Molecular basis of plasticity in the visual cortex

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Sensory experience is known to shape the maturation of cortical circuits during development. A paradigmatic example is the effect of monocular deprivation on ocular dominance of visual cortical neurons. Although visual cortical plasticity has been widely studied since its initial discovery by Hubel and Wiesel >40 years ago, the description of the underlying molecular mechanisms has lagged behind. Several new findings are now beginning to close this gap. Recent data deepen our knowledge of the factors involved in the intercellular communication and intracellular signaling that mediate experience-dependent plasticity in the developing visual cortex. In addition, new findings suggest a role for the extracellular matrix in inhibition of ocular-dominance plasticity in the adult visual cortex.

Development of the visual cortex is strongly influenced by visual experience during short periods of postnatal development called critical periods. During these periods of heightened plasticity, experience can produce permanent and extensive modifications of cortical organization. If during the critical period one eye is deprived of patterned vision, as is the case following unilateral congenital cataract or experimental monocular deprivation, there is an irreversible reduction of visually driven activity in the visual cortex through the deprived eye, which is reflected by a dramatic shift in the ocular-dominance distribution of cortical neurons in favour of the non-deprived eye in all mammals tested [1,2]. Following monocular deprivation, visual acuity and contrast sensitivity for the deprived eye (tested either behaviourally or electrophysiologically) develop poorly (amblyopia) and there is a loss of depth perception. Similar effects can be produced by abnormal alignment of the two eyes (strabismus). The loss of depth perception has been directly related to the loss of binocular cells in the visual cortex, whereas the loss of visual acuity has been attributed both to the total decrease of neurons driven by the deprived eye and to a loss of those neurons with the smallest receptive fields [3]. It has to be said that abnormalities in the dominance of the deprived eye and in the spatial properties of visual cortical neurons alone do not explain the full range of visual deficits in amblyopia [3], and that ocular-dominance plasticity and development of vision might be based on different cellular mechanisms [4,5]. However, there seems to be a close link between critical-period duration and maturation of some visual functions: for instance, the closure of the critical period for monocular deprivation roughly coincides with completion of visual acuity development in several species, including rodents, monkeys and humans [1], suggesting that the development of visual function and the progressive reduction of ocular-dominance plasticity are two aspects of the same process – namely, the maturation of the visual cortex.

Experience shapes the development and maintenance of visual cortical circuits through activity-dependent mechanisms that seem to follow Hebb's principle, a hypothesis first put forth to explain ocular-dominance plasticity but then extended to explain experience-dependent development of other visual functions. Hebb's principle states that if electrical activity in a set of afferent fibers is temporally correlated with the activity of the postsynaptic neuron, then the afferents will be allowed to maintain and even expand the connections with it. However, if the activity is not temporally correlated, the afferent fibers will loose their hold on the postsynaptic neuron.

Plasticity in the visual cortex declines with age. Adult visual cortex still responds to experience with plastic changes, as shown by the effects of perceptual learning [6] and of retinal lesions [7], with similar Hebbian rules governing these changes as are in force during critical periods. However, the extent of plasticity is reduced in the adult with respect to the young: monocular deprivation or strabismus in adults produce no effect, and recovery from amblyopia is also very limited once the critical period is terminated.

The cellular and molecular mechanisms that control the developmental plasticity of visual cortical connections and restrict experience-dependent plasticity to short critical periods are still unclear. This article reviews recent advances in this field.

NMDA receptors

The first modifications induced by experience in visual cortical circuits are likely to be changes in synaptic efficacy. Ever since the discovery of NMDA receptors, these synaptic receptors have been implicated in experience-dependent

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plasticity. Their characteristic of being both transmitter- and voltage-dependent, and their coupling via Ca^{2+} influx to plasticity-related intracellular signalling, has led to the notion that they might be a neural implementation of Hebbian synapses.

Involvement of NMDA receptors in developmental visual cortical plasticity has been initially suggested by the observation that block of NMDA receptors blocks the effects of monocular deprivation [8]. A difficulty with pharmacological block of NMDA receptors can be that it significantly affects visually driven activity, but the use of different NMDA receptor antagonists [9] or antisense oligonucleotides to reduce expression of the NMDAR1 subunit has overcome this problem, showing that it is possible to block the effects of monocular deprivation without affecting visual responses [10] and confirming NMDA-receptor involvement in visual cortical plasticity.

NMDA receptors are developmentally regulated and their expression is modified by electrical activity. In particular, their subunit composition varies in the visual cortex, from a dominant presence of receptors containing the subunit 2B to a high presence of receptors containing the subunit 2A, with a time course paralleling that of functional visual cortical development and the critical period. Expression of the 2A subunit correlates with the progressive shortening of NMDA current. Dark rearing, which delays critical-period closure and impairs development of functional properties of the visual cortex and of visual acuity, delays the developmental shortening of NMDA-receptor currents and of subunit 2A expression, suggesting that the 2B-to-2A switch is related to visual cortical development and, possibly, to the closure of the critical period [1].

However, recent results have shown that in mice with deletion of the NMDA-receptor 2A subunit, the sensitivity to monocular deprivation is restricted to the normal critical period, thus suggesting that expression of the 2A subunit is not essential to delineate the time course of the critical period of ocular-dominance plasticity [5] and might be related to other features of visual cortical plasticity.

Neurotrophins

Several observations have suggested that neurotrophins control visual cortical plasticity during the critical period. Initially, it was shown that exogenous supply of neurotrophins in the visual cortex strongly affects the ocular-dominance plasticity induced by monocular deprivation [1,11]. In these studies, the effects of neurotrophins on ocular dominance plasticity were sometimes accompanied by alteration of other properties of visual cortical neurons, such as their pattern of discharge and orientation selectivity [12,13], possibly owing to the high concentration of exogenous neurotrophins. Other studies, which followed the opposite course of antagonizing the action of endogenous neurotrophins, have clearly shown that neurotrophins are important for normal visual cortical development and plasticity [14,15]. More recently, Huang *et al.* [16] generated a mouse overexpressing brain-derived neurotrophic factor (BDNF) in the visual cortex, maintaining a normal cellular pattern of BDNF expression and release. In this mouse, BDNF overexpression accelerates both the development of visual acuity and the time course of ocular dominance and synaptic plasticity, thus supporting a crucial role for neurotrophins in visual cortical development and plasticity.

What are the mechanisms of action of neurotrophins in controlling experience-dependent visual cortical plasticity? Neurotrophin production and release depend on electrical activity and, in particular, depend on visual activity [11]. In turn, neurotrophins can modulate electrical activity and synaptic transmission at both presynaptic and postsynaptic levels [17,18]. They can have both fast actions, for instance by increasing transmitter release [19,20] or by directly depolarizing neurons [21], and slow actions, by modulating gene expression [18] (Fig. 1a,b). BDNF also enhances visual cortical synaptic plasticity [11]. This reciprocal regulation between neurotrophins and neural activity might provide a means by which active neuronal connections are selectively strengthened. Indeed, neurotrophins seem to require the presence of electrical activity to exert their actions [11,19,22].

Recently, Konnerth and colleagues have demonstrated that the coincidence between weak synaptic activity and localized BDNF application, which by themselves do not lead to long lasting changes in synaptic efficacy, induces long-lasting potentiation of synaptic transmission, suggesting that neurotrophins operate in synergy with electrical activity in promoting synaptic plasticity [23]. It is interesting to note that, although BDNF can promote the phosphorylation of the transcription factor cAMP-response-element-binding protein (CREB) (Fig. 2), it evokes only weak CREB-mediated gene expression unless it is coupled with electrical activity [24].

Several studies on neurotrophin-receptor expression and on the effects of neurotrophins on visual cortical neurons or afferents to the visual cortex have indicated that different neurotrophins act on different neuronal targets [11]. Therefore, the synergy between neurotrophins and activity has to be considered to be specific for each neurotrophin and the neuronal populations that are its targets. The possible sites of action of neurotrophins in visual cortical plasticity are illustrated in Fig. 1c.

A strong link between BDNF and intracortical inhibition has been recently suggested by the finding that development of intracortical GABA-mediated inhibition is accelerated in BDNF-overexpressing mice [16], suggesting that BDNF controls the time course of the critical period by accelerating the maturation of GABA-mediated inhibition (Box 1).

A final consideration is necessary. The local supply of neurotrophins has been proposed as possible therapy for neurological and neurodegenerative diseases. The clear demonstration that neurotrophins so strongly affect cortical plasticity and can disrupt activity of cortical neurons warns that their supply could elicit as yet unpredictable side effects, as has been recently pointed out by Thoenen [25].

Intracortical inhibition

Recently, it has become clear that inhibition not only is a 'brake' for excitation but also has an important role in sculpting the pattern of electrical activity. This action

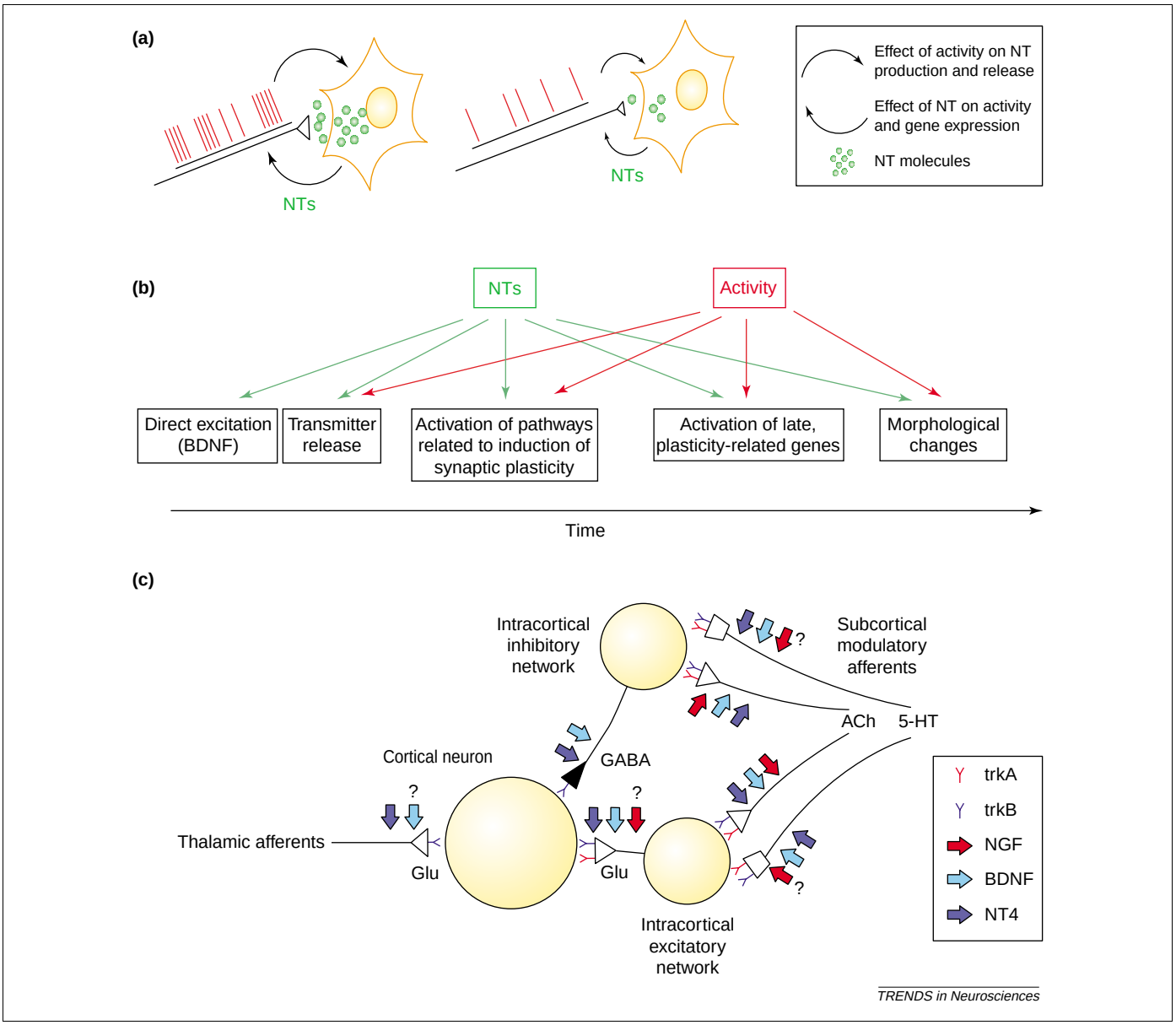

Fig. 1. Neurotrophin action in visual cortical plasticity. (a) Production and release of neurotrophins (NTs) is under the control of electrical activity (red bars representing action potentials): a more active afferent (left) would activate more effectively the postsynaptic neuron, therefore evoking a stronger release of neurotrophins. The reverse would be true for less active afferents (right). Released neurotrophins exert then their actions on the presynaptic neuron, in synergy with activity. The specificity of neurotrophin action is determined by the fact that the released neurotrophins exert their actions only on neurons that are active. The less active neuron not only evokes a smaller release of neurotrophins but also has a weaker action exerted on it by the released neurotrophins. Neurotrophins can also be released by the presynaptic neuron and act on the postsynaptic neuron, again in synergy with activity. (b) Schematic time scale of neurotrophin actions. No distinction is made between presynaptic or postsynaptic sites of action. The scheme suggests that neurotrophins and activity act in synergy in producing several effects, some of which are very fast and some of which are slower. Direct excitation of the postsynaptic neuron has been described for brain-derived neurotrophic factor (BDNF) in several types of cortical neurons. Also for BDNF, however, the induction of synaptic plasticity requires coincidence with activity. Based on Refs [1,18–23]. (c) Possible targets of neurotrophin actions in the control of visual cortical plasticity. Neurotrophins seem to play their roles by acting on different targets: each neurotrophin has a particular subset of targets among the intracortical neurons and the cortical afferents. Neurotrophin 4 (NT4) but not BDNF regulates lateral geniculate nucleus soma size [94,95]; BDNF but not nerve growth factor (NGF) promotes GABA release [96] in the visual cortex [19] and regulates neuropeptide expression in interneurons. These differential actions are accounted for by the distribution of neurotrophin receptors (trkA for NGF, and trkB for BDNF and NT4) on visual cortical neurons and on afferents to the visual cortex, although a difference in the intracellular signalling that is activated by binding of NT4 and BDNF to trkB has been postulated [97] and might explain the differences between BDNF and NT4 action. Question marks indicate action of a neurotrophin on a target that is not well established yet. Some targets are common to all neurotrophins, for instance the cholinergic (ACh) afferents from the basal forebrain; some targets are specific, such as GABAergic intracortical inhibitory neurons (targets of NT4 and BDNF), the serotonergic (5-HT) afferents from the Raphe nucleus (targets of BDNF and possibly NT4) and the glutamatergic (Glu) thalamic afferents (targets of NT4).

contributes to the detection of imbalance of activity between the afferents to a cortical neuron. A failure of the postsynaptic neuron to evaluate the timing of arrival of its synaptic inputs is bound to be a failure in plasticity.

Indeed, Hensch *et al.* have shown that inhibitory interactions are necessary for the manifestation of experience-dependent plasticity. In transgenic mice lacking the 65-kDa isoform of the GABA-synthesizing enzyme GAD (GAD65), experience-dependent plasticity in response to monocular deprivation is deficient. Normal plasticity in these animals can be rescued if GABA transmission is enhanced in the visual cortex by means of benzodiazepines [26].

Development of inhibition seems also to be a determinant of the critical period [16,27]. The results

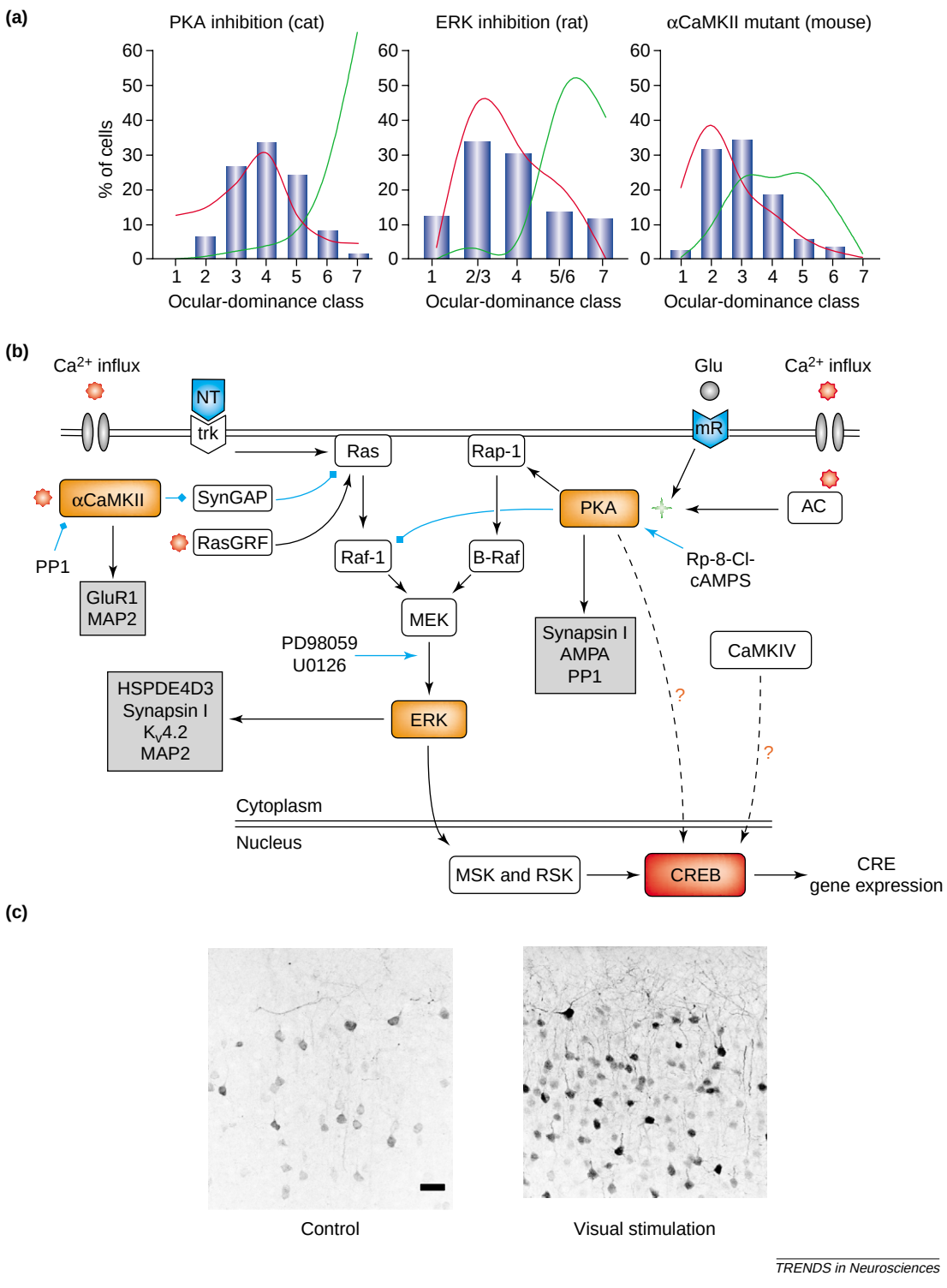

Fig. 2. Ocular-dominance plasticity and intracellular signalling. (a) Pharmacological block of cAMP-dependent protein kinase (PKA) or extracellular-signal-regulated kinase (ERK), or the genetic suppression of α Ca^{2+}/calmodulin-dependent protein kinase II (αCaMKII) autophosphorylation, blocks the ocular-dominance shift that normally follows monocular deprivation. Curves indicate the ocular-dominance distribution in normally reared animals (red) or after monocular deprivation (green). The bar diagrams show the ocular-dominance distribution after monocular deprivation and concurrent inhibition of the specified kinase. Cells in classes 7 and 1 are monocular and driven only by the ipsilateral (non-deprived) or contralateral (deprived) eye, respectively. Cells in classes 2 and 3 and classes 5 and 6 are binocular and preferentially driven by the contralateral or the ipsilateral eye, respectively. Class-4 cells are equally driven by the two eyes. (b) PKA, ERK and αCaMKII are mutually connected by a complex network of interactions. Black arrows indicate activation; blue lines indicate inhibition. Blue arrows indicate the site of action of the PKA and ERK blockers used in the experiments illustrated in (a) (Rp-8-Cl-cAMPs to block PKA, and PD98059 and U0126 to block ERK). PKA is activated in response to increased levels of cAMP (green four-pointed star) and, therefore, integrates activity of metabotropic glutamate (Glu) receptors (mR) and Ca^{2+}-dependent adenylate cyclase (AC; Ca^{2+} represented by red eight-pointed star). ERK integrates the signals carried by neurotrophins (NT) and electrical activity because its phosphorylation is regulated both by neurotrophins, through the trk–Ras pathway [98,99], and by electrical activity [100] (not shown). αCaMKII is particularly enriched in the postsynaptic density and is rapidly recruited by influx of Ca^{2+} through NMDA receptors. After phosphorylation of the autonomy site Y286, a process of autophosphorylation on this tyrosine residue maintains αCaMKII activation independently of intracellular Ca^{2+}. In this way, the transient activation produced by the coincidence detection operated by NMDA receptors is converted into a longer-lasting molecular signal. Notice how PKA can have either an excitatory or inhibitory effect on the ERK pathway, depending on the availability of the substrates Rap-1 or Raf-1 [51,101].

Box 1. Inhibitory circuitry and control of the critical period

Intracortical GABA-mediated inhibition exerts strong control on the critical period for ocular-dominance plasticity. In transgenic mice lacking the 65-kDa isoform of the GABA-synthesizing enzyme GAD (GAD65), experience-dependent plasticity in response to monocular deprivation is deficient [26]. Normal plasticity in these animals can be rescued at any age if GABA-mediated transmission is enhanced in the visual cortex by means of benzodiazepines [26,28]. Thus, if intracortical inhibition is reduced, critical-period onset is delayed, suggesting that there is an inhibitory threshold to be surpassed before the critical period can start [102]. In Fig. Ia, this is schematized by the red rightward arrow, which indicates a delayed time course of the critical period. If intracortical inhibition is precociously increased, either by early diazepam administration [28] or by overexpression of brain-derived neurotrophic factor (BDNF) [16,27], the critical-period starts earlier. The precocious development of GABA-mediated inhibition in BDNF-overexpressing mice determines also the precocious closure of the critical period (Fig. Ib), suggesting that a second inhibitory threshold that causes critical-period closure is reached during development [16,27,102]. The effects of precocious increase in inhibition are schematized by the blue leftward arrow in Fig. Ia, which indicates early onset and closure of the critical period. The accelerated closure of the critical period in BDNF-overexpressing mice is accompanied by accelerated development of visual acuity [16] (Fig. Ib), indicating that maturation of intracortical inhibition affects visual cortical development as a whole.

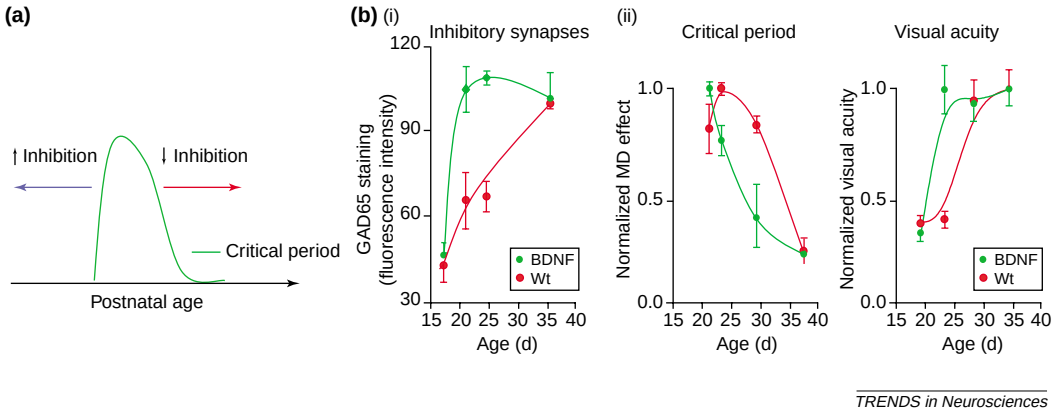

Fig. I. Relationship between intracortical inhibition and critical period in the visual cortex. (a) The effects of a decreased level (↓) or of a precocious increase (↑) of inhibition on the time course of the critical period (green line). The blue arrow indicates accelerated onset and closure; the red arrow indicates the reverse. (bi) Staining for GAD65, the synthetic enzyme for the inhibitory neurotransmitter GABA, in the visual cortex of wild-type mice (Wt) and transgenic mice overexpressing brain-derived neurotrophic factor (BDNF). GAD65 expression in the presynaptic boutons of GABAergic interneurons was quantified around the soma of the target neurons. In BDNF-overexpressing mice, maturation of GABAergic synapses is accelerated. (ii) The critical period for monocular deprivation (MD) and development of visual acuity in wild type mice and transgenic mice. Notice that the accelerated maturation of GABA-mediated inhibition caused by precocious expression of BDNF in transgenic mice determines early closure of the critical period and accelerated development of visual acuity. Using data from Ref. [16].

obtained in mice with precocious BDNF expression (Box 1) clearly show that accelerated development of GABA-mediated inhibition results in an early opening and closure of the critical period. This point is further strengthened by the work of Fagiolini and Hensch [28] showing that precocious enhancement of inhibitory tone by early administration of diazepam to the visual cortex accelerates opening of the critical period (Box 1).

Intracellular signalling of cortical plasticity

How do central neurons integrate electrical activity and neurotrophin signalling to control plasticity of cortical circuitry? A flurry of recent experiments has identified three kinases that are necessary for shift of ocular-dominance during monocular deprivation: cAMP-dependent protein kinase (PKA), extracellular-signal-regulated kinase (ERK) and α Ca^{2+}/calmodulin-dependent protein kinase II (αCaMKII) [29–31] (Fig. 2a). Each kinase is activated by a specific pattern of extracellular signals and is tightly woven within a network of mutual interactions, as detailed in Fig. 2b.

The possible targets of PKA, ERK and αCaMKII after visually driven activation are at two different levels: the cytoplasm and the nucleus. In the first case, we can envisage a local and rapid action of these kinases and that, upon their activation, they phosphorylate substrates that are crucial for synaptic transmission, neuronal excitability and morphological stabilization. The list of possible targets is continuously expanding, underlining the complexity of the action of these kinases on neuronal function (Table 1).

Because the PKA, ERK and αCaMKII pathways vary in the signal integration that leads to their activation and in their downstream targets, it is somewhat surprising that interfering with the activation of any of these pathways

Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) is an activator of cAMP-response-element-binding protein (CREB) that has been well characterized in a variety of cellular models, but no information is available on CaMKIV in the visual cortex. Because the pharmacological block of ERK completely suppresses visually driven cAMP-response-element (CRE)-mediated gene expression, it is likely that such activation is operated by ERK [52]. For PKA, αCAMKII and ERK, lists of proteins that these kinases activate directly are shown in grey boxes. Abbreviations: GluR1, glutamate-receptor subunit 1; HSPDE4D3, an isoform of human cAMP-specific phosphodiesterase; K_v4.2, a voltage-gated K^+ channel; MAP2, microtubule-associated protein 2; MEK, ERK kinase; MSK, mitogen-and-stress-activated kinase; PP1, protein phosphatase 1; RSK, ribosomal S6 kinase; SynGAP, synaptic GTPase-activating protein. Dashed lines with question marks represent interactions of unknown significance in the context of visual cortical plasticity. (c) Representative fields from the visual cortex stained for phosphorylated ERK in a dark-reared rat (control) and in a rat that was exposed to a normal environment for 15 min. Scale bar, 50 μm.

Table 1. Possible targets of PKA, ERK and αCaMKII in synaptic plasticity[a]

Kinase	Possible targets	Refs
PKA	Synapsin I (for facilitation of secretion)	[79]
	Regulation of AMPA receptors	[53,54,80–82]
	Regulation of $GABA_A$ receptors	[83–85]
	Inhibition of PP1 (which is responsible for α-CaMKII dephosphorylation)	[32,35,86]
ERK	Synapsin I (for facilitation of secretion)	[20,87,88]
	$K_V4.2$ (a rapidly inactivating K^+ channel; activation of ERK facilitates spike backpropagation)	[60,89]
	MAP2 (for reversible control of dendrite morphology)	[90]
	HSPDE4D3 (a cAMP phosphodiesterase)	[36]
αCaMKII	AMPA receptors	[91–93]
	MAP2 (for reversible control of dendrite morphology)	[90]

[a]Abbreviations: αCaMKII, α Ca^{2+}/calmodulin-dependent protein kinase II; ERK, extracellular-signal-regulated kinase; HSPDE4D3, an isoform of human cAMP-specific phosphodiesterase; MAP2, microtubule-associated protein 2; PKA, cAMP-dependent protein kinase; PP1, protein phosphatase 1.

causes the same end result: the suppression of the ocular-dominance shift after monocular deprivation. This could be due to the extensive overlap and cross talk of these pathways, so that the blockade of a single kinase reverberates on the entire network. The complexity of these interactions is illustrated by many examples [32]. For instance, αCaMKII phosphorylates synaptic GTPase-activating protein (synGAP), a major component of the NMDA-receptor protein complex [33], thus reducing its inhibitory action on Ras, leading to an increase of ERK activation [34]. Similar excitatory interactions exist between PKA and αCaMKII [32,35], and between ERK and PKA [36]. It is easy to see how the block of any of these kinases can lead to a depression spreading through the entire signalling network.

Regulation of gene expression

The first steps of neural plasticity, which are changes in synaptic efficacy that do not require new protein synthesis, are followed by long-lasting changes in neuronal circuitry that require gene expression and protein synthesis. It is now clear that this is true also for ocular-dominance plasticity in the visual cortex [37,38]. Thus, the pattern of kinase activation has to be translated into a pattern of gene expression, probably through the activation of transcription factors. How can the crucial kinase–transcription-factor interactions be individuated? Several transcription factors, such as early-growth-response 1 (egr1/zif 268), are regulated by visual activity [39,40]. However, the condition of being visual-activity-dependent does not necessarily imply that the activation of a specific transcription factor is necessary for ocular-dominance plasticity, as exemplified by egr1/zif 268: mice with this factor knocked out exhibit a normal response to monocular deprivation [41].

An important hint leading to the molecular identity of the transcription factors necessary for plasticity is offered by the recent finding that the activation of CREB is necessary for ocular-dominance plasticity [37,42,43]. To cause CREB phosphorylation, activated kinases must translocate to the nucleus, where they start the expression of genes under the cAMP-response-element (CRE) promoter, with the consequent production of gene transcripts essential for establishment and maintenance of plastic changes [44]. Both PKA and ERK are well-characterized activators of CREB [45,46], although the ability of αCaMKII to translocate into the nucleus and directly activate CREB is far less certain [47–49]. Another activator of CREB is Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) [50] but the role of this factor in the visual system is unknown.

What is the pathway responsible for CRE-mediated gene expression activated by visual stimulation? This question can only be answered by *in vivo* studies on behaving animals because the details of the PKA–ERK interaction depend strongly on the cellular context [51]. Recently, it has been shown that patterned vision is a powerful activator of ERK in neurons of the visual cortex (Fig. 2c). Visually induced ERK activation relies, at least partially, on the cAMP–PKA system, and pharmacological block of ERK phosphorylation completely suppresses CRE-mediated gene expression after visual stimulation [52]. This is a strong indication that ERK is the final effector linking extracellular signals with gene expression in the visual system during the critical period. A rough scheme that would account for our present knowledge about the plasticity-related signalling can be designed as follows: NMDA coincidence detection activates αCaMKII, possibly helped by the co-occurring activation of PKA and the consequent inhibition of the αCaMKII phosphatase, protein phosphatase 1 (PP1) [32,35]. Locally activated αCaMKII acts on local targets, such as AMPA receptors [53,54], contributing to further depolarization. Finally, ERK detects the simultaneous and stabilized activation of PKA and αCaMKII, integrates these signals with those of the neurotrophin signalling cascades, and controls CRE-mediated gene expression and the induction of long-lasting modification of cortical circuitry.

Extracellular environment and visual cortical plasticity

Downstream effectors that implement the program initiated by the signalling mechanisms described in the preceding section are largely unknown; however, recent results indicate that removal of factors present in the extracellular environment is necessary for the experience-dependent modification of visual cortical circuits. The extracellular protease tissue plasminogen activator (tPA) is induced by electrical activity as an immediate-early

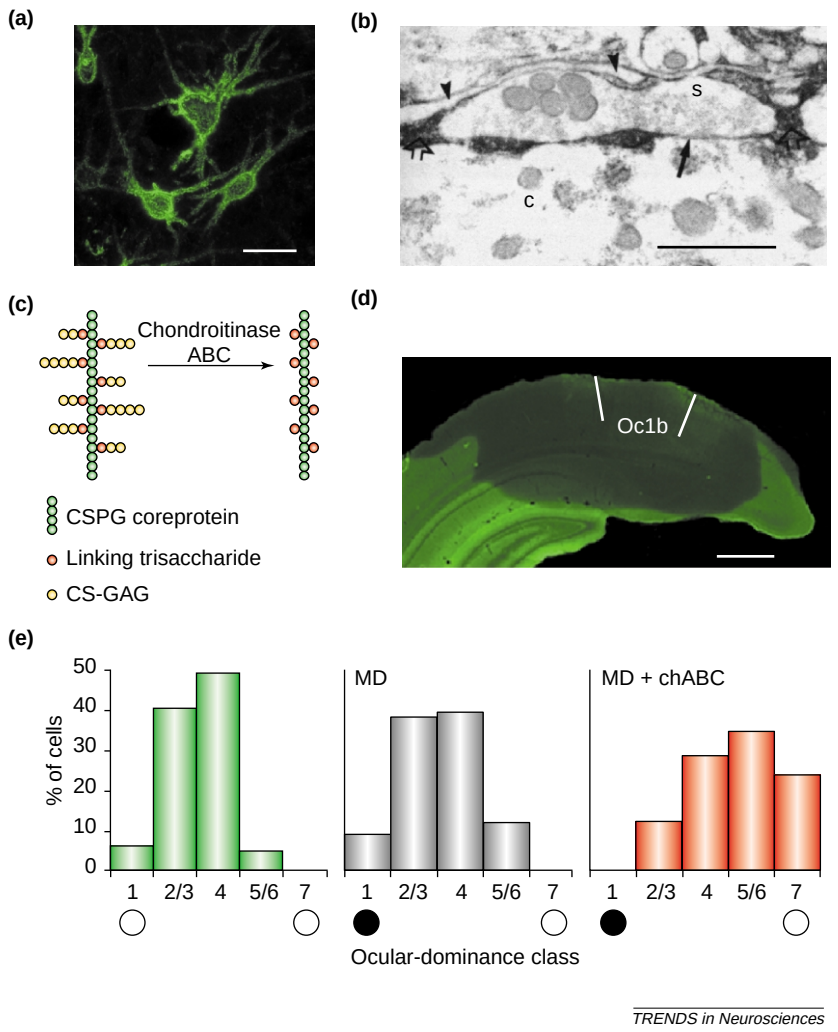

Fig. 3. Relationship between chondroitin-sulfate proteoglycans (CSPGs) and adult visual cortical plasticity. (a) CSPGs form a perineuronal net that ensheaths visual cortical neurons in adult rats. (b) Utrastructural analysis of the localization of the CSPG aggrecan (open arrows) shows perisynaptic localization. The arrow indicates synaptic contact and the arrowheads indicate astrocytic processes. Reproduced, with permission, from Ref. [70]. Abbreviations: c, neuronal cell body; s, synaptic vesicles. (c) Treatment with chondroitinase ABC degrades the chondroitin-sulfate glycosaminoglycans (CS-GAGs) from CSPG. This degradation results in major disruptions to the macromolecular heterophilic interactions that hold the perineuronal net together. (d) Immunostaining for the CSPG neurocan shows that the treatment of the adult visual cortex with chondroitinase ABC removes CSPGs from the whole binocular subfield of the adult visual cortex (area Oc1b) and from neighbouring cortical areas. (e) In adult rats (age >100 postnatal days), monocular deprivation (MD; black and white circles indicate the ocular-dominance class corresponding to the deprived and non-deprived eyes, respectively) does not cause a shift of ocular dominance. When adult rats were treated with chondroitinase ABC (chABC), monocular deprivation elicited a significant shift of ocular dominance towards the non-deprived eye. Panels (d) and (e) reproduced, with permission, from Ref. [63], © (2002) American Association for the Advancement of Science (http://www.sciencemag.org). Scale bars, 40 μm (a), 1 μm (b) and 1 mm (d).

gene [55] and its proteolytic activity in the visual cortex is increased during monocular deprivation [56]. The first investigations on the role of tPA in visual cortical plasticity indicated that its pharmacological inhibition attenuates the ocular-dominance shift induced by monocular deprivation [57] and prevents the effects of reverse suture (a form of plasticity in which a previously deprived eye is reopened while the contralateral, previously open eye is monocularly deprived). In young animals, this procedure is normally able to revert the effects of the previous monocular deprivation, but reverse suture was ineffective in kittens treated with tPA inhibitors [58]. The implications of these pharmacological studies have been deepened by analysis of the effects of monocular deprivation on tPA-knockout mice. These mice displayed an impaired ocular-dominance shift that could be rescued by exogenous tPA [56]. tPA has a wide spectrum of possible molecular targets, including extracellular-matrix proteins [59], growth factors [60], membrane receptors [61] and cell-adhesion molecules [62], and the available information is not sufficient to dissect which of these actions of tPA are relevant for inhibition of plasticity. Recent data, however, suggest that at least part of the inhibitory action of the extracellular environment could reside in components of the extracellular matrix [63]. The authors studied chondroitin-sulfate proteoglycans (CSPGs), a class of glycoproteins that are major components of the extracellular matrix of the CNS. These molecules comprise a core protein and chondroitin-sulfate glycosaminoglycan (CS-GAG) chains. CSPGs are abundantly expressed in the CNS, where they are used mainly to create barriers. Thus, in the developing nervous system, barriers between the two sides of the brain contain large amounts of CSPGs [64]. CSPGs are inhibitory for axonal sprouting and after

injury they are upregulated in the CNS, with the effect of blocking axon regeneration [65].

In the adult CNS, CSPGs are typically condensed in lattice-like structures, designated perineuronal nets (PNNs; Fig. 3a), which completely ensheath neuronal cell bodies and dendrites. PNNs are fenestrated at sites of synaptic contact, where they assume a perisynaptic localization [66,67] (Fig. 3b). In the visual cortex, the process of condensation of CSPGs into PNNs begins during late development and is completed after the end of the critical period [63,68–70]. Dark rearing, which is known to prolong the critical period for ocular-dominance plasticity [1], also prevents PNN formation, as assessed by staining for CS-GAG chains with Wisteria Floribunda Agglutinin [63], and by immunostaining for neurocan [63] and with CAT-301 [71], CAT-315 and CAT-316, which are antibodies that recognize glycovariants of aggrecan [72,73].

The correlation between CSPG maturation and critical-period closure [74] suggested that CSPGs could hinder ocular-dominance plasticity in the adult visual cortex [70]. A direct demonstration of this theory comes from the recent analysis of the effects of degradation of CS-GAG chains *in vivo* with the enzyme chondroitinase ABC [63] (Fig. 3c,d). This treatment destabilizes PNNs and causes their disappearance from the adult visual cortex. Removal of CSPGs was able to reactivate ocular-dominance plasticity in monocularly deprived adult rats (Fig. 3e), suggesting that developmental maturation of PNNs could contribute to the progressive reduction of plasticity that occurs in the visual cortex at the end of the critical period.

The mechanisms by which CSPGs inhibit plasticity in the adult visual cortex are still unknown. However, the inhibitory action of CSPGs on axonal sprouting suggests that degradation of PNNs could restore plasticity by removing substrates that are non-permissive for the generation or rearrangement of synaptic connections. Experiments in the somatosensory cortex have suggested that plasticity of dendritic spines is at the core of plasticity of the somatotopic map during development [75,76]. In the adult somatosensory cortex, dendritic spines are still dynamic and changes in spine turnover can be activated during experience-dependent plasticity. Indeed, long-term two-photon imaging of dendritic spines coupled with electron microscopy has shown a change in the dynamics of synaptic contacts in whisker-deprived mice [77]. Surprisingly, this highly dynamic scenario seems not to be present in the adult mouse visual cortex. Indeed, the half-life of spines of layer-V visual pyramidal neurons is >13 months [78]. It is tempting to speculate that the developmental maturation of an extracellular matrix that is non-permissive for synaptic rearrangement could cause the remarkable structural stability of the adult visual cortex.

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