CHAPTER 7

A Kinase with a Vision:

Role of ERK in the Synaptic Plasticity of the Visual Cortex

Gian Michele Ratto and Tommaso Pizzorusso

Introduction

We look at these written words with two eyes, their neuronal representations are elaborated separately in the two retinae and they are conveyed to two separate zones of the thalamus. The segregation in eye specific structures is broken only in the primary visual cortex, where neurons responsive to both eyes can be finally found. The cortical circuitry that brings together information from the two eyes is exquisitely tuned during the early post natal life, in a critical period in which synaptic changes are driven by the electrical activity evoked by the visual stimulation. Though the plasticity of binocular vision has served as a model for the study of synaptic plasticity for over 40 years, the identity of the molecular mechanisms involved in this process has remained elusive. Recently, we have offered evidences, gathered both in vivo and in vitro, suggesting that the Extracellular-signal Regulated Kinase 1/2 (ERK1/2) plays a crucial role in the control of this form of plasticity.

In this chapter we will at first describe ocular dominance in visual cortex and how it is shaped by visual activity during development. Then we will analyze the factors that are known to influence visual plasticity and their transduction pathway. Finally, we will show how the activation of this intracellular machinery is necessary for visual cortical plasticity in vitro and in vivo.

Critical Period for Ocular Dominance

Most of the richness and complexity of the external world is conveyed to us by the sense of vision. The architecture of the visual system complicates gradually from lower mammalian to primates and man, in a way corresponding to the increasing importance of vision in respect of the other sensorial modalities. Still, many functional features of the visual system are shared at all levels of phylogenetic development, both in terms of the neuronal architecture and in terms of the developmental processes leading to the mature system. Among these conserved themes, one of the most striking feature is the fact that the visual system gradually learns to see during a definite period of development. During this time window most parameters determining the performances of the visual system improve gradually, in correspondence of a progressive tuning of cortical circuitry. This period, aptly named critical period, begins roughly at eye opening and lasts for a few weeks or months depending on the species, and culminating to a duration of a few years in humans. At this time the circuitry of the visual system is prone to changes according to the visual experience impinging on the subject, as it is exemplified by the

Brain Repair, edited by Mathias Bähr. ©2006 Eurekah.com
and Kluwer Academic / Plenum Publishers.

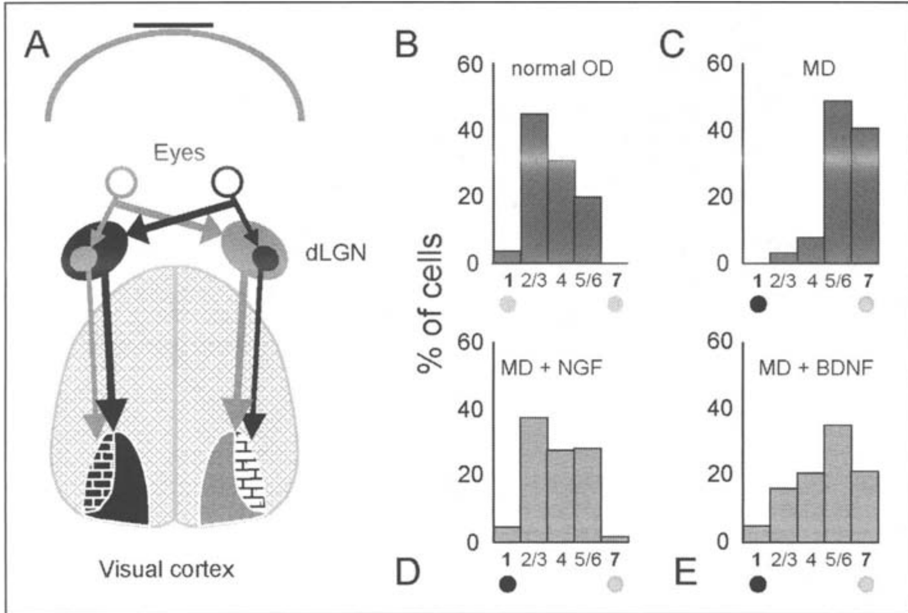

Figure 1. Organisation of the mammalian visual system, and definition of ocular dominance. A) Each eye projects to both sides of the thalamus with unequal weight. In rodents, 95% of the retinal fibres occupy most of the controlateral thalamus, while the remaining fibers project to a small volume of the ipsilateral thalamus. These two zones are still segregated, in the sense that post-synaptic thalamic neurons respond to either one or the other eye. The thalamo-cortical projections converge on the binocular portion of the visual cortex, and thus cortical neurons are responsive to stimuli presented to both eyes. B) The histogram is used to classify the ocular dominance of cortical neurons, from a score of 1 (responsive only to the controlateral eye) to 7 (responsive only to the ipsilateral eye). C) The monocular deprivation during the critical period (post natal day 21 to about 32 in rats and mice) cause a dramatic shift of ocular dominance, which can be partially rescued with exogenous infusion of NGF or BDNF (D and E respectively).

maturation of the ocular dominance of cortical neurons. The information incoming from the two eyes is initially segregated in two separate pathways that converge only at the level of the binocular portion of the primary visual cortex (Fig. 1A). Not until this stage we find neurons responsive to visual stimuli presented to both eyes. Accordingly, cortical neurons can be classified in base of their ocular dominance, with a score that defines the degree of control of each eye in determining the cell responsiveness (Fig. 1B). A score of 1 or 7 indicates that the eye is responsive only to the contralateral or ipsilateral eye respectively, score 4 indicates equal responsiveness to both eyes. The ocular dominance in a mature rodent is described by the histogram 1B. Clearly the contralateral eye is somewhat dominant, but there is a significative contribution by the ipsilateral eye also. If during the critical period one eyelid is sutured (monocular deprivation) the ocular dominance distribution shifts drastically toward the non deprived eye (Fig. 1C).[1]

Factors Critical for Ocular Dominance: Electrical Activity and Neurotrophins

In the quest for the mechanisms controlling the plasticity of ocular dominance, we must understand first how monocular deprivation is translated into the neuronal alphabet of spike trains. The suture of the eyelid does not sink the retina in uniform darkness since light changes

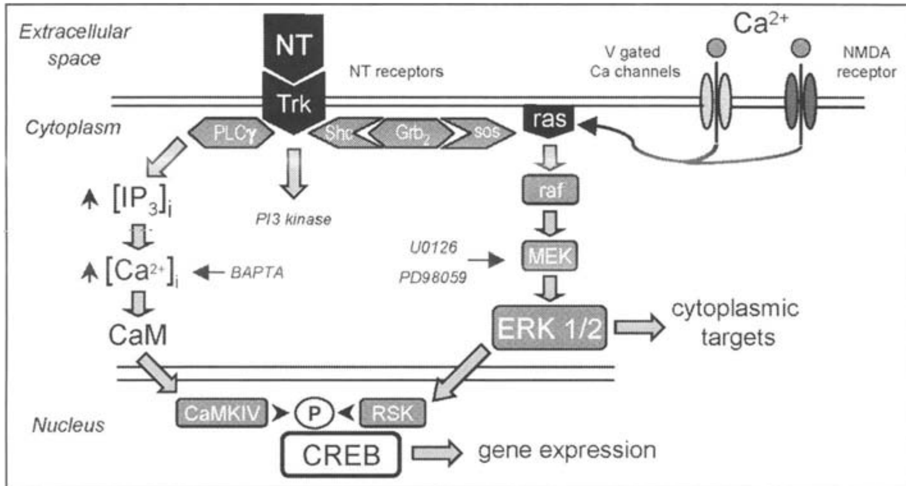

Figure 2. Signal transduction pathway associated with neurotrophins and electrical activity. Neurotrophins transduction is started at the Tyrosine Kinase receptors. There are three flavours of receptors (TrkA, B and C) with different preferential affinity for the various neurotrophins: NGF (TrkA), BDNF (TrkB), NT3 (TrkC) and NT4 (TrkB). The phosphatidylinositol 3-kinase pathway mediates the control of neuronal survival, and it is not considered here. Electrical activity triggers the ras-ERK pathway in a Ca-dependent way. After its phosphorylation ERK 1/2 can control gene expression and exert local effects on cytoplasmatic or membrane-bound substrates.

are attenuated but still discernible; what is taken away from the visual world is the presence of spatial contrast. The thalamic fibers carrying the input originating from the deprived eye miss the high frequency trains characteristic of the perception of edges. Thus, the strengthening of the normal eye at expenses of the deprived eye, can be interpreted as an expansion of the synaptic target of the active fibers against the terminals with depressed electrical activity. Another factor that contributes to regulate this competitive process between the two eyes, is the availability of neurotrophins in the visual cortex.[2-4] Indeed, the involvement of neurotrophins on monocular deprivation is directly demonstrated by the fact that their exogenous administration during deprivation prevents the shift of ocular dominance caused by the unbalance of electrical activity[5-8] (Fig. 1D-E). Furthermore, the duration of the critical period for monocular deprivation is shortened in mice overexpressing BDNF.[9]

These observations reveal that monocular deprivation is a play with two actors on stage: electrical activity and neurotrophins, with a central role played by BDNF. Therefore it is reasonable to assume that the mechanisms controlling plasticity of ocular dominance must sit somewhere between the intracellular signaling pathways activated by neurotrophins and those which convert visually driven activity into long-lasting changes of cortical circuitry. A possible scheme of molecular interactions between neurotrophin signaling and activity dependent signaling is represented in Figure 2. Most of the physiological functions of neurotrophins begin with their binding with the tyrosine kinase receptors (Trk receptor).[10] Consequently, the receptor self-phosphorylates and exposes consensus sites for various Trk substrates. Two pathways that have been well characterized in vitro are especially interesting, because they converge on the transcription factor CREB (cAMP Response Element Binding protein) that, upon activation, begins CRE-mediated transcription. The offspring of this episode of gene expression has an important role for memory and learning in Drosophila, Aplysia, and mouse.[11-13]

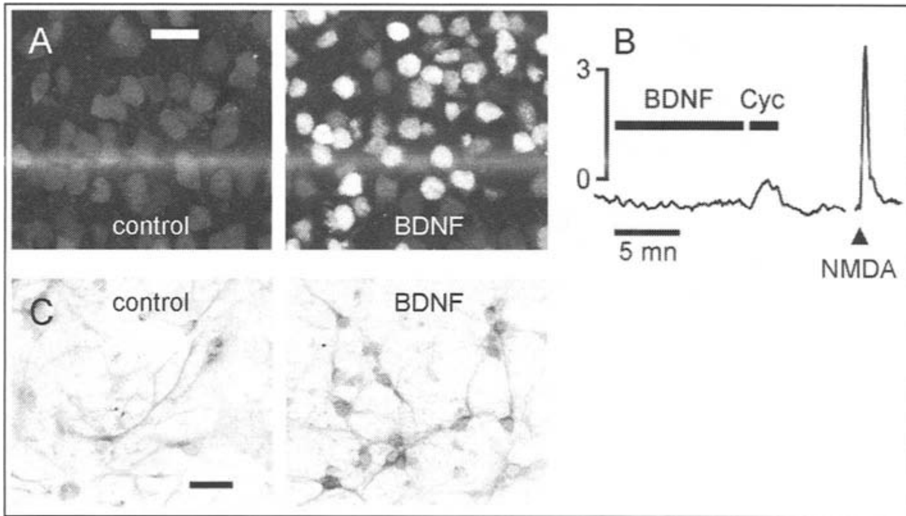

Figure 3. Effects of BDNF on neurons of the visual cortex. A) A 1 hr exposure of a slice of visual cortex to BDNF (200 ng/ml) caused a strong phosphorylation of the response element CREB (bar 20 μm). B) In the same conditions Ca-imaging at the confocal microscope failed to show any response to BDNF, but the cells were responsive to brief pulses of Cyclopiazonic acid (50 μM) and of NMDA (20 μM). C) BDNF (50 ng/ml for 30 min) is a powerful activator of ERK 1/2, as shown by the strong pERK immunofluorescence in cell body and dendrites (bar 50 μm).

The first pathway leading from Trk to CREB passes through the activation of phospholipase Cγ, the production of Inositol 3-phosphate (IP_3) and the consequent release of calcium from internal stores.[14] At the end of this cascade, CREB phosphorylation would be brought about by the Ca-dependent activation of CaMK IV.[15] The second pathway does not involve a Ca change, and the Trk receptors are linked by the complex of adapter proteins shc-Grb-sos to the ras-ERK 1/2 cascade. Eventually, these molecular stepping-stones lead to the cell nucleus since phosphorylated ERK causes CREB activation by means of the intermediate kinase RSK. In these schemes coalesce experimental evidences gathered mainly in vitro, and do not reflect necessarily what occurs in vivo. For example, the data regarding a direct action of neurotrophins on intracellular calcium are rather controversial, and have been obtained mainly in culture as discussed elsewhere.[16] In the next section we will show that, in the visual cortex, BDNF causes CREB activation by means of the Ca–indepent cascade only.

Activation of ERK 1/2 Is Necessary for BDNF-Induced Phosphorylation of CREB

Our initial goal is to show what pathways are involved in mediating BDNF action in the visual cortex. Simply put, we must answer to the following questions:

1. Does BDNF activate CREB in neurons of the visual cortex?
2. Does BDNF cause an increase of intracellular calcium and/or activate ERK 1/2 to mediate the activation of CREB?

Experiments executed on both cultured neurons and acute slices obtained from the visual cortex show that BDNF activates CREB, strongly arguing for an involvement of CREB in mediating the action of BDNF in the visual system. This is demonstrated by immunoreaction with an antibody raised against CREB phosphorylated at the Ser 133 residue (Fig. 3A). Exposing cortical slices to BDNF in similar conditions, we performed Ca-imaging experiments to

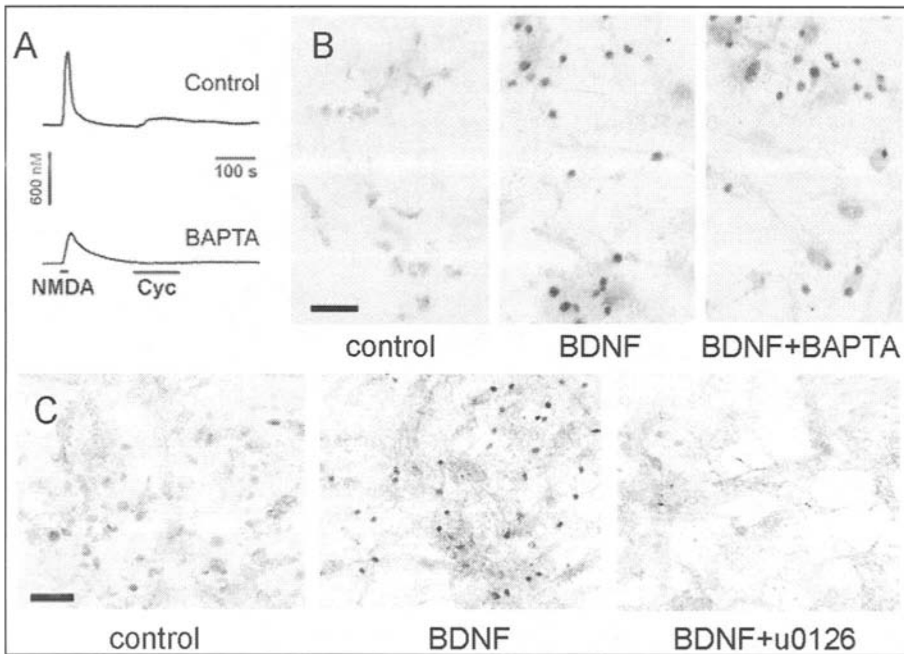

Figure 4. BDNF causes CREB phosphorylation in a Ca-independent way but requires ERK activation. A) Incubation with the cell permeable form of the calcium chelator BAPTA quenched the Ca transient caused by release from the intracellular stores (cyclopiazonic acid 50 μM), and strongly reduced the size of the NMDA response (20 μM). B) In the same conditions, BDNF (50 ng/ml for 15 min) still caused a strong phosphorylation of CREB in cultured neurons (bar 50 μm). C) In contrast, treatment with the MEK inhibitor U0126 completely blocked CREB phosphorylation (bar 50 μm).

detect any calcium increase triggered by the neurotrophin. In a set of remarkably uneventful experiments, we recorded hundreds of cells and we observed a Ca-change only in an handful of neurons (13 out of over 1000 cells), even if the imaging system was sensitive enough to reveal the tiny Ca transient unmasked by the SERCA inhibitor Cyclopiazonic acid (Fig. 3B). More was to be found on the other branch of the cascade departing from the Trk receptor and leading to the nucleus, since BDNF proved to be a powerful activator of ERK 1/2. This is shown by the strong increase in phospho-specific immunostaining of cultured neurons, after exposure to BDNF (Fig. 3C).

The necessity of either pathways can be demonstrated by blocking them with specific agents and looking at the effects on CREB phosphorylation. The two tools in our hands were the Ca chelator BAPTA, to interrupt the Ca-dependent pathway, and the molecule U0126, a specific blocker for the ERK kinase MEK.[17,18] In neurons loaded with a cell-permeant form of BAPTA, the calcium changes due to the release from intracellular stores were virtually suppressed, and even the much larger changes caused by the influx of external calcium through the NMDA receptors were heavily depressed (Fig. 4A). Therefore, given the high affinity and binding speed of BAPTA with Ca, this treatment should effectively quench any Ca change that might have been left undetected in the imaging experiments. In these conditions the pCREB staining after exposure to BDNF was virtually unaffected (Fig. 4B). This data, together with the Ca imaging experiments, prove that Ca is not a key effector in the pathway between TrkB activation and CREB phosphorylation in visual neuron. The opposite situation holds true for

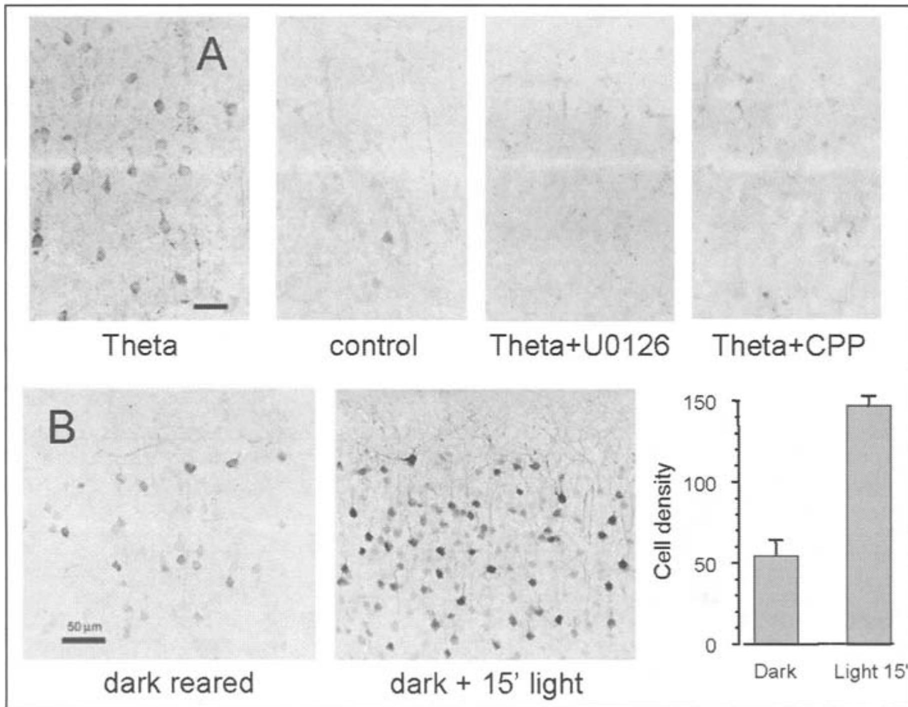

Figure 5. Neuronal activity causes ERK phosphorylation. A) A theta burst delivered to the white matter activated ERK in the visual cortex 5 min after stimulation. A similar control slice that did not receive the theta stimulation was much more weakly stained. ERK activation by the theta burst was blocked by U0126 (20 µM) and by a similar concentration of the NMDA antagonist CPP (bar 40 µm). B) ERK phosphorylation was also induced by a 15 min exposure to light after a period or dark rearing.

the ras-ERK pathway: pre-incubation of cortical neurons with the MEK blocker U0126, completely suppressed the pCREB induction operated by stimulation with BDNF (Fig. 4C). Therefore, BDNF dependent CREB phosphorylation requires ERK activation and it is independent on changes of intracellular Ca.[16]

ERK 1/2 Is Phosphorylated by Activity and Visual Stimulation in the Cortex

The surprise of meeting a Ca-independent process does not last long, since Ca comes back on scene when we study the pathway linking electrical activity to CREB phosphorylation. Indeed, activity-mediated CREB phosphorylation is quantitatively dependent on intracellular calcium increases caused by influx through voltage sensitive channels and NMDA receptors.[19-22] While the details of this regulation are still elusive, it is likely that the Ca influx acts on the ras-ERK pathway at the level of ras and /or raf. The involvement of NMDA receptors on activity dependent ERK phosphorylation is demonstrated in Figure 5: slices from the visual cortex received a special pattern of stimuli from an extracellular electrode. The pattern, named theta burst, consists of short bursts at high frequency (100 Hz), repeated at 5Hz frequency. Fifteen minutes after stimulation, staining with pERK antibody revealed a strong level of phosphorylation that required Ca influx through the NMDA receptor, since ERK activation was completely blocked by the NMDA receptor antagonist CPP (Fig. 5A). It could be argued that

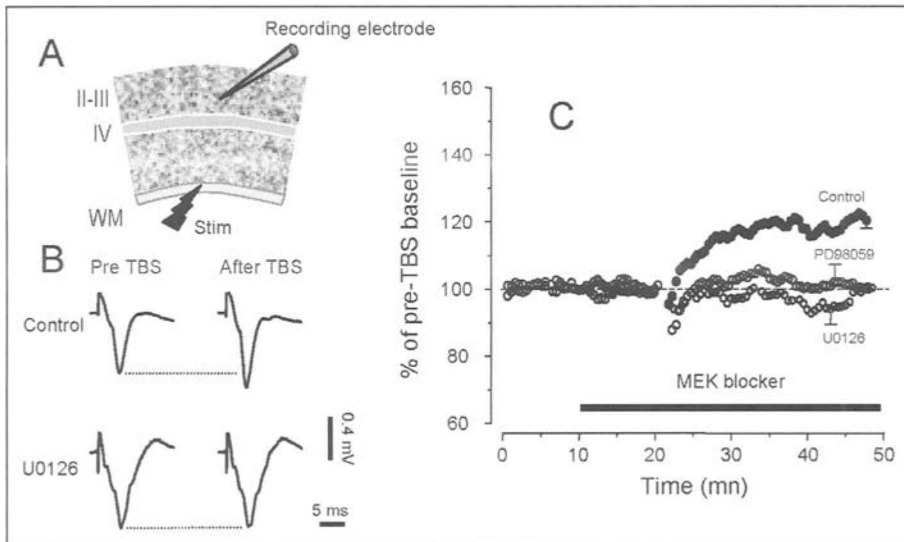

Figure 6. ERK activation is necessary for LTP induction in visual cortex. A) Experiments were performed on slices from the visual cortex of rats within the critical period. The stimulating electrode is placed in the white matter (WM), and the recordings are obtained from neurons in the superficial layers. B) The artefact caused by the stimulation is followed by the downward field response. After theta burst the response shows potentiation in control conditions, but not after incubation with U0126. C) The amplitude of the field response is tested every 30 s before and after the TBS and plotted in function of time. Each point represents averages from 18, 5 and 7 different slices recorded in control, PD98059 and U0126 respectively.

the in vitro stimulation is different from the pattern of electrical activity that is evoked in the cortex by visual stimuli. Thus, we desired to test whether visual stimulation would bring about ERK phosphorylation in behaving animals. Rats were kept in darkness for 3 days, and then were exposed to light. Twenty minutes afterward, the animals were sacrificed and processed for immunohistochemistry with the pERK antibody. Figure 5B shows that exposure to visual environment caused a robust activation of neurons in the visual cortex.[23,23a]

In conclusion, since ERK acts as a convergence point between electrical activity and neurotrophins, it appears to be in a strategic position to translate the regulatory actions of activity and neurotrophins into changes of cortical circuitry. Of course, it remains to be demonstrated a direct link between ERK activation and the regulation of synaptic strength.

ERK Activation Is Required for Synaptic Plasticity in Vitro and in Vivo

In models of synaptic plasticity, such as long term potentiation (LTP) or depression (LTD), it has been shown that the changes of neuronal connectivity are the result of a complex chain of events involving calcium entry through NMDA receptors and voltage-gated calcium channels, activation of protein kinases, gene expression and protein synthesis (see refs. 24,25 for recent reviews). In acute slices of visual cortex it is possible to stimulate the incoming excitatory fibers by means of extracellular electrodes and the evoked post-synaptic activity can be recorded from the superficial layers (Fig. 6A). LTP of the thalamo-cortical circuit can be induced by applying a theta burst stimulation in the white matter. Since this form of LTP is present only during the critical period, it has been suggested that this form of synaptic plasticity might be implicated in the activity-dependent refinement of cortical circuitry occurring during the critical period.[26]

Test stimuli were delivered every 30 sec by an electrode placed on the white matter, while recording the evoked field response from layer II/III. The presentation of a theta burst induced a potentiation of the response amplitude of about 20% in control conditions (Fig. 6B,C). If the theta was preceded by a 10 min incubation with the MEK inhibitors U0126 or PD98059, the potentiation was completely suppressed. It is interesting to notice that the time course of the response amplitude of slices treated with the inhibitors, differs from controls immediately after TBS delivery (Fig. 6C). The rapidity of the onset of the effect of MEK blockage suggests that ERK action is required for some mechanism of potentiation that, at least initially, is independent on gene transcription. Separate experiments have shown that the inhibitory effect of U0126 on LTP were not caused by reduced responses to the theta burst or by reduced activation of the NMDA receptors that are required for the induction of this form of LTP.[23]

To test the role of ERK pathway in visual plasticity, we needed a way of delivering the MEK inhibitors to the cortex during monocular deprivation. The drugs can be supplied by osmotic minipumps with their outlet placed immediately in front of the binocular visual cortex. Treatment was provided for one week at the peak of the critical period, as schematized in Figure 7A. After seven days of treatment the MEK inhibitor was still effective. This was proven in an experiment in which electrical activity was strongly increased by acute infusion with picrotoxin, a blocker of $GABA_A$ receptor. This treatment caused a robust ERK phosphorylation in control cortex but not in the cortex treated with U0126 (Fig. 7B). At this point we are finally ready to study whether activation of the ERK cascade is necessary for the experience-dependent plasticity occurring during monocular deprivation. Indeed, block of ERK activation by U0126 or PD98059 prevented MD effects (Fig. 7C). In normal P28 rat the overwhelming majority of visual cortical cells are binocular, with a clear dominance of the controlateral eye, and one week of deprivation at this age should cause a massive shift of responsiveness to the normal eye. This plastic change is clearly prevented by the blockage of the ERK pathway.

Beyond ERK: Mechanisms Controlling Synaptic Plasticity in the Visual Cortex

What are the cellular mechanisms critical for the plasticity of ocular dominance and that are blocked by the inhibition of the ras-ERK pathway? From the molecular point of view, the possible targets of ERK after its visually driven activation are at two different levels: at the nucleus and at the cytoplasm. In the first case activated ERK translocates to the nucleus where it can start CRE-mediated gene expression, with the consequent production of gene transcripts essential for establishment or maintenance of plastic changes.[12,27-29a] Indeed, recent observations by us and others, suggest that protein synthesis is necessary for ocular dominance plasticity, and that visual activity regulates CRE-mediated gene expression.[30,23a] The second scheme envisions a local action of ERK that, upon its activity/neurotrophin dependent activation, phosphorylates certain substrates that are critical for synaptic transmission or neuronal excitability. Such an intriguing possibility, is consistent with the observed distribution of the pERK staining that is very strong in dendrites and not only at the cell body, and with the rapid effect of ERK blockade after theta. Recent data show that there are at least three possible sites for acute ERK action: First, in Aplysia ERK is required for the downregulation and internalization of the adhesion molecule Ap-CAM, a key step in the induction of long term facilitation.[31] Second, ERK can act at synaptic level, since it has been shown that synapsin I has consensus sequences for ERK.[32] Furthermore, ERK phosphorylates synapsin I in response to neurotrophis,[33] with consequent effects on glutamate release.[34] Finally, ERK can phosphorylate the potassium channel Kv4.2,[35] which is one of the main determinant of the rapidly inactivating potassium current. Given the importance of this conductance in determining spike duration, neuronal excitability and spike back-propagation, it is conceivable that this might be

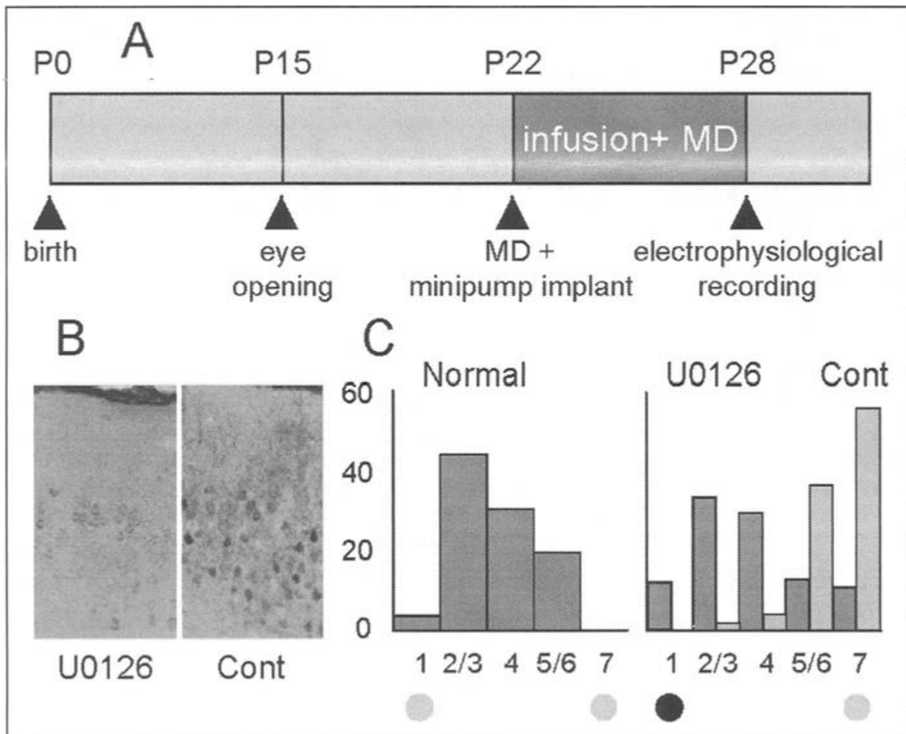

Figure 7. ERK activation is necessary for ocular dominance shift after monocular deprivation. A) Time schedule of experimental treatment. The period of monocular deprivation coincides with the duration of infusion and it is located at the peak of the critical period. B) At the end of the treatment the cortex was exposed and perfused with 1 mM of picrotoxin. At this concentration the electrical activity increased of about a factor four, and this was reflected by ERK induction, which was still blocked on the cortex treated with U0126 (250 μM). C) Average distributions of the ocular dominance for 6 control rats (histogram at left), and 11 rats that were monocularly deprived (right). Seven of the deprived rats were implanted with a minipump delivering U0126. Similar results were obtained with the second MEK inhibitor, PD98059.

another key target for ERK actions on synaptic connectivity. Further details of the mechanisms controlling the acute and long term effects of ERK on neuronal plasticity are still no more than hypotheses.

References

1. Wiesel TN, Hubel DH. Comparison of the effects of unilateral and bilateral eye closure on cortical unit responses in kittens. J Neurophysiol 1995; 28(6):1029-1040
2. Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. Science 1996; 274:1133-1138.
3. Pizzorusso T, Maffei L. Plasticity in the developing visual system. Curr Opin Neurol 1996; 9:122-125.
4. McAllister AK, Katz LC, Lo DC. Neurotrophins and synaptic plasticity. Annu Rev Neurosci 1999; 22:295-318.
5. Maffei L, Berardi N, Domenici L et al. Nerve growth factor (NGF) prevents the shift in ocular dominance distribution of visual cortical neurons in monocularly deprived rats. J Neurosci 1992; 12:4651-4662.

6. Riddle DR, Lo DC, Katz LC. NT-4-mediated rescue of lateral geniculate neurons from effects of monocular deprivation. Nature 1995; 378:189-191.
7. Galuske RA, Kim DS, Castren E et al. Brain-derived neurotrophic factor reversed experience-dependent synaptic modifications in kitten visual cortex. Eur J Neurosci 1996; 8:1554-1559.
8. Lodovichi C, Berardi N, Pizzorusso T et al. Effects of neurotrophins on cortical plasticity: same or different? J Neurosci 2000; 20:2155-2165.
9. Huang ZJ, Kirkwood A, Pizzorusso T et al. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 1999; 98:739-755.
10. Patapoutian A, Reichardt LF. Trk receptors: mediators of neurotrophin action. Curr Opin Neurobiol 2001; 11(3):272-280
11. Bourtchuladze R, Frenguelli B, Blendy J et al. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell 1994; 79:59-68.
12. Silva AJ, Kogan JH, Frankland PW et al. CREB and memory. Annu Rev Neurosci 1998; 21:127-148.
13. West AE, Chen WG, Dalva MB et al. Calcium regulation of neuronal gene expression. Proc Natl Acad Sci U S A 2001; 25:11024-11031.
14. Zirrgiebel U, Ohga Y, Carter B et al. Characterization of TrkB receptor-mediated signaling pathways in rat cerebellar granule neurons: Involvement of protein kinase C in neuronal survival. J Neurochem 1995; 65:2241-2250.
15. Finkbeiner S, Tavazoie SF, Maloratsky A et al. CREB: a major mediator of neuronal neurotrophin responses. Neuron 1997; 19:1031-1047.
16. Pizzorusso T, Ratto GM, Putignano E et al. Brain-derived neurotrophic factor causes cAMP response element-binding protein phosphorylation in absence of calcium increases in slices and cultured neurons from rat visual cortex. J Neurosci 2000; 15;20(8):2809-2816.
17. Favata MF, Horiuchi KY, Manos EJ et al. Identification of a novel inhibitor of mitogen-activated protein kinase kinase. J Biol Chem 1998; 273:18623-18632.
18. Roberson ED, English JD, Adams JP et al. The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. J Neurosci 1999; 19:4337-4348.
19. Ghosh A, Greenberg ME. Calcium signaling in neurons: molecular mechanisms and cellular consequences. Science 1995; 268:239-247.
20. Deisseroth K, Bito H, Tsien RW. Signaling from synapse to nucleus: postsynaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity. Neuron 1996; 16:89-101.
21. Fields RD, Eshete F, Stevens B et al. Action potential-dependent regulation of gene expression: Temporal specificity in Ca^{2+}, cAMP-responsive element binding proteins, and mitogen-activated protein kinase signaling. J Neurosci 1997; 17:7252-7266.
22. Grewal SS, York RD, Stork PJ. Extracellular-signal-regulated kinase signalling in neurons. Curr Opin Neurobiol, 1999; 9:544-553.
23. Di Cristo G, Berardi N, Cancedda L et al. Requirement of ERK activation for visual cortical plasticity. Science. 2001; 22; 292:2337-2340.
23a. Cancedda L, Putignano E, Impey S et al. Patterned vision causes CRE-mediated gene expression in the visual cortex through PKA and ERK. Neurosci 2003; 23(18):7012-20.
24. Elgersma, Y, Silva AJ. Molecular mechanisms of synaptic plasticity and memory. Curr Opin Neurobiol 1999; 9:209-213.
25. Sweat JD. Toward a molecular explanation for long-term potentiation. Learning & Mem 1999; 6:399-416.
26. Kirkwood A, Lee HK, Bear MF. Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. Nature 1995; 375:328-31.
27. Sgambato V, Pages C, Rogard M et al. Extracellular signal-regulated kinase (ERK) controls immediate early gene induction on corticostriatal stimulation. J Neurosci 1998; 18:8814-25.
28. Impey S, Obrietan K, Storm DR. Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. Neuron 1999; 23:11-14.
29. Pham TA, Impey S, Storm DR et al. CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. Neuron 1999; 22:63-72.

29a. Berardi N, Pizzorusso T, Ratto GM et al. Molecular basis of plasticity in the visual cortex. Trends Neurosci 2003; 26:369-378.
30. Taha SA, Stryker MP. Protein synthesis is required for ocular dominance plasticity. Soc for Neurosci Abs 2000; 322.12.
31. Bailey CH, Kaang BK, Chen M et al.Mutation in the phosphorylation sites of MAP kinase blocks learning- related internalization of apCAM in Aplysia sensory neurons. Neuron 1997; 18:913-924.
32. Matsubara M, Kusubata M, Ishiguro K et al. Site-specific phosphorylation of synapsin I by mitogen-activated protein kinase and Cdk5 and its effects on physiological functions. J Biol Chem 1996; 271:21108-21113.
33. Jovanovic JN, Benfenati F, Siow YL et al. Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions. Proc Natl Acad Sci U S A 1996; 93:3679-3683.
34. Jovanovic JN, Czernik AJ, Fienberg AA et al. Synapsins as mediators of BDNF-enhanced neurotransmitter release. Nat Neurosci 2000; 3(4):323-329
35. Adams JP, Anderson AE, Varga AW et al. The A-type potassium channel Kv4.2 is a substrate for the mitogen-activated protein kinase ERK. J Neurochem 2000; 75:2277-2287.