Inhibitory threshold for critical-period activation in primary visual cortex

Michela Fagioli & Takao K. Hensch

Laboratory for Neuronal Circuit Development, Brain Science Institute RIKEN, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan

Neuronal circuits across several systems display remarkable plasticity to sensory input during postnatal development1–10. Experience-dependent refinements are often restricted to well-defined critical periods in early life, but how these are established remains mostly unknown. A representative example is the loss of responsiveness in neocortex to an eye deprived of vision11–14. Here we show that the potential for plasticity is retained throughout life until an inhibitory threshold is attained. In mice of all ages lacking an isof orm of GABA (γ-aminobutyric acid) synthetic enzyme (GAD65), as well as in immature wild-type animals before the onset of their natural critical period, benzodiazepines selectively reduced a prolonged discharge phenotype to unmask plasticity. Enhancing GABA-mediated transmission early in life rendered mutant animals insensitive to monocular deprivation as adults, similar to normal wild-type mice. Short-term presynaptic dynamics reflected a synaptic reorganization in GAD65 knockout mice after chronic diazepam treatment. A threshold level of inhibition within the visual cortex may thus trigger, once in life, an experience-dependent critical period for circuit consolidation, which may otherwise lie dormant.

The term ‘critical period’ refers to a cascade of functional and anatomical events in the brain, which ultimately consolidate synaptic connections into their final wiring patterns. Once activated within visual cortex, this machinery bestows a transient sensitivity to brief monocular deprivation, which is very low just after eye opening, peaks around four weeks, and rapidly declines over the next days (rats; mice, Fig. 1b) or weeks (cat; monkey; human). The critical period, however, is not a simple age-dependent maturational process, but is rather a series of events itself controlled in a
use-dependent manner. For example, rearing in the dark from birth delays the critical period, leaving the cortex in an immature state that can be altered by sensory perturbations even into adulthood\cite{11,12}. These results suggest that the intrinsic critical-period machinery may lie dormant until it is run down after the onset of visual experience; yet, the endogenous mechanism that triggers transient sensitivity to sensory perturbation remains unknown.

One possibility is that excitatory and inhibitory circuit elements reach an optimal balance once in life during which plasticity may occur. We have identified an animal model that does not respond to a brief monocular deprivation during the typical plastic period in wild-type mice\cite{13}. Moreover, at this age the slight reduction of GABA release in these mice lacking the synaptic isoform of the GABA\textsubscript{A} synthetic enzyme glutamic acid decarboxylase (GAD65 knockout) could be entirely compensated with benzodiazepine agonists. Such drugs enhance postsynaptic responses at active inhibitory synapses\cite{14} and restore ocular dominance plasticity\cite{15}. We considered whether these same mice could be made to exhibit plasticity at any age, or only during the so-called critical period. For example, a four-day period of monocular occlusion was initiated in adult GAD65 knockout mice receiving diazepam or vehicle infusion directly into one visual cortex through an osmotic minipump. Responsiveness shifted significantly in favour of the open eye only when diazepam was present during the deprivation (Fig. 1a). This plasticity did not differ from that observed previously at postnatal day, P28 (Fig. 1c; ref. 13).

This is the first example, to our knowledge, of an animal model raised with normal visual experience that retains the full potential for ocular dominance plasticity into adulthood. As in other species\cite{2±4,16}, mice express a transient sensitivity to monocular deprivation only early in life\cite{5}. When tested with four-day deprivations (Fig. 1b, left), plasticity is observed beginning one-week after eye-opening (P14) for about 10 days (P23–33). Long-term deprivations (LTMD, 15 days) similarly produced robust shifts in ocular dominance only when they spanned this same 10-day period (Fig. 1b, right). In contrast, GAD65 knockout mice, which do not normally respond to brief monocular deprivation on their own\cite{2,17}, could be made to exhibit this plasticity before, during and after the wild-type critical period when treated with diazepam (Fig. 1c, left). Similarly, LTMD produced significant shifts in ocular dominance in GAD65 knockout animals as old as four months (Fig. 1c, right). The fact that only an extreme imbalance of input (LTMD) or monocular tetrodotoxin injections as shown previously\cite{13}) could induce plasticity without diazepam in GAD65 knockout mice strongly supports the idea that intracortical inhibition in these mutants is perpetually at a threshold for expressing plasticity.

During brief monocular deprivation, subtle pharmacological enhancement of the open probability and channel conductance of
the GABA<sub>A</sub> receptor with benzodiazepine agonists<sup>14</sup> is sufficient to express ocular dominance plasticity. Indeed, saturating doses of diazepam only modestly decrease the integrated local field potential response in both GAD<sub>65</sub> knockout and wild-type mice to a similar extent (Fig. 2a). Basic receptive field properties, such as size, retinotopy, response strength, habituation, spontaneous activity, orientation or direction preference were unaffected by GAD<sub>65</sub> deletion, as reported previously<sup>11</sup>. We further examined the development of these features in mice to determine which might be linked to inhibitory mechanisms in accord with ocular dominance plasticity. For example, the number of cells exhibiting an orientation bias matured steadily after eye opening but was insensitive to slight alterations of inhibition by either GAD<sub>65</sub> deletion or diazepam infusion (Fig. 2b). These properties are similarly unaffected by diazepam in kitten visual cortex (T.K.H. and M.P. Stryker, unpublished data).

Instead, a prolonged discharge of neurons upon visual stimulation, first identified at P28 in GAD<sub>65</sub> mutants<sup>13</sup>, was found throughout life in these animals (Fig. 2c, right). A similar hyperexcitability as light-bar stimuli exited the cell’s receptive field was also prominent in young wild-type mice but dropped off sharply during the critical period for plasticity (Fig. 2c, left). As expected from the reduction of integrated synaptic field potentials (Fig. 2a) that underlie spike generation, prolonged discharge whenever present was significantly reduced by chronic diazepam infusion in vivo. Thus, ocular dominance plasticity and prolonged discharge appear to be tightly co-regulated by inhibition independent from orientation selectivity. However, we cannot rule out the possibility of subtle, higher order inhibitory effects (for example, cross-orientation), which have not yet been described in rodent visual cortex. Intracortical inhibition is generally believed to mature around the end of the first postnatal month<sup>15–16</sup>. Only a particular subcircuitry may be involved, if at all<sup>17,18</sup>, in determining individual receptive field properties.

If the presence of prolonged discharge indicates a potential for plasticity, then it should be possible to unmask ocular dominance plasticity in wild-type mice before the natural critical period, but not after (Fig. 2c, left). Diazepam or vehicle solution was infused one day before and throughout a four-day period of monocular eyelid suture in young (P15–20; Fig. 3a) or adult (> P45; Fig. 3b) wild-type mice. Only young animals treated with diazepam responded to monocular deprivation. Premature enhancement of inhibition, therefore, revealed the presence of a molecular machinery for plasticity which became unavailable for use in adults. These results strongly suggest that a certain level of intracortical inhibition is the endogenous trigger for activating the cascade of events underlying synapse consolidation in the critical period.

To test this idea, we attempted to restore a critical period to the GAD<sub>65</sub> knockout mouse. We mimicked a 10-day period of sensitivity to monocular deprivation by intracranial diazepam injection, then assayed for plasticity later in life (Fig. 4a, left). In wild-type mice, LTMD after the critical period had little or no effect on ocular dominance (Fig. 4a, right; Fig. 1b, right). Similarly, adult GAD<sub>65</sub> knockout mice that had previously been exposed to diazepam no longer exhibited plasticity. This was clearly distinct from vehicle-treated (Fig. 4b) or untreated mutants (Fig. 1c, right). The typical contralateral bias of ocular dominance was unaffected by prolonged diazepam treatment alone (contralateral bias index (CBI) = 0.72 ± 0.02 for 5 mice before, and 0.73 ± 0.01 for 3 mice after infusion). Instead, ocular dominance would have shifted had one eye been deprived concurrently, as already shown (Fig. 1c, ref. 13). Thus, a transient, early elevation of inhibition occluded future plasticity in GAD<sub>65</sub> knockout mice, apparently re-introducing a unique window of sensitivity to sensory experience.

The specific cellular events that are triggered to actually consolidate circuit connectivity during the critical period are still unclear. We have previously shown that synaptic mechanisms of long-term potentiation (LTP) or depression (LTD) are intact in GAD<sub>65</sub> knockout mice, despite their immunity to brief monocular deprivation<sup>3</sup>. Moreover, enhancing inhibitory transmission impairs LTP and LTD<sup>19–21</sup>, whereas the opposite is true of experience-dependent plasticity in vivo<sup>13</sup>. Short-term synaptic dynamics have been proposed to better reflect cortical reorganization due to sensory experience<sup>22</sup>. To determine whether bursts, rather than single impulses (Fig. 2a), are processed differently in GAD<sub>65</sub> mutants in vitro as in vivo (Fig. 2c), we recorded the synaptic response to brief trains of stimuli along vertical pathways onto layer 2/3 neurons. Short-term depression (STD) typical of these connections was significantly reduced during 5 and 10 Hz trains in GAD<sub>65</sub> knockout mice but was restored to wild-type levels by prolonged diazepam treatment in vivo (Fig. 4c). Acute bath application of diazepam did not alter the magnitude of this presynaptic depression (97 ± 2% and 98 ± 4% of pre-diazepam values at 5Hz, 6 wild-type and 4 knockout slices, respectively). Thus, chronic restoration of inhibitory–excitatory balance to GAD<sub>65</sub> knockout...
mice may promote proper maturation of short-term synaptic dynamics, which could serve as a ‘readout’ of critical-period activation.

Vision is thought to initiate an irreversible developmental process of transient sensitivity to monocular deprivation\(^5\)-\(^1\). We have identified an animal model in which this program fails to be executed despite normal visual input. A particular inhibitory–excitatory balance within cortex may trigger the machinery for synapse consolidation, which would otherwise lie dormant. Through subtle genetic or pharmacological modulation of inhibition, it was possible to alter timing of this critical period, but it could only be initiated once in life. Thus, usage of plasticity mechanisms itself sows the seeds for terminating sensitivity to sensory perturbation, making reorganization progressively more difficult once large-scale organization of connections takes place\(^2\). Only modest plasticity can be re-induced in adults\(^2\)-\(^4\) by artificially tapping into processes downstream of the local circuit interactions that endogenously trigger them. Future work must elaborate which homeostatic events\(^2\) are activated to set into place circuits that are no longer malleable later in life. Understanding the developmental regulation of spatio-temporal synaptic dynamics and prolonged discharge properties within visual cortex may yield deeper insight into what underlies synapse consolidation during the critical period.

Methods

Visual cortex electrophysiology in vivo

Mice carrying a functional disruption of GAD65 were generated by the insertion of a neomycin-resistance cassette into exon 1 of the gene on the C57Bl/6 background, as described\(^2\). Electro-physiological recordings were performed under Nembutal (50 mg kg\(^{-1}\)), Abbot/chlorpromethine (0.2 mg: Sigma) anaesthesia using standard techniques\(^2\)-\(^4\). For each animal, 5 to 8 single units (> 75 µm apart) were recorded in each of 4 to 6 vertical penetrations spaced evenly (> 200 µm intervals) across the medio-lateral extent of primary visual cortex to map the monocular and binocular zones and avoid sampling bias. Cells were assigned ocular dominance scores using a 7-point classification scheme\(^5\). For each binocular zone, a CBI was calculated according to the formula CBI = \(\frac{(n_r - n_l)}{n_r + n_l} + \frac{2}{3}(n_r - n_l)/(1/3)n_r + 2n_l\), where \(n_r\) and \(n_l\) = number of cells of ocular dominance score equal to \(r\) and \(l\), respectively. This weighted average of the bias toward one eye or the other takes values from 0 to 1 for complete ipsilateral or contralateral eye dominance, respectively.

Monocular deprivation and drug infusion

In monocular deprivation experiments, eyelid margins were trimmed and sutured under halothane anaesthesia for 4 (brief deprivation) or 15 days (long-term deprivation). All recordings were obtained contralateral to the deprived eye and blind to drug treatment. Local rescue experiments were carried out in adult mice with low-flow osmotic mini-pumps (0.5 µl h\(^{-1}\); Alzet 1007, Alza) containing diazepam (DZ: 2mg ml\(^{-1}\); Wako) or vehicle solution (50% propylene glycol; Wako) connected to cannulae (30 ga.) implanted stereotaxically into one hemisphere under sterile surgical conditions 1 or 2 days before eyelid suture\(^1\). In small young mice, drug or vehicle solutions were injected daily into both lateral ventricles (1.5 µl per hemisphere), starting one day before deprivation until the day before recording, or to control timing over a 10-day period to restore a ‘critical period’. Intraventricular injections yield similar results as direct cortical mini-pump infusion\(^1\), were completed within 5 min under halothane anaesthesia, and put little stress upon the animals.

Visual cortex electrophysiology in vitro

Coronal slices (350 µm thick) of mouse visual cortex were cut blind to genotype and incubated (32–33°C) in equilibrated (95% O\(_2\)-5% CO\(_2\), pH 7.2) artificial cerebral spinal fluid (ACSF) as described\(^6\). The ACSF contained (in millimolar): 119 NaCl, 2.5 KCl, 1.3 MgSO\(_4\), 10 NaH\(_2\)PO\(_4\), 26.2 NaHCO\(_3\), 2.5 CaCl\(_2\) and 11 glucose. Slices were transferred to a recording chamber superfused with the same ACSF and maintained at 32–33°C. Half-maximal field potential amplitudes evoked by a bipolar glass stimulating electrode filled with ACSF were recorded through a glass electrode filled with 1M NaCl (1–3 MΩ) inserted into layer 2/3 of the binocular zone. To measure diazepam sensitivity, baseline responses to white noise stimulation were recorded at a rate of 0.1 Hz for at least 15 min before applying a saturating dose of diazepam (15 µM; Wako) to the bath.

Short-term plasticity experiments

To assay short-term presynaptic dynamics, stable, baseline responses to layer 4 stimulation were recorded at a rate of 0.2 Hz for at least 5 min, then trains (30 pulses) of various frequencies were applied with more than 1-min rest interval between each. Up to six frequencies were tested randomly during an experiment, and each train was repeated five times. Baseline responses were carefully monitored throughout to ensure that no long-term changes in synaptic efficacy occurred. At the end of each experiment, non-NMDA and NMDA glutamate receptor antagonists, 10 µM CNQX (Tocris) and 50 µM D-APV (Tocris), respectively, were applied to the bath to confirm the synaptic component of the extracellular response. For analysis, maximum negative field potential amplitude was measured from the synaptic component of the raw response. Five responses before, all responses during, and five responses immediately after each stimulus train were measured and normalized to the baseline preceding each train. Steady-state depression was taken as the mean response evoked by the last 5 stimuli of the 30-pulse train. Augmentation after the completion of each train was routinely observed at the ages studied (P28–33). Chronic diazepam-treated mice received intraventricular injections as described (> 6 days) before slicing. In acute diazepam (15 µM) experiments, short-term plasticity was first measured in control ACSF then again after switching to perfusion medium containing the drug.

Received 8 November 1999; accepted 12 January 2000.

10. Crepel, F. Regulation of spatio-temporal synaptic dynamics and prolonged discharge properties within visual cortex may yield deeper insight into what underlies synaptic consolidation during the critical period.

Acknowledgements

We thank K. Hartman for contributing to Fig. 4c, N. Mataga for comments, and S. Fujishima for genotyping and maintenance of the GAD65 mouse colony re-derived from original heterozygote breeding pairs which were kindly provided by S. Baekkeskov and S.F. Kash.

Correspondence and requests for materials should be addressed to T.K.H. (e-mail: hersch@postman.riken.go.jp).