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Materials and Methods
Figs. S1 to S4
References

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Specific $GABA_A$ Circuits for Visual Cortical Plasticity

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Weak inhibition within visual cortex early in life prevents experience-dependent plasticity. Loss of responsiveness to an eye deprived of vision can be initiated prematurely by enhancing γ-aminobutyric acid (GABA)–mediated transmission with benzodiazepines. Here, we use a mouse "knockin" mutation to α subunits that renders individual GABA type A ($GABA_A$) receptors insensitive to diazepam to show that a particular inhibitory network controls expression of the critical period. Only α1-containing circuits were found to drive cortical plasticity, whereas α2-enriched connections separately regulated neuronal firing. This dissociation carries implications for models of brain development and the safe design of benzodiazepines for use in infants.

Experience-dependent plasticity shapes the early postnatal brain, as exemplified by the loss of responsiveness to an eye briefly deprived of vision during a critical period, which results in severe amblyopia (poor visual acuity) (*1*). This behavioral sensitivity is reflected in the neuronal firing of single units in the primary visual cortex of mammals, including mice (*2*, *3*). Critical-period onset can be delayed indefinitely if release of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) is kept low by gene-targeted disruption of the synaptic isoform of its synthetic enzyme, glutamic acid decarboxylase 65 (GAD65) (*4*). Conversely, the natural plasticity profile is accelerated by prematurely enhancing inhibition (*4*, *5*).

It remains unclear, however, whether overall inhibitory tone or a specific network controls critical-period onset. The diversity of GABA cells in the neocortex complicates local circuit analysis. Although neuronal morphology and biochemistry are heterogeneous, synaptic connections are precisely targeted (*6*). We have used the infusion of benzodiazepine agonists concurrent with monocular deprivation (MD) to prematurely trigger ocular dominance plasticity (Fig. 1A). These drugs enhance in a use-dependent manner specific GABA type A ($GABA_A$) receptor–mediated currents whose benzodiazepine sensitivity is determined by a particular α-subunit complement (*7*, *8*). This allows us to analyze here whether particular GABA circuits underlie visual cortical plasticity.

We first attempted critical-period acceleration in wild-type (C57BL/6) mice, using the subtype-selective benzodiazepine receptor agonist zolpidem (*9*). No plasticity typically occurs after a 4-day period of MD just after eye opening (Fig. 1, A and B). Instead, a strong ocular dominance shift in favor of the open eye was induced in the presence of zolpidem (Fig. 1) (χ^2 test, $P < 0.0001$ versus vehicle), as observed previously for the broad spectrum agonist diazepam (DZ) (*4*). Receptors sensitive to zolpidem include α1, α2, and α3 subunits, while the α5 subtype is less sensitive by a factor of 10,000 (*9*). The ability to shift critical-period onset with this drug at low concentration (~ 0.1 to 1 μM in cortex upon diffusion) (*10*, *11*), therefore, indicates little role for α5-containing receptors in triggering visual cortical plasticity.

Of nearly 20 identified $GABA_A$ receptor subunits (*7*), just four (α1, α2, α3, and α5), together with an obligatory γ2 subunit, contribute critical amino acid residues to the benzodiazepine binding site. Mutation of a histidine (H) to an arginine (R) renders individual GABA receptors insensitive to DZ, as occurs naturally for the α4 or α6 subtypes (*8*). Selective targeting of the homologous site in each of the α subunits produces specific impairments of the sedative, anxiolytic, and motor behavioral effects of DZ (*12*–*14*). We tested the DZ-induced critical-period acceleration paradigm in the three separate α1(H101R), α2(H101R), and α3(H126R) knockin mouse lines described previously.

Of the neocortical inhibitory interneurons, chandelier cells represent a unique example of synapse specificity, forming the fundamental source of input onto pyramidal cell axon initial segments (*6*), where $GABA_A$ receptor α2 subunits are preferentially localized (*15*–*17*). In both GAD65 knockout and pre–critical period wild-type mice, where plasticity fails to occur, single units in the primary visual cortex fire excess spikes that outlast the visual stimulus (*4*, *10*). Because axo-axonic contacts are ideally situated to regulate such a prolonged discharge phenotype, we first examined whether this particular GABA subcircuit may directly underlie visual cortical plasticity. Brief MD just after eye opening in α2(H101R) knockin mice still produced premature ocular dominance shifts when combined with DZ but not with vehicle injections (Fig. 2, A and B) (χ^2 test, $P <$ 0.0001 versus vehicle).

In contrast, prolonged discharge was not corrected by benzodiazepine treatment in α2(H101R) mutant mice. The other two α3 and α1 knockin lines, as well as zolpidem treatment of wild-type mice, exhibited a normal regulation of this spiking phenotype in vivo (Fig. 2C). Thus, the disruption of neural coding in and of itself does not predict whether ocular dominance shifts will occur. Although α2-subunit-enriched (e.g., chandelier cell "cartridge") synapses may control prolonged discharge, other GABAergic connections must drive visual cortical plasticity.

We, therefore, turned our attention to α1-containing circuits. The expression of the α1 subunit in primary visual cortex is

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regulated by age and visual experience in a manner that is correlated with the critical period in kittens (*18*) and mice (*19*). Indeed, brief MD failed to produce ocular dominance shifts in pre–critical period α1(H101R) mutant mice even in the presence of DZ (Fig. 3, A and B) (χ^2 test, $P > 0.9$ versus vehicle). In contrast, allowing these animals to grow up to the peak of their natural critical period (about postnatal day P25) revealed a robust plastic response to MD without any drug treatment (Fig. 3C) (χ^2 test, $P < 0.001$ versus no MD). Thus, the relevant GABAergic transmission develops normally (*8*), and plasticity machinery is intact despite the mutation in the α1 subunit. Plasticity in response to DZ in pre–critical period α3(H126R) mutant mice was similarly unaltered despite the high expression of this receptor subunit early in life (Fig. 4D) (*20*). Of the four α subunits that bestow benzodiazepine sensitivity to the $GABA_A$ receptor (*7*, *8*), only the α1 subunit dictated whether shifts of contralateral eye bias could be produced prematurely with DZ (Fig. 3D).

Total deletion of the α1 subunit by standard gene knockout (*21*) causes massive compensatory changes in other $GABA_A$ receptor subunits that are not seen in the α1(H101R) knockin line (*8*, *12*, *14*). Extensive immunohistochemical analysis revealed no changes in laminar or subcellular targeting of individual α1, α2, or α3 subunits in cortex (Fig. 4 and figs. S2 to S5) (*12–14*). The α5(H105R) mutation was not studied here because of zolpidem efficacy in wild-type animals (Fig. 1) and potential changes in expression as reported in the hippocampus (*22*). Notably, α2-subunit clusters were observed at normal density surrounding axon initial segments, even in α2(H101R) mice that failed to reduce prolonged discharge in response to DZ while plasticity was still triggered (fig. S5). The double dissociation of proper spike regulation without plasticity in α1(H101R) mice compels a reconsideration of the interrelationship between neural coding and visual cortical plasticity (*23*).

Our results are harmonious with recent spike-timing–dependent models of synaptic refinement (*24*). Poor regulation of action potential discharge at the axonal initiation site (e.g., chandelier cells) would not affect these models of plasticity in the dendritic arbor as long as the fidelity of back-propagation through the soma is enforced (e.g., basket cells) (*25*). While behavioral effects of DZ can be attributed to regional expression differences of α1, α2, and α3 subunits in cortex, limbic areas, and medullary brainstem, respectively (*14*), the rescue of visual plasticity occurs locally within neocortical circuits (*10*). $GABA_A$ receptors are not found on thalamic afferent terminals (*26*), but α subunits are localized to distinct postsynaptic sites on pyramidal cells as reported for hippocampus (*6*, *15–17*).

$GABA_A$ receptor–α1 subunits are preferentially enriched at somatic synapses receiving input from parvalbumin (PV)–positive large basket-cell terminals (*16*). Predominantly α2 receptors are instead sorted to other somatic contacts (e.g., cholecystokinin-positive). Maturation of PV-positive interneurons is correlated with critical-period expression (*5*). Impaired ocular dominance plasticity by gene-targeted removal of a unique potassium channel ($K_v3.1$) contributing to PV-cell fast-spiking behavior directly mimics the GAD65 knockout mouse phenotype in a cell-type–specific manner (*27*). Large basket cells in particular extend a wide, horizontal axonal

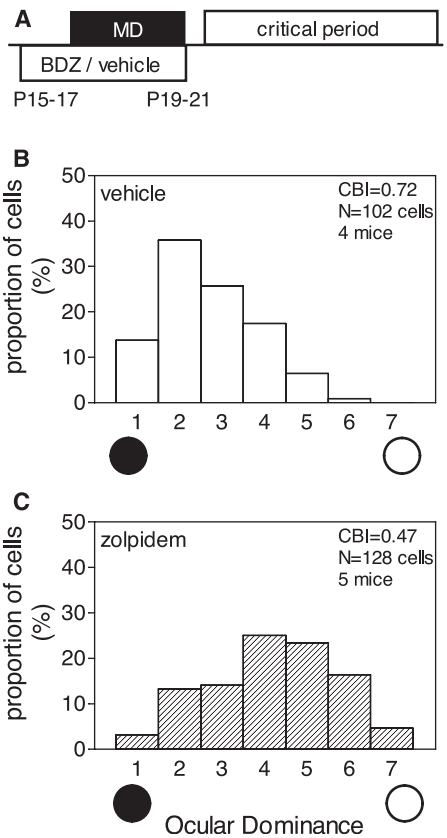

Fig. 1. A benzodiazepine (BDZ) receptor subset triggers visual cortical plasticity. (**A**) Critical-period acceleration paradigm. Monocular deprivation (MD) just after eye opening (P15 to P17) for 4 days (pre–critical period) typically yields no amblyopia or change of single-unit responses in primary visual cortex (*2*, *3*). Plasticity can be triggered prematurely by concomitant administration of BDZ agonists (*4*). (**B**) Vehicle treatment does not affect the typical contralateral eye bias of rodent visual cortex, which is resistant to MD in pre–critical period wild-type animals. Individual cell discharge was assigned an ocular dominance score from group 1 (purely contralateral) to group 7 (ipsilateral). The Contralateral Bias Index (CBI) in the upper right corner is a weighted average of the total distribution ranging from 0 to 1 for complete ipsilateral or contralateral eye dominance, respectively (*11*). Number of animals and cells as indicated. (**C**) Robust premature shift of responsiveness (and decrease in CBI) by zolpidem injection (100 μM, intracerebroventricularly) concurrent with MD mimics the effect of the broad-spectrum BDZ agonist diazepam (*4*). At this dose (~0.1 to 1 μM), $GABA_A$ α5 subunits are not engaged within visual cortex, indicating a role for α1-, α2-, or α3-containing receptors (*9*). Mean CBI ± SEM = 0.47 ± 0.04 versus 0.72 ± 0.02; five zolpidem and four vehicle-treated mice, respectively; $P < 0.003$, *t* test.

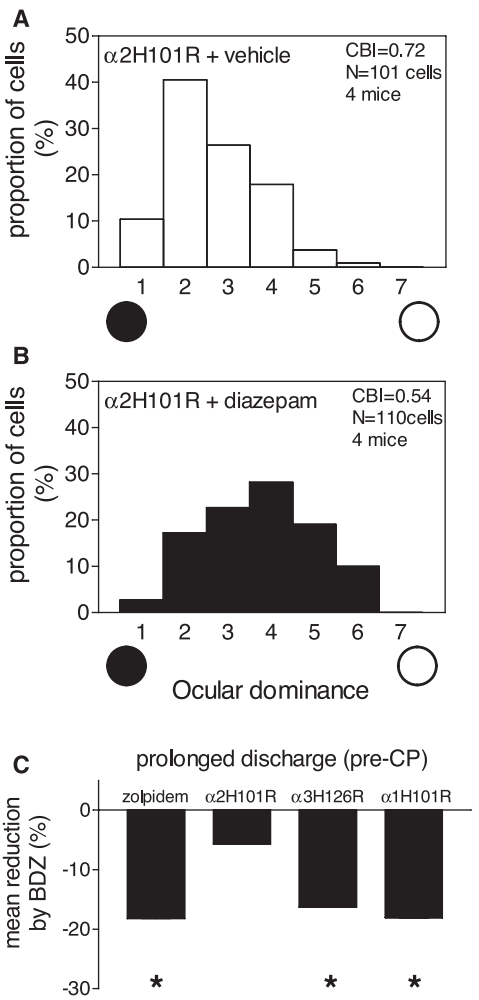

Fig. 2. $GABA_A$ α2 subunits regulate neural spiking but do not trigger visual cortical plasticity. (**A**) Vehicle treatment of α2(H101R) knockin mice shows typical lack of MD response in pre–critical period animals. (**B**) Diazepam remains effective in producing ocular dominance shifts in young α2(H101R) mice. (**C**) Prolonged discharge characteristic of immature visual cortex (*4*, *10*) is significantly reduced by zolpidem in C57BL/6 mice or by diazepam in α3(H126R) and α1(H101R) mutants, but not in α2(H101R) mutants. *, $P < 0.05$, *t* test versus respective vehicle-treated mutant.

arbor that can span ocular dominance columns in cat visual cortex (*28*). Moreover, coupled networks of PV-positive cells offer a system exquisitely sensitive to timing that could detect and pass along synchronized signals in a columnar manner (*29*). Indeed, by discriminating input coming from the two eyes, long-range inhibitory modulation sculpts the spacing of nascent ocular dominance columns in developing cat visual cortex (*30*).

Our results present a cellular basis (fast-spiking large basket cells) for critical-period plasticity triggered by inhibition in the visual cortex and explain the benzodiazepine side effect of premature plasticity in the developing brain (*4*). The special function of neocortical $GABA_A$ receptor α1 subunits suggests constraints on drugs designated for use in human infants.

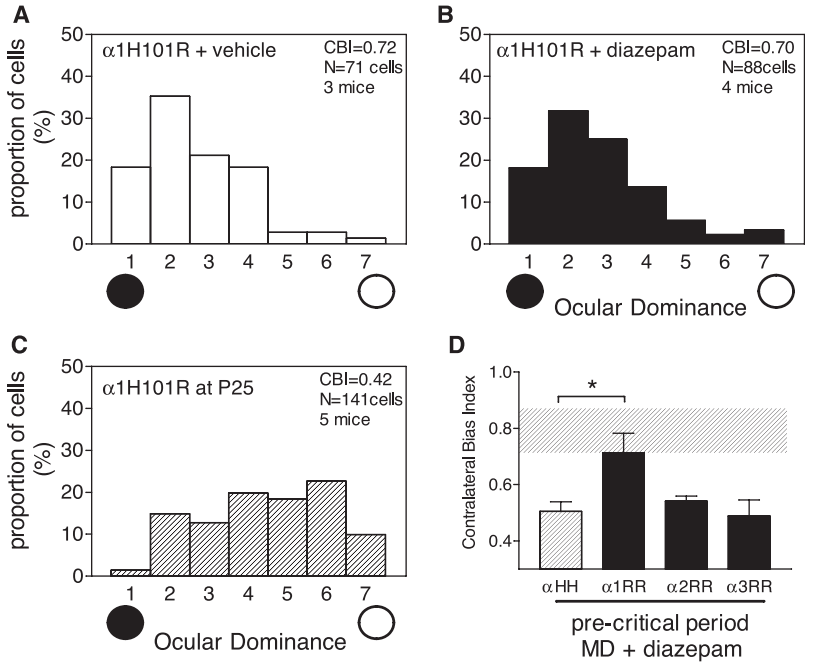

Fig. 3. $GABA_A$ α1-containing receptors specifically drive visual cortical plasticity. (**A**) Vehicle treatment of pre–critical period α1(H101R) mice yields no plasticity in response to MD. (**B**) Plasticity fails to be induced by MD with DZ injection of pre–critical period α1(H101R) mice. (**C**) Robust ocular dominance shifts are observed in α1(H101R) mice even without DZ during the usual peak of the critical period (4-day MD starting at P25). (**D**) Only α1 knockin mice show no significant reduction of CBI from the nondeprived range (shaded) by conjoint MD and DZ treatment in pre–critical period animals. αHH, control mice ($N = 5$); α1RR, α1(H101R) homozygotes ($N = 4$ mice); α2RR, α2(H101R) homozygotes ($N = 4$ mice); α3RR, α3(H126R) homozygotes ($N = 3$ mice). *, $P < 0.05$, *t* test.

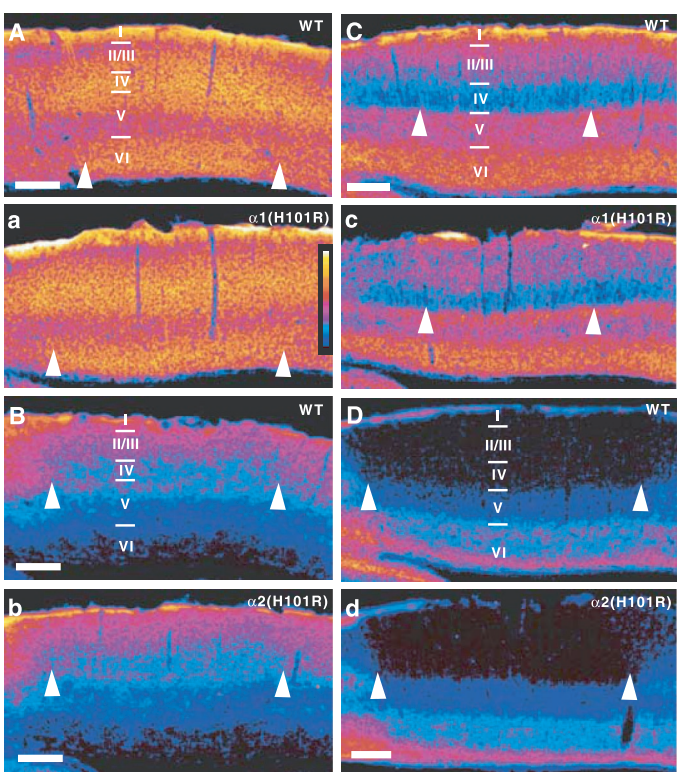

Fig. 4. Normal localization of $GABA_A$ α subunits in visual cortex (V1) of α knockin mice. Regional and laminar distribution of the α1, α2, α3, and α5 subunits, respectively, in occipital cortex of wild-type mice (**A** to **D**) is unchanged in the point mutants (**a** to **d**) and delineates the medial and lateral boundaries of V1 by abrupt changes in staining intensity in layers III, IV, and VI (arrowheads), as seen in color-coded images from immunoperoxidase staining (*11*, *17*). (See figs. S2 to S5 for higher magnification.) Scale, 100 μm.

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Figs. S1 to S5
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