 were normalized by dividing them against each GAPDH or N-tubulin values from the same thalamus. Western blots typically were run with multiple wild-type and KΔD−/− samples; to compare results from different western blots, the intensity measurement for each sample thalamus was normalized against the average value derived from all wild-type bands on that same blot. The GluR1/GluR2 ratio for each thalamus was then determined. Finally, an average GluR1/GluR2 ratio was calculated from each individual thalamus (Extended Data Fig. 7c). Mouse cortical cultures were made as described.4,11 Wild-type or KΔD−/− cortical neurons were isolated at E16.5, and cultured on 6-cm tissue culture plates for 14 days in vitro. Plates were then washed twice with PBS and cells were lysed directly on the plates with lysis buffer (150 mM NaCl, 50 mM Tris–HCl pH 7.5, 0.25% sodium deoxycholate, 1% NP-40, 1 mM EGTA, 1 mM PMSF, 1% Pefabloc SC PLUS (Roche)) for 10 min. Lysates were then centrifuged at 12,000g for 10 min. Supernatants were normalized by BioRad Protein Assay (BioRad), and equivalent amounts of protein from each sample (20 μg per sample) were electroblotted to PVDF membranes. Western blots were performed with antibodies to GluR1, GluR2, GAPDH, and/or N-tubulin, as described above. Gels were scanned and bands quantified using Image J. Values were then normalized by dividing them by GAPDH or N-tubulin values, and an average GluR1/GluR2 ratio was calculated from each individual sample (Extended Data Fig. 7f). Non-parametric Mann–Whitney U-test (Prism software) was used to calculate P values for all analyses (two-tailed).

**Data analysis and statistics for electrophysiology experiments.** Data analysis as well as graph plotting were done using Clampfit 10.2 (Axon Instruments) and the OriginLab Corporation. Cages containing mice of different genotype were housed in separate cages. Animals from each experiment were relabelled and randomized so that experimenter did not know genotype. Experiments were then analysed blinded to genotype unless stated in figure legends. In addition, Student’s t-test was used for data with normal distribution and Mann–Whitney U-test for data with non-normal distribution. Shapiro–Wilks test was conducted for normality test. N = number of animals; n = number of cells. Average data are presented as mean ± s.e.m., unless stated otherwise. For synaptic plasticity experiments, t-test was used. Two-tailed analysis was used throughout the study.

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Extended Data Figure 1 | Comparison of retinogeniculate synaptic responses in wild type versus $K^\alpha D^{\beta-/-}$.

a, b, Examples of minimal stimulation for wild type (open circles) and $K^\alpha D^{\beta-/-}$ (filled circles). Plot of EPSC peak versus number of stimulations (grey box represents failures, >50%). c, No difference in onset latency of SF-AMPA between all genotypes. Onset latency of SF-AMPA was estimated using minimal stimulation as time (ms) to reach 10% of peak AMPA from stimulation artefact (wild type: $3.0 \pm 0.3$ (n = 12 cells/N = 6 animals); $K^\alpha D^{\beta-/-}$: $2.7 \pm 0.1$ (n = 23/N = 8); $K^\alpha D^{\beta-/-}\cdot NSEDb^+$: $3.0 \pm 0.2$ (n = 17/N = 7); $K^\alpha D^{\beta-/-}\cdot NSEDb^-$: $2.6 \pm 0.2$ (n = 19/N = 5); P > 0.5, t-test). d, Cumulative probability histogram shows no difference in Max-AMPA between wild type and $K^\alpha D^{\beta-/-}$ (filled circle). Wild type versus $K^\alpha D^{\beta-/-}$: 1 Hz: 82.3 ± 2.6 (n = 10) versus 77.4 ± 3.4 (n = 8); 10 Hz: 44.9 ± 2.3 (n = 9) versus 42.8 ± 3.9 (n = 9); 20 Hz: 37.1 ± 2.1 (n = 10) versus 37.3 ± 2.8 (n = 9) (P > 0.1 for each).

e WT, EPSC1 500 pA 20 ms

f $K^\alpha D^{\beta-/-}$ EPSC1 200 pA 20 ms

SR95531 (20 μM) + APV (100 μM)

g No CTZ

d WT, EPSC2 1 Hz 20 Hz

e WT, EPSC2 1 Hz 20 Hz

h CTZ 20 μM

Extended Data Figure 1 | Comparison of retinogeniculate synaptic responses in wild type versus $K^\alpha D^{\beta-/-}$. a, b, Examples of minimal stimulation for wild type (open circles) and $K^\alpha D^{\beta-/-}$ (filled circles). Plot of EPSC peak versus number of stimulations (grey box represents failures, >50%). c, No difference in onset latency of SF-AMPA between all genotypes. Onset latency of SF-AMPA was estimated using minimal stimulation as time (ms) to reach 10% of peak AMPA from stimulation artefact (wild type: $3.0 \pm 0.3$ (n = 12 cells/N = 6 animals); $K^\alpha D^{\beta-/-}$: $2.7 \pm 0.1$ (n = 23/N = 8); $K^\alpha D^{\beta-/-}\cdot NSEDb^+$: $3.0 \pm 0.2$ (n = 17/N = 7); $K^\alpha D^{\beta-/-}\cdot NSEDb^-$: $2.6 \pm 0.2$ (n = 19/N = 5); P > 0.5, t-test). d, Cumulative probability histogram shows no difference in Max-AMPA between wild type and $K^\alpha D^{\beta-/-}$. Inset: mean ± s.e.m. Wild type: 2.6 ± 0.4 nA (n = 14/N = 6); $K^\alpha D^{\beta-/-}$: 2.9 ± 0.4 nA (n = 22/N = 8); P > 0.1, Mann–Whitney U-test. e–h, Presynaptic release probability at $K^\alpha D^{\beta-/-}$ retinogeniculate synapses is similar to wild type at P20–24. e, f, Examples of EPSCs evoked by paired-pulse stimulation of optic tract (20 Hz) recorded in whole-cell mode in individual LGN neurons from wild type (e) versus $K^\alpha D^{\beta-/-}$ (f). g, h, Paired-pulse depression (PPD) (%) (EPSC 2/EPSC 1) over varying intervals. g, Wild type (open circle) versus $K^\alpha D^{\beta-/-}$ (filled circle) without cyclothiazide (CTZ), a blocker of AMPA receptor desensitization. Wild type versus $K^\alpha D^{\beta-/-}$: 1 Hz: 82.3 ± 2.6 (n = 10) versus 77.4 ± 3.4 (n = 8); 10 Hz: 44.9 ± 2.3 (n = 9) versus 42.8 ± 3.9 (n = 9); 20 Hz: 37.1 ± 2.1 (n = 10) versus 37.3 ± 2.8 (n = 9) (P > 0.1 for each). h, Wild type (open circle) versus $K^\alpha D^{\beta-/-}$ (filled circle) with CTZ (20 μM). Wild type versus $K^\alpha D^{\beta-/-}$: 1 Hz: 79.0 ± 2.4 (n = 9) versus 81.8 ± 1.1 (n = 7); 10 Hz: 59.2 ± 3.0 (n = 8) versus 57.4 ± 3.7 (n = 7); 20 Hz: 58.5 ± 2.3 (n = 8) versus 56.6 ± 4.1 (n = 7) (P > 0.1 for each). N = 4 for wild type; N = 3 for $K^\alpha D^{\beta-/-}$ for g, h. There was no significant difference in PPD between wild type and $K^\alpha D^{\beta-/-}$, but note significant decrease of PPD + 20 μM CTZ versus 0 μM CTZ application for both wild type and $K^\alpha D^{\beta-/-}$ at 10 Hz and 20 Hz (P < 0.05). t-test. mean ± s.e.m. n = cells/N = animals.
Extended Data Figure 2 | Intact spatio-temporal pattern of retinal waves in \( K^D^{+/−} \) mice at P5–12. a, Correlation indices as a function of inter-electrode distance for all cell pairs for wild type (grey) and \( K^D^{+/−} \) (black) at P5–P8. Data points correspond to mean values of medians from individual data sets and error bars represent s.e.m. b–e, Summary of temporal firing patterns for retinas isolated from wild-type (open squares) and \( K^D^{+/−} \) (filled squares) mice at P5–P8 (stage II, cholinergic waves) and P10–P12 (stage III, glutamatergic waves). Open circles correspond to the mean values of individual retinas. b, Interburst interval for stage II (in min): wild type: 1.9 ± 0.2; \( K^D^{+/−} \): 1.6 ± 0.2; stage III: wild type: 1.0 ± 0.3; \( K^D^{+/−} \): 0.9 ± 0.1. c, Firing rate during burst for stage II (in Hz): wild type: 8.6 ± 1.9; \( K^D^{+/−} \): 8.4 ± 1.7; stage III: wild type: 19.7 ± 2.1; \( K^D^{+/−} \): 18.6 ± 2.2. d, Burst duration for stage II (in seconds): wild type: 4.2 ± 0.2; \( K^D^{+/−} \): 3.8 ± 0.1; stage III: wild type: 2.6 ± 0.6; \( K^D^{+/−} \): 2.1 ± 0.2. e, Mean firing rate for stage II (in Hz): wild type: 0.5 ± 0.1; \( K^D^{+/−} \): 0.5 ± 0.2; stage III: wild type: 1.0 ± 0.1; \( K^D^{+/−} \): 0.8 ± 0.1, mean ± s.e.m. (\( P > 0.05 \) for each, \( t \)-test, \( N = 6 \) animals for each group, except when \( N = 5 \) for stage III wild type; non-blind experiments).
Extended Data Figure 3 | Rescue of H2-Dk expression in brain of KdDk−/−;NSkD− mice. a, Diagram of breeding strategy to generate KdDk−/−;NSkD− mice. KdDk−/− (white indicates absence of H2-Dk) were crossed to NSkD mice (black indicates presence of H2-Dk in both body and brain). From F1 offspring, KdDk−/− mice were selected and crossed to KdDk−/− mice further, generating KdDk−/− mice (black brain with white body indicates rescue of H2-Dk expression in brain alone) and KdDk−/− littermate controls (white body, white brain).

b, H2-Dk-specific primers. Solid arrows: forward (exon 2) and reverse (exon 3) for H2-Dk mRNA as well as unspliced proRNA (exon, i; intron). Dotted arrows: NSkD-A (forward, NSkD promoter region) and NSkD-B1 and/or B2 (reverse, exon 2) for genotyping and mRNA detection. c, RT–PCR showing rescue at P10 in thalamus of KdDk−/−;NSkD− mice cDNAs. Wild-type thalamus (WT(T)) shown as positive control; KdDk−/− thalamus (KdDk−/−(T)) and spleen (KdDk−/−(S)) as negative controls, and various organs from KdDk−/−;NSkD− mice (spleen (S), liver (L), gut (G), thalamus (T), hippocampus (H), cortex (C), retina (R)) were used as templates. d, Quantitative PCR comparing relative H2-Kk/H2-Dk gene expression in wild type, KdDk−/− and KdDk−/−;NSkD− thalami. Left: results show small but highly significant rescue of H2-Dk mRNA expression in KdDk−/−;NSkD− (KdDk−/−: 0.00003 ± 0.00002, KdDk−/−;NSkD−: 0.0035 ± 0.00044 relative to wild type: 1.0056 ± 0.032, ***P < 0.0001). Each point represents average relative gene expression for one mouse. Right: raw H2-Kk/H2-Dk Ct values for each genotype (wild type: 27.4 ± 0.1; KdDk−/−: 44.3 ± 0.4; KdDk−/−;NSkD−: 35.9 ± 0.3; ***P < 0.001), one-way ANOVA, mean ± s.e.m. N = 5 animals for wild type, 7 for KdDk−/−, 10 for KdDk−/−;NSkD−. e, Rescue of H2-Dk protein in KdDk−/−;NSkD− brains at P60. Western blot of immunoprecipitation from whole brain (three pooled brains) lysate from KdDk−/− and KdDk−/−;NSkD−. H2-Dk-specific signal from KdDk−/−;NSkD− appears below IgG band.
Extended Data Figure 4 | Cumulative probability distribution for SF-AMPA and Max-AMPA recorded at retinogeniculate synapses according to H2-D<sup>β</sup> genotype. a, b, Max-AMPA (a) and SF-AMPA (b) showed similar cumulative probability histograms between wild type (black line), K<sup>o</sup>D<sup>β/β</sup>;NSED<sup>β/β</sup> (dashed grey line), K<sup>o</sup>D<sup>β/β</sup>;NSEDb<sup>1</sup> (dashed black line) and K<sup>o</sup>D<sup>β/β</sup>;NSEDb<sup>2</sup> (grey line). Number of experiments are the same as in the main text, except for Max-AMPA: for K<sup>o</sup>D<sup>β/β</sup>;NSED<sup>β/β</sup>: n = 18 cells/N = 5 animals; for K<sup>o</sup>D<sup>β/β</sup>;NSEDb<sup>1</sup>: n = 16/N = 7 (P > 0.05, Mann-Whitney U-test). Fibre fraction calculated from Max-AMPA and SF-AMPA measurements is similar between wild-type and K<sup>o</sup>D<sup>β/β</sup>;NSED<sup>β/β</sup> mice (Fig. 2d).
Extended Data Figure 5 | Neuronal H2-Db expression in KdDb<sup>+/−</sup> mice rescues impaired eye-specific axonal segregation at P34. a, Coronal sections of dLGN of KdDb<sup>+/−; NSEDb</sup><sup>−/−</sup> (top row) and KdDb<sup>+/−; NSEDb</sup><sup>+/+</sup> (rescue; bottom row) showing pattern of RGC axonal projections from the two eyes after intraocular tracer injections of CTB AF594 (red channel; contralateral eye injected) or AF488 (green channel; ipsilateral eye injected). Left: thresholded fluorescent images of dLGN at 60% maximum signal intensity (see Fig. 2e). Right: overlap of RGC projections (white pixels) from ipsilateral and contralateral eyes displayed for 20%, 40% and 60% maximal threshold for KdDb<sup>+/−; NSEDb</sup><sup>−/−</sup> (top) and KdDb<sup>+/−; NSEDb</sup><sup>+/+</sup> (bottom). Overlap = pixels labelled in both red and green channels. b, Mean percentage dLGN area ± s.e.m. pixel overlap for KdDb<sup>+/−; NSEDb</sup><sup>−/−</sup> (filled squares; N = 3) versus KdDb<sup>+/−; NSEDb</sup><sup>+/+</sup> (open triangles; N = 4): 0% threshold: 8.3 ± 1.1 versus 1.2 ± 0.3; 20% threshold: 12.2 ± 1.5 versus 2.9 ± 0.7; 40% threshold: 17.9 ± 1.8 versus 5.3 ± 0.9; 60% threshold: 21.1 ± 1.8 versus 7.2 ± 0.9; 80% threshold: 23.2 ± 1.3 versus 11.1 ± 1.2; 100%: 25.3 ± 1.9 versus 14.0 ± 1.5 (P < 0.05, two-way ANOVA). See Methods and ref. 47.
Extended Data Figure 6 | Intact release probability at \( K^4D^{-/-} \) retinogeniculate synapses before eye opening. Paired-pulse stimulation was delivered to the optic tract at 10 Hz, similar to the natural firing frequency of RGCs (Extended Data Fig. 2c), and whole-cell recordings were made from LGN neurons in slices aged between P8–13. Paired-pulse stimulation resulted in synaptic depression, represented as EPSC 2 divided by EPSC 1 (%). In 0 mM CTZ (left panel): wild type: 67.0 ± 2.9 (n = 11/N = 4); \( K^4D^{-/-} \): 64.2 ± 2.8 (n = 7/N = 2). In 20 μM CTZ (right panel): wild type: 48.6 ± 4.9 (n = 8/N = 3); \( K^4D^{-/-} \): 46.6 ± 2.5 (n = 7/N = 2) (P > 0.1 for each, t-test), mean ± s.e.m. 20 mM BAPTA containing Cs\(^+\)-internal solution was used for this experiment due to prolonged kinetics of EPSCs in \( K^4D^{-/-} \). The identical paired-pulse ratios between wild type and \( K^4D^{-/-} \) are consistent with the conclusion that presynaptic release probability is intact at P8–13 retinogeniculate synapses in \( K^4D^{-/-} \) mice. (See also Extended Data Fig. 1e–h for similar conclusion at P20–24, after synapse elimination is largely complete.)
Extended Data Figure 7 Normal NMDA/AMPA ratio but increased Ca²⁺-permeable AMPA receptors at retinogeniculate synapses in $K^+/D^-$ mice. a, b, NMDA/AMPA ratio is unchanged in $K^+/D^-$ mice. a, NMDA/AMPA ratio (%): peak $I_{\text{AMPA}}$ measured at $-70 \text{ mV}$ (20 μM SR95531) versus peak $I_{\text{NMDA}}$ at $+40 \text{ mV}$ (20 μM SR95531 + 20 μM DNQX): wild type: $61 \pm 6.8$ (n = 10/N = 4); $K^+/D^-$: $70.6 \pm 12.1$ (n = 7/N = 3) (P > 0.1, t-test) mean ± s.e.m. b, Example recordings from individual neurons for wild type (left) and $K^+/D^-$ (right). APV (100 μM) was added at the end of each experiment to confirm NMDA-mediated synaptic currents. D600 in pipette.

c, Example showing effect of NASPM (100 μM bath) on $I_{\text{AMPA}}$: note significant blockade of $I_{\text{AMPA}}$ in $K^+/D^-$. Grey line, before NASPM; black line, after NASPM (5 traces averaged for single cell). SR95531 (20 μM) added to bath for a–c. d, Examples for $I_{\text{AMPA}}$ normalized to EPSC amplitude at $-40 \text{ mV}$. Note reduction in EPSC amplitude at $+40 \text{ mV}$ in $K^+/D^-$ but not wild type. 100 μM APV + 20 μM SR95531 in bath. Spermine (100 μM) and D600 (100 μM) in pipette. Ages: P8–13. Experimenter was aware of genotype due to obvious differences in time course of EPSCs and effects of NASPM.

e, f, Example western blot (left) and GluR1/GluR2 ratio (right) of P22 thalamus; wild type: 1.0 ± 0.1 (N = 12); $K^+/D^-$: 1.3 ± 0.1 (N = 13) (P = 0.07), f, Example western blot (left) and GluR1/GluR2 ratio (right) of cultured cortical neurons; wild type: 1.0 ± 0.1 (N = 4); $K^+/D^-$: 2.3 ± 0.7 (N = 4) (*P = 0.03). Mann–Whitney U-test for e, f, n = cells/N = animals.
Extended Data Figure 8 | NASPM-dependent rescue of LTD in K<sup>δ</sup>D<sup>−/−</sup> LGN at P8–13. a, Summary of all 1,100 ms latency experiments: EPSC peak amplitude (% change from baseline) versus time (n = 7/N = 7 for 0 μM NASPM; n = 5/N = 5 for 20 μM NASPM). Grey bar = induction period (Methods). 1 min data binning. b, Average of per cent change (mean ± s.e.m.); K<sup>δ</sup>D<sup>−/−</sup> 0 μM NASPM: 105 ± 8.2 (N = 7); K<sup>δ</sup>D<sup>−/−</sup> + 20 μM NASPM: 72.5 ± 2.2 (N = 5). **P < 0.01, t-test, n = cells/N = animals.
Extended Data Figure 9 | Neuronal H2-Dβ expression decreases Ca2+ permeability of AMPA receptors at retinogeniculate synapses in KβDβ−/−:NSED−/− mice. a, NASPM blockade of I_{AMPA} is significantly reduced in KβDβ−/−:NSED+/+ mice. Example trace of NASPM effect on I_{AMPA} recorded from KβDβ−/−:NSED−/− (left) or KβDβ−/−:NSED+/+ (right) individual LGN neuron. Grey line, before NASPM; black line, after NASPM application (5 traces averaged for single cell); 20 μM SR95531 in bath. b, Internal spermine-dependent block of I_{AMPA} at positive membrane potentials is rescued in KβDβ−/−:NSED−/− LGN neurons. Example recordings for I_{AMPA} normalized to EPSC amplitude at −40 mV from individual neurons. Note reduction in EPSC amplitude at +40 mV in KβDβ−/−:NSED−/−, but restored to wild-type level in KβDβ−/−:NSED+/+. 100 μM APV + 20 μM SR95531 in bath. Spermine (100 μM) and D600 (100 μM) in internal solution.