LECTURE

Developmental changes and ocular dominance plasticity in the visual cortex

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Abstract. There is a shift in ocular dominance of cells recorded in the visual cortex which occurs after closure of one eye during a critical period lasting from eye opening to puberty. Three criteria distinguish factors that are crucially related to ocular dominance plasticity: 1) the factor should be more concentrated or active at the peak of the critical period; 2) dark rearing, which makes the cortex less plastic early in the critical period and more plastic late in the critical period, should have a similar effect on the factor, and 3) antagonists or inhibitors of the factor should block ocular dominance plasticity. The second criterion can be used to distinguish activity-related factors that may simply increase or decrease with development from factors that are more specifically related to plasticity. Two factors currently fulfill these criteria, namely N-methyl-D-asparate (NMDA) receptors and protein kinase A (PKA). PKA and NMDA receptors are linked through calcium, since calcium influx through the NMDA receptor increases the production of cyclic AMP by calcium-sensitive adenylate cyclase, which in turn activates PKA. PKA is specifically involved, since protein kinase G and protein kinase C antagonists do not inhibit ocular dominance plasticity. However, NMDA agonists and PKA activators by themselves are not known to bring back plasticity. Thus there may be two or more pathways for ocular dominance plasticity acting in parallel with each other: for example, metabotropic glutamate receptors may act in parallel with NMDA receptors to change calcium levels within the cell. (Keio J Med 50 (3): 192–197, September 2001)

Key words: plasticity, NMDA, cAMP, protein kinase A, visual cortex

Introduction

Connections in the visual cortex can be modified by visual experience early in life. This is a means to compensate for ocular or motor defects, such as when the eyes point in different directions (strabismus), one eye is out of focus (anisometropia), or one or both eyes have a cataract (stimulus deprivation). The mechanism of compensation is that vision in one eye is suppressed and/or loses acuity.

The anatomical and physiological effects involved are now quite clearly understood from experiments in animals using monocular deprivation, where the eyelids of one eye are closed for a period of time. Connections from the deprived eye decay,[1,2] and the percentage of cells in the visual cortex that can be driven by the deprived eye is reduced,[3] so that after substantial deprivation, the animal becomes blind in that eye.[4] The current question of interest is: how does the afferent activity reaching the cortex from the two eyes lead to the decay of connections and synapses between the deprived eye and cortical cells, and to the expansion of connections from the non-deprived eye? What are the intermediate steps?

There are three criteria for a factor that is closely related to ocular dominance plasticity. The first is that the concentration or activity of the factor should follow the critical period for ocular dominance plasticity. The critical period starts after eye opening, and ends around puberty. In the cat, which is the animal used for most of our experiments, it starts around 3 weeks of age, is most sensitive at 4–6 weeks of age, and ends around 9

months of age.[5-8] At the peak of the critical period, monocular deprivation for a day or two has an effect. The second criterion is that rearing in the dark should affect plasticity and the factor in a similar fashion. Rearing in the dark is known to increase plasticity later in the critical period,[9] and to reduce it early in the critical period.[10] Thus 5–6 week old dark-reared cats are less plastic than normal, 8–9 weeks old dark-reared cats are equally plastic, and 12–20 week old dark-reared cats are more plastic than normal. This second criterion is particularly important, because it distinguishes factors related to activity, which simply increases with age, from factors related to plasticity.

The third criterion is that antagonists or blockers of the factor should reduce or abolish plasticity. The most common experiment is to measure the ocular dominance of a sample of cells in the visual cortex, and construct an ocular dominance histogram, which is shifted from normal by monocular deprivation.[3] Numerous treatments have been shown to reduce this ocular dominance shift. The list includes N-methyl-D-aspartate (NMDA) receptor antagonists,[11,12] protein kinase A (PKA) antagonists,[13] depletion of noradrenaline and acetylcholine,[14] and infusion of growth factor compounds,[15,16] or class I MHC antigens[17] *inter alia*. Very few treatments block the ocular dominance shift altogether, one of the few being tetrodotoxin, infused so that it blocks the afferent activity from the retina.[18] However, at the present time, there are only two factors known to fulfill all three of the criteria listed above, NMDA receptors and protein kinase A. This article will summarize the evidence in relation to these two factors.

NMDA Receptors

NMDA receptors were an early candidate as a factor involved in plasticity, because they are ligand-gated by glutamate, and also voltage sensitive.[19,20] The voltage sensitivity of NMDA receptors on a postsynaptic cell increases the probability that the cell will fire when the presynaptic cell fires. This increases plasticity according to Hebb's postulate that simultaneous firing of pre- and postsynaptic cells will strengthen the synapse in between.[21]

We have shown that B_{max} for NMDA receptors in the visual cortex follow the critical period for plasticity in the visual cortex (Fig. 1).[22] There is also a drop in the NMDA contribution to the visual response in layers IV, V and VI,[23] which occurs at the same time as the afferents from the lateral geniculate to the cortex are segregating into bands for the left and right eyes. The drop in this NMDA contribution is delayed by rearing in the dark,[24] just as ocular dominance segregation is delayed by rearing in the dark.[25]

The NMDA antagonist amino phosphonobutyric

NMDA receptor binding follows critical period

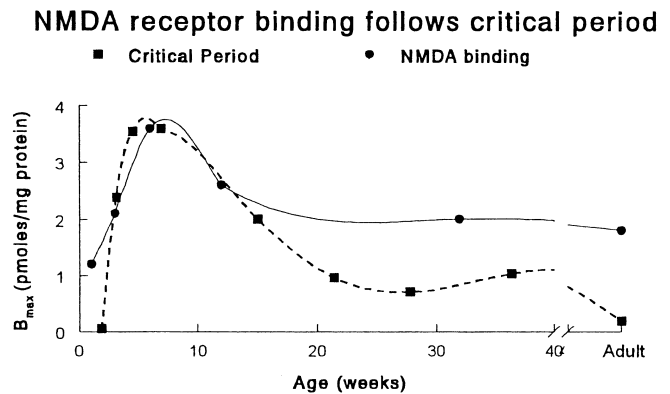■ Critical Period   ● NMDA binding

Fig. 1   NMDA receptor binding to membranes from cat visual cortex follows the critical period for ocular dominance plasticity in cat visual cortex.

acid (APV) reduces ocular L-dominance plasticity.[11,26] This experiment was criticized on the grounds that APV might reduce activity sufficiently that it would be just like TTX, which would change the interpretation of the experiment.[27] We therefore repeated the experiment using the NMDA channel blocker, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK-801). We found that this drug, injected into the leg muscle, reduced ocular dominance plasticity at a dose of 0.1 mg/kg (Fig. 2).[12] This dose abolishes the response to NMDA in the visual cortex, without affecting the visual response, or the response to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (Fig. 3). Higher doses of MK801 may affect the visual response, but there is a dose that affects plasticity with little effect on activity. A similar result has been obtained using antisense oligonucleotides for NMDA receptors.[28] Thus NMDA receptors fulfill the three criteria listed above for a factor specifically related to ocular dominance plasticity in the visual cortex.

Protein Kinases

Several protein kinases have been implicated in plasticity in the hippocampus. This includes PKA,[29,30] protein kinase C (PKC),[31] and protein kinase G (PKG).[32,33] PKA has also been implicated in learning in *Drosophila*.[34]

PKA is activated by cyclic AMP (cAMP), and levels of cAMP produced by the metabotropic glutamate agonist ACPD in the visual cortex follow the critical period closely (Fig. 4).[35] This is primarily due to developmental changes in levels of cAMP up to the age of 15 weeks, while the drop to zero between 15 weeks and adult is due to the drop in metabotropic glutamate receptors.[36] Levels of cAMP are lower than normal in 5

Control MD animals

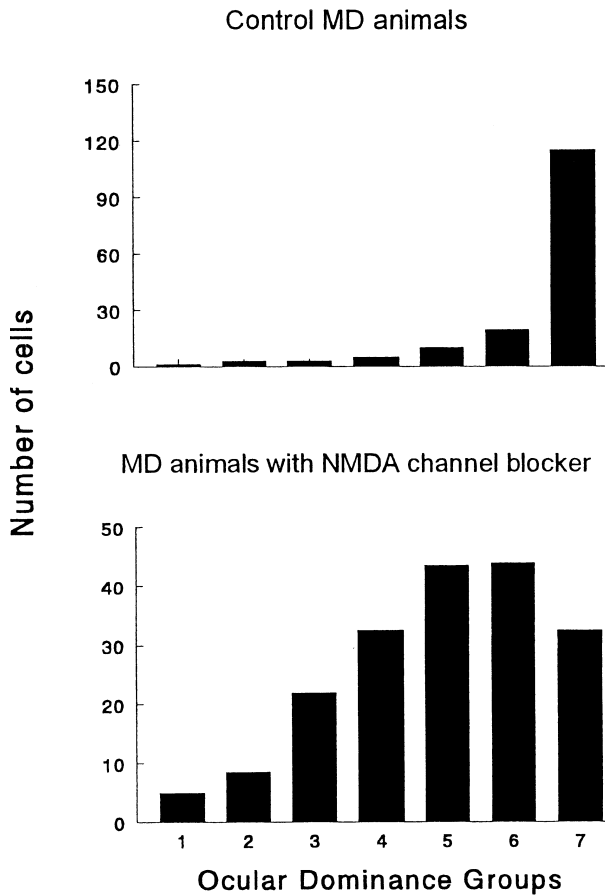## MD animals with NMDA channel blocker

Fig. 2 Ocular dominance histograms from cats monocularly deprived (MD) for 5 days at 4–5 weeks of age (top) showing a shift towards the open eye, compared with MD cats treated with injections of 0.1 mg/kg MK-801 in the muscle twice daily. The MK-801 reduced the ocular dominance shift.
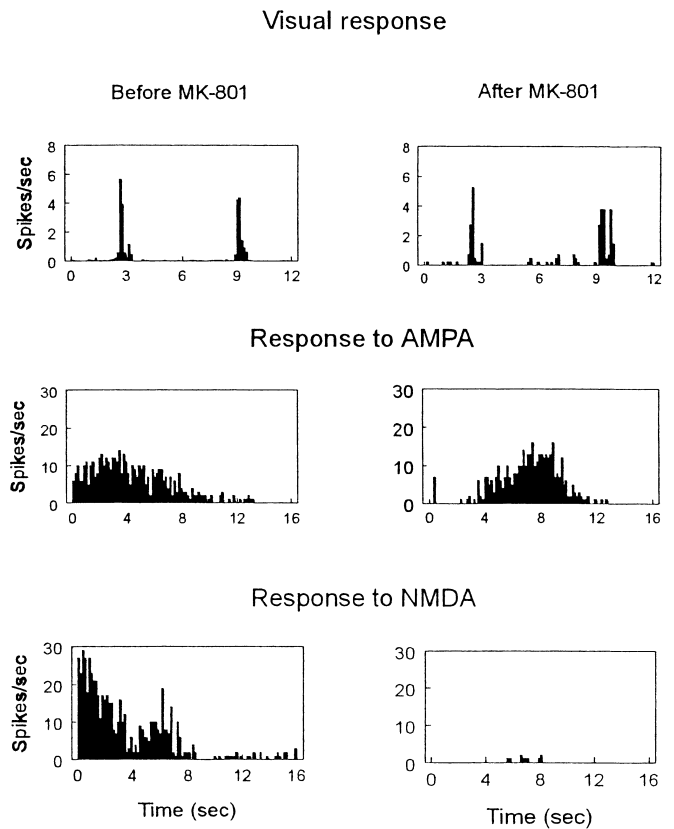
Visual response

Response to AMPA

Response to NMDA

Fig. 3 A 0.1 mg/kg injection of MK-801 abolishes the response to iontophoresis of NMDA in the visual cortex, with little effect on the visual response, or the response to iontophoresis of AMPA.

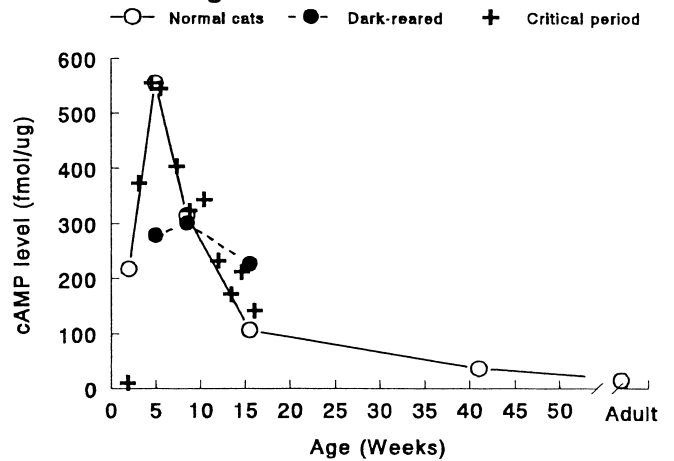## cAMP follows OD plasticity in light and dark-reared cats

Fig. 4 Levels of cAMP produced by the general metabotropic glutamate agonist, ACPD, in the cat visual cortex, follow the critical period for ocular dominance plasticity closely. Levels are lower in dark-reared animals at 5 weeks of age, and higher in dark-reared animals at 15 weeks of age.

week dark-reared animals, similar to normal at 9 weeks of age, and higher than normal at 15 weeks of age,[35] just as ocular dominance plasticity is less than normal, equal to, and greater than normal at these ages.[10]

We have also found that inhibitors of PKA reduce ocular dominance shifts sufficiently that they are almost totally blocked (Fig. 5).[13] This result is also obtained without having a significant effect on visual responses or signal/noise ratio in the visual cortex. However, ocular dominance shifts are not reduced or blocked by inhibitors of PKG or inhibitors of PKC (Fig. 5). The result with inhibitors of PKG agrees with previous results, showing that inhibitors of nitric oxide synthase also do not affect ocular dominance shifts (nitric oxide produces cGMP, which activates PKG).[37,38] Thus there is a distinct difference between plasticity in the visual cortex and plasticity in the hippocampus as far as the role of protein kinases is concerned.
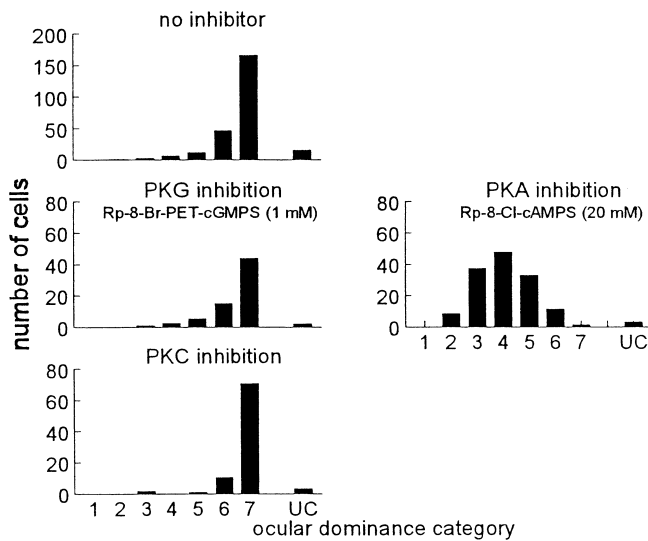
Fig. 5 Ocular dominance shift in 25–31d cats monocularly deprived for 5 days (top left). Infusion of a PKA inhibitor (Rp-8-Cl-cAMPS at 20 mM from an Alzet osmotic minipump) blocks the ocular dominance shift (right). Infusion of a PKG inhibitor (Rp-8-Br-PET-cGMPS at 1 mM) does not (middle left), nor does infusion of a PKC inhibitor (either chelerythrine chloride at 166 : M, or an inhibitory myristolated peptide at 4 mM – see bottom left).

Restoration of Plasticity

All of these results lead to the following hypothesis: that activity coming from the two eyes releases glutamate in the cortex, which activates NMDA receptors, which lets calcium into the cell, which activates calcium-stimulated adenylate cyclase, which produces cAMP, which activates PKA and leads to protein synthesis for the degradation of some synapses and the formation of others. If this simple hypothesis were true, then application of a PKA agonist or an NMDA receptor agonist late in the critical period might accentuate plasticity. This result has been found to be not true in our laboratory with application of the agonist Sp-8-Cl-cAMPS at 15 weeks of age.[13] Other authors have seen a change in binocularity, although not a shift from one eye to the other, with application of forskolin and dibutyryl cAMP, which both activate PKA.[39] The experiment has not been tried with agonists acting at the NMDA receptor.

There are several reasons why restoration of plasticity might be difficult. It may not be sufficient simply to activate all PKAs or all NMDA receptors: the PKAs or NMDA receptors might have to be activated at the correct place at the correct time. Moreover, there may be pathways acting in parallel with each other. For example, NMDA receptors and voltage-gated calcium channels both let calcium into the cell, and metabotropic glutamate receptors release it from intracellular stores. We found that MK801, at doses that did not affect the visual response, reduced the ocular dominance shift but did not block it altogether. This suggests that other routes to change in calcium concentration may also play a role. On the other hand, PKA antagonists did block the ocular dominance shift totally. This is consistent with our observation that antagonists to other protein kinases did not affect the ocular dominance shifts. Levels of calcium and cAMP may be a point in the biochemical pathways for ocular dominance plasticity where signals converge, as has been suggested for plasticity in the hippocampus.[40]

Summary and Future Directions

In summary, we have shown that both NMDA receptors and levels of cAMP produced by metabotropic glutamate receptors follow the critical period for ocular dominance plasticity in the cat visual cortex closely. Rearing in the dark affects these substances, as it does ocular dominance plasticity. Antagonists to NMDA receptors reduce ocular dominance plasticity while antagonists to the protein kinase activated by cAMP, protein kinase A, block ocular dominance plasticity altogether. We suggest from this that various receptors and channels converge to affect calcium in the cell, and calcium in turn affects cAMP. If this is true, then the calcium-stimulated adenylate cyclases, AC1 and AC8, which are found primarily in the brain, should also be a crucial factor that will block ocular dominance plasticity totally, rather than just reduce it. We are currently testing this with double mutants of AC1 and AC8 kindly provided by Dr Daniel Storm.

The next question is: how does activation of PKA lead to protein synthesis for degradation of some synapses and production of others? This is an important question where research is just starting to make a contribution. PKA is known to have both immediate and long-term actions on synaptic function. In the short term, PKA can up or down-regulate the activity of both excitatory and inhibitory neurotransmitter receptors through direct interactions.[41,42] In the longer term, PKA is likely to modulate the expression of specific genes by directly controlling the activity of transcription factors such as the calcium/cAMP response element binding protein (CREB).[43] Alternately, PKA could control gene transcription by acting through the phosphorylation of other kinases such as the mitogen-activated protein kinases that have been shown to control gene expression[44] and that have been implicated in synaptic plasticity in other regions of the brain.[45,46] Indeed, there is evidence that CREB-mediated gene transcription in the visual cortex of CRE-lacZ transgenic mice is upregulated by periods of monocular deprivation.[47] Beyond that, not much is known. The

field is currently an exciting one, because it has relevance to clinical problems, is relevant to plasticity in other parts of the nervous system, and is being done in a system where behavior, anatomy, and physiology can be correlated. While much has been done, there are clearly a lot of open questions.

References

1. LeVay S, Stryker MP, Shatz CJ: Ocular dominance columns and their development in layer IV of the cat's visual cortex: a quantitative study. J Comp Neurol 1978; 179: 223–244

2. LeVay S, Wiesel TN, Hubel DH: The development of ocular dominance columns in normal and visually deprived monkeys. J Comp Neurol 1980; 191: 1–51

3. Wiesel TN, Hubel DH: Single cell responses in striate cortex of kittens deprived of vision in one eye. J Neurophysiol 1963; 26: 1003–1017

4. Dews PB, Wiesel TN: Consequences of monocular deprivation on visual behaviour in kittens. J Physiol 1970; 206: 437–455

5. Hubel DH, Wiesel TN: The period of susceptibility to the physiological effects of unilateral eye closure in kittens. J Physiol 1970; 206: 419–436

6. Olson CR, Freeman RD: Profile of the sensitive period for monocular deprivation in kittens. Exp Brain Res 1980; 39: 17–21

7. Jones KR, Spear PD, Tong L: Critical periods for effects of monocular deprivation: differences between striate and extrastriate cortex. J Neurosci 1984; 4: 2543–2552

8. Daw NW, Fox K, Sato H, Czepita D: Critical period for monocular deprivation in the cat visual cortex. J Neurophysiol 1992; 67: 197–202

9. Cynader M, Mitchell DE: Prolonged sensitivity to monocular deprivation in dark-reared cats. J Neurophysiol 1980; 43: 1026–1040

10. Mower GD: The effect of dark rearing on the time course of the critical period in cat visual cortex. Brain Res Dev Brain Res 1991; 58: 151–158

11. Kleinschmidt A, Bear MF, Singer W: Blockade of "NMDA" receptors disrupts experience-dependent plasticity of kitten striate cortex. Science 1987; 238: 355–358

12. Daw NW, Gordon B, Fox KD, Flavin HJ, Kirsch JD, Beaver CJ, Ji Q, Reid SN, Czepita D: Injection of MK-801 affects ocular dominance shifts more than visual activity. J Neurophysiol 1999; 81: 204–215

13. Beaver CJ, Ji Q, Fischer QS, Daw NW: Cyclic AMP-dependent protein kinase mediates ocular dominance shifts in cat visual cortex. Nat Neurosci 2001; 4: 159–163

14. Bear MF, Singer W: Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature 1986; 320: 172–176

15. Carmignoto G, Canella R, Candeo P, Comelli MC, Maffei L: Effects of nerve growth factor on neuronal plasticity of the kitten visual cortex. J Physiol 1993; 464: 343–360

16. Gillespie DC, Crair MC, Stryker MP: Neurotrophin-4/5 alters responses and blocks the effect of monocular deprivation in cat visual cortex during the critical period. J Neurosci 2000; 20: 9174–9186

17. Huh GS, Boulanger LM, Du H, Riquelme PA, Brotz TM, Shatz CJ: Functional requirement for class I MHC in CNS development and plasticity. Science 2000; 290: 2155–2159

18. Stryker MP, Harris WA: Binocular impulse blockade prevents the formation of ocular dominance columns in cat visual cortex. J Neurosci 1986; 6: 2117–2133

19. Mayer ML, Westbrook GL, Guthrie PB: Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. Nature 1984; 309: 261–263

20. Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A: Magenesium gates glutamate-activated channels in mouse central neurones. Nature 1984; 307: 462–465

21. Hebb DO: The Organization of Behaviour. New York, Wiley, 1949

22. Gordon B, Daw NW, Parkinson D: The effect of age on binding of MK-801 in the cat visual cortex. Brain Res Dev Brain Res 1991; 62: 61–67

23. Fox K, Sato H, Daw N: The location and function of NMDA receptors in cat and kitten visual cortex. J Neurosci 1989; 9: 2443–2454

24. Fox K, Daw N, Sato H, Czepita D: Dark-rearing delays the loss of NMDA-receptor function in kitten visual cortex. Nature 1991; 350: 342–344

25. Swindale NV: Absence of ocular dominance patches in dark-reared cats. Nature 1981; 290: 332–333

26. Bear MF, Kleinschmidt A, Gu QA, Singer W: Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. J Neurosci 1990; 10: 909–925

27. Miller KD, Chapman B, Stryker MP: Visual responses in adult cat visual cortex depend on N-methyl-D-aspartate receptors. Proc Natl Acad Sci USA 1989; 86: 5183–5187

28. Roberts EB, Meredith MA, Ramoa AS: Suppression of NMDA receptor function using antisense DNA block ocular dominance plasticity while preserving visual responses. J Neurophysiol 1998; 80: 1021–1032

29. Frey U, Huang YY, Kandel ER: Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science 1993; 260: 1661–1664

30. Weisskopf MG, Castillo PE, Zalutsky RA, Nicoll RA: Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP. Science 1994; 265: 1878–1882

31. Akers RF, Lovinger DM, Colley PA, Linden DJ, Routtenberg A: Translocation of protein kinase C activity may mediate hippocampal long-term potentiation. Science 1986; 231: 587–589

32. Zhuo M, Hu Y, Schultz C, Kandel ER, Hawkins RD: Role of guanylyl cyclase and cGMP-dependent protein kinase in long-term potentiation. Nature 1994; 368: 635–639

33. Reyes-Harde M, Potter BVL, Galione A, Stanton PK: Induction of hippocampal LTD requires nitric-oxide-stimulated PKG activity and Ca2+ release from cyclic ADP-ribose-sensitive stores. J Neurophysiol 1999; 82: 1569–1576

34. Dubnau J, Tully T: Gene discovery in Drosophila: new insights for learning and memory. Annu Rev Neurosci 1998; 21: 407–444

35. Reid SN, Daw NW, Gregory DS, Flavin H: cAMP levels increased by activation of metabotropic glutamate receptors correlate with visual plasticity. J Neurosci 1996; 16: 7619–7626

36. Reid SN, Romano C, Hughes T, Daw NW: Developmental and sensory-dependent changes of phosphoinositide-linked metabotropic glutamate receptors. J Comp Neurol 1997; 389: 577–583

37. Reid SN, Daw NW, Czepita D, Flavin HJ, Sessa WC: Inhibition of nitric oxide synthase does not alter ocular dominance shifts in kitten visual cortex. J Physiol 1996; 494: 511–517

38. Ruthazer ES, Gillespie DC, Dawson TM, Snyder SH, Stryker MP: Inhibition of nitric oxide synthase does not prevent ocular dominance plasticity in kitten visual cortex. J Physiol 1996; 494: 519–527

39. Imamura K, Kasamatsu T, Shirokawa T, Ohashi T: Restoration of ocular dominance plasticity mediated by adenosine 3′,5′-

monophosphate in adult visual cortex. Proc R Soc Lond B Biol Sci 1999; 266: 1507–1516

40. Bailey CH, Bartsch D, Kandel ER: Toward a molecular definition of long-term memory storage. Proc Nat Acad Sci USA 1996; 93: 13445–13452
41. Blackstone C, Murphy TH, Moss SJ, Baraban JM, Huganir RL: Cyclic AMP and synaptic activity dependent phosphorylation of AMPA-preferring glutamate receptors. J Neurosci 1994; 14: 7585–7593
42. Poisbeau P, Cheney MC, Browning MD, Mody I: Modulation of synaptic GABAA receptor function by PKA and PKC in adult hippocampal neurons. J Neurosci 1999; 19: 674–683
43. Montminy M: Transcriptional regulation by cyclic AMP. Annu Rev Biochem 1997; 66: 807–822
44. Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G, Storm DR: Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. Neuron 1998; 21: 869–883
45. English JD, Sweatt JD: A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. J Biol Chem 1997; 272: 19103–19106
46. Impey S, Obrietan K, Storm DR: Making new connections: role of ERK/MAP kinase signalling in neuronal plasticity. Neuron 1999; 23: 11–14
47. Pham TA, Impey S, Storm DR, Stryker MP: CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. Neuron 1999; 22: 63–72