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Extracellular Matrix and Visual Cortical Plasticity: Freeing the Synapse

Minireview

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The effects of monocular deprivation (MD) on the ocular dominance of visual cortical neurons are a paradigmatic example of experience-dependent plasticity. Here we review recent data showing that extracellular matrix (ECM) plays an important role in the control of experience-dependent plasticity both in the developing and adult visual cortex.

A classical test for the plasticity of visual cortical connections is monocular deprivation (MD) (Berardi et al., 2003). In the mammalian binocular visual cortex, neurons are activated to different degrees by visual stimuli presented to one eye or the other, a property called ocular dominance (OD). If vision is normal for both eyes during development, the majority of visual cortical neurons are binocular. If one eye is occluded during development, visual cortical neurons become dominated by the nondeprived eye. This change in OD is taken as a sensitive index of plasticity of visual connections. OD plasticity is particularly high during a critical period of postnatal development and declines with age (Berardi et al., 2000; Hensch, 2004).

The first step in the cascade of events leading to the full shift of OD is the detection of the imbalance of activity between the afferents to a cortical neuron. At this stage, intracortical inhibition and NMDA receptors are the key players: block of NMDA receptors blocks MD effects (Bear et al., 1990; Daw et al., 1999; Roberts et al., 1998), while transgenic mice lacking the 65 kDa isoform of the GABA synthesizing enzyme (GAD65) show a deficient response to MD that can be rescued if GABA transmission is enhanced by benzodiazepines (Hensch et al., 1998).

A second step is the activation of inter- and intracellular signaling triggering both local changes in synaptic transmission (Heynen et al., 2003) and changes of gene expression. The signaling molecules likely involved are neurotrophins and the kinases PKA, ERK, and CaMKII. These pathways converge on gene transcription involving the transcription factor CREB (Berardi et al., 2003). Ultimately, this molecular machinery leads to long-lasting changes in neuronal circuitry, both functional and structural.

Up to a couple of years ago, structural changes were considered to be inessential for the full shift in OD. Indeed, a shift in OD distribution, which was assumed to be initiated in layer IV, is evident very shortly after MD onset and is saturated after a few days both in cats and rodents (Gordon and Stryker, 1996; Olson and Freeman, 1975). Anatomical changes in thalamocortical arborization, terminating in layer IV, are evident only much later, a week in the cat and more than a month in the mouse (Antonini et al., 1999; Antonini and Stryker, 1993). Thus, structural changes where thought to consolidate the functional ones, making OD changes more stable and more difficult to revert.

Recently, it has been shown that the initial physiological changes induced by MD in cortical circuits take place outside layer IV (Trachtenberg et al., 2000), and analysis of intracortical connectivity has suggested that anatomical rearrangements in response to altered visual experience can be found after 2 days in strabismic cats (Trachtenberg and Stryker, 2001). The necessity for protein synthesis in the visual cortex, but not in the LGN, at very early stages of the changes in OD (Taha and Stryker, 2002) strengthens the hypothesis that rapid anatomical rearrangements in extragranular layers, but not in layer IV, might accompany the very first changes in OD.

Two nicely related papers, published in this issue of *Neuron*, have now clarified this point, showing that these rapid morphological changes of intracortical circuitry occur at the level of dendritic spines. Indeed a brief MD during the critical period causes rapid changes in spine motility (Oray et al., 2004) and density (Mataga et al., 2004) in the binocular portion of the primary visual cortex, which is precisely where OD changes take place. In addition, the effect of a brief MD on spine dynamics is not present in layer IV, in striking correlation with the observation that the first changes in OD occur outside layer IV (Trachtenberg et al., 2000). The relation between a rapid change in spine density and the changes in OD is further strengthened by the observation that in GAD65 KO mice brief MD are ineffective in shifting OD (Hensch et al., 1998) or decreasing spine density (Mataga et al., 2004). Diazepam treatment, which rescues OD plasticity, also rescues spine plasticity (Mataga et al., 2004).

Spine plasticity could therefore be the earliest structural component of the effects of MD during the critical period, and its occurrence would be almost contextual with the functional changes in OD.

The mechanisms by which spine plasticity is controlled are still unclear. Factors that modify the actin cytoskeleton, such as the Rho family of small GTPases, are able to control spine dynamics, which is regulated by the rate of actin polymerization (Matus et al., 2000). However, it is becoming clear that the extracellular environment, and in particular the ECM, plays an important role in controlling spine dynamics and visual cortical plasticity.

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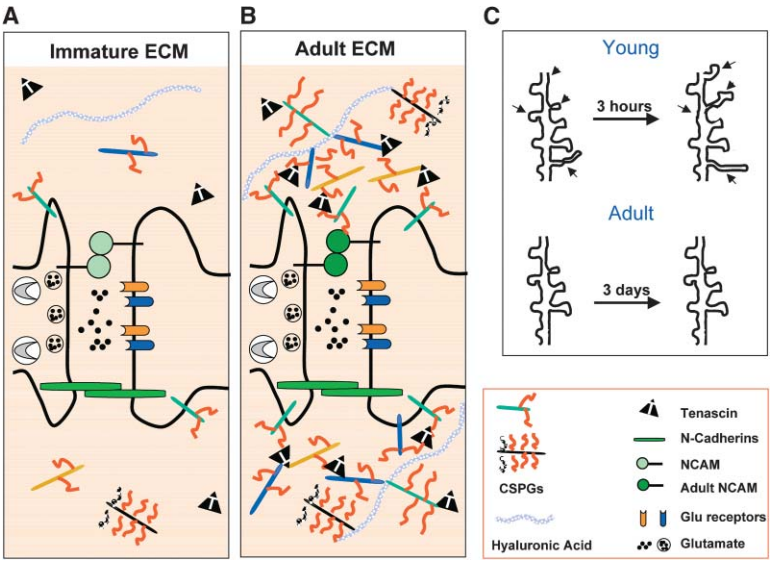

Figure 1. Changes in ECM and Spine Dynamics during Visual Cortex Development

(A and B) Developmental changes in ECM in the visual cortex. Note that as development proceeds the molecular composition of the ECM changes and ECM components (CSPGs, hyaluronic acid, tenascins) condense to form nets around neural processes (see text for details). (C) Representation of dendritic spine dynamics in the visual cortex (modified from Grutzendler et al., 2002). In young mice (1 month old) during the critical period, spines are highly dynamic (changes in spines are marked by arrows); in the adult, they are very stable.

ECM and Visual Cortical Plasticity

A substantial amount of brain volume consists of extracellular space interposed between brain cells. This space is filled with a matrix of molecules that are linked between themselves and with membrane bound molecules. These interactions are key determinants of the mechanical properties of brain tissue and are also able to activate intracellular signaling pathways. A number of studies, recently reviewed by Dityatev and Schachner (2003), have involved elements of this network, such as integrins, cadherins, NCAM, tenascins, and heparin-sulfate proteoglycans, in synaptic plasticity (LTP and LTD) and in learning and memory processes. Recent studies have shown an important role for key components of the brain ECM, the chondroitin-sulfate proteoglycans (CSPGs), in OD plasticity of the visual cortex.

CSPGs are major components of the ECM of the CNS and comprise a core protein and chondroitin-sulfate glycosaminoglycan chains. CSPGs are inhibitory for axonal sprouting, and after injury they are upregulated in the CNS, with the effect of blocking axon regeneration (Bradbury et al., 2002; Silver and Miller, 2004). During development, CSPGs condense at high concentration in lattice-like structures, designated perineuronal nets (PNNs), which completely ensheath visual cortical neurons (Figures 1A and 1B). PNNs are fenestrated at sites of synaptic contact, where they assume a perisynaptic localization (Dityatev and Schachner, 2003). The process of condensation of CSPGs into PNNs begins during late development and is completed after the end of the critical period. Dark rearing, which prolongs critical period closure, also prolongs CSPG condensation (Hockfield et al., 1990). Degradation of CSPGs from the adult visual cortex with the enzyme chondroitinase ABC reactivates ocular dominance plasticity in monocularly deprived adult rats, suggesting that the CSPG-enriched adult ECM exerts a powerful inhibitory control on OD plasticity (Pizzorusso et al., 2002).

This control could occur at the level of inhibitory interneurons, around which most of the current studies localize PNNs, or at the level of excitatory neurons, as suggested by the presence of CSPG-containing nets also around pyramidal neurons and their spines (Hockfield et al., 1990). Spine dynamics in the visual cortex declines over development. A recent in vivo study showed that filopodia-like dendritic protrusions, extending and retracting over hours, are abundant in young animals but quite rare in the adult (Grutzendler et al., 2002) (Figure 1C). A developmental decline has also been observed for spine motility (Konur and Yuste, 2004; Majewska and Sur, 2003; Oray et al., 2004). Mataga et al., show that MD in adult mice is totally ineffective in reducing spine density (Mataga et al., 2004). These results indicate that spines, initially plastic during development, become remarkably stable in the adult. The inhibitory nature of the mature ECM could be one of the factors at the basis of this remarkable stability.

The influence of the extracellular environment on OD plasticity is not limited to the adult cortex and occurs also during the critical period. Pharmacological inhibition of the extracellular protease tPA hampers visual cortical plasticity (Mataga et al., 1996; Muller and Griesinger, 1998), and MD is ineffective in mice with genetic deletion of the *tPA* gene (Mataga et al., 2002). Plasticity can be rescued in tPA knockout mice by the exogenous administraton of tPA during the period of MD. The link between tPA and experience-dependent plasticity is strengthened by the observation that, in wild-type animals, MD elicits a fast and transient increase of tPA activity (within 2 days from MD onset) during the critical period but not in the adult (Mataga et al., 2002).

The released tPA increases extracellular proteolysis directly or by the activation of plasmin from the zymogen plasminogen. These proteases have a wide spectrum of targets, including CSPGs (Wu et al., 2000), growth factors and neurotrophins (Pang et al., 2004; Yuan et al., 2002), membrane receptors (Nicole et al., 2001), and cell adhesion molecules (Endo et al., 1999), and the available information is not sufficient to dissect which of these targets must be cleaved by tPA/plasmin for plasticity to proceed. However, converging data reported in Oray et al. (2004) and Mataga et al. (2004)

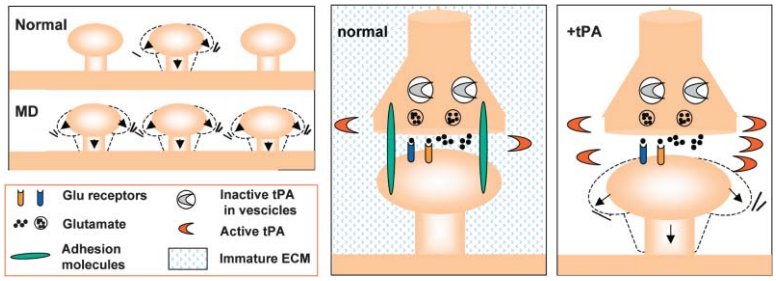

Figure 2. Visual Experience and Extracellular Proteolysis Control Spine Dynamics

(Left) The basal level of spine motility present during the critical period is increased by 2 days of monocular deprivation. (Right) Exogenous tPA, by cleavage of adhesion molecules and other components of the immature ECM, increases spine motility. The effects of monocular deprivation on spine motility occludes further tPA effects, suggesting that tPA is a mediator of MD effects on spine dynamics.

clearly make a case for these tPA/plasmin targets being key regulators of dendritic spine dynamics in the visual cortex. Oray et al. applied tPA on visual cortical slices and observed a dramatic increase of spine motility in all cortical layers (Figure 2). Then, tPA was applied to slices obtained from MD animals and it was found that the effects of tPA were not additive with the effects of MD, suggesting that tPA is a mediator of MD action on spine motility. Mataga et al. show that tPA action is also needed for MD-induced changes in spine density. Counting spines on dendrites of layer III pyramids, the authors find that the decrease of spine density caused by 4 days of MD is not present in tPA knockouts and this effect could be rescued by exogenous tPA.

What could be the mechanism by which tPA promotes spine retraction? Previous work has shown that an increase in tPA activity could lead to activity-dependent growth of synapses (Baranes et al., 1998), production of perforated postsynaptic spines (Neuhoff et al., 1999), and increased LTP (Baranes et al., 1998; Madani et al., 1999). This led to the idea that tPA could be needed to make space for new synaptic contacts (Baranes et al., 1998; Mataga et al., 2002). The newly obtained data suggest a different picture in which tPA is permissive for structural plasticity but does not specify the sign of the plastic change. In this model (Figure 3, top), MD causes the release of tPA, and tPA reaches the synapses corresponding to both the deprived and the nondeprived eye. The sign of the ensuing change, whether it will be the retraction or protrusion of spines, will then be spatially and temporally dependent upon activity and the local molecular environment. Active synapses would be protected from the elimination triggered by tPA, while inactive synapses would loose their postsynaptic spines. To explain this, the authors propose that activity would endow active synapses with forms of adhesion molecules which are insensitive to tPA, while the adhesion molecules of inactive synapses could be cleaved by tPA\plasmin, causing initially spine motility and, eventually, spine retraction (Mataga et al., 2004). A spatially restricted release of tPA inhibitors by active synapses could also participate to preserve them. It is worth remembering that activation of tPA in response to MD is secondary to detection of activity imbalance and thus dependent upon a correct balance between intracortical inhibition and excitation (Mataga et al., 2002, 2004). Thus, tPA release would signal the presence of the unbalanced input activity, but the repertoire of adhesion molecules of each synapse would determine whether that synapse should be eliminated or preserved. No

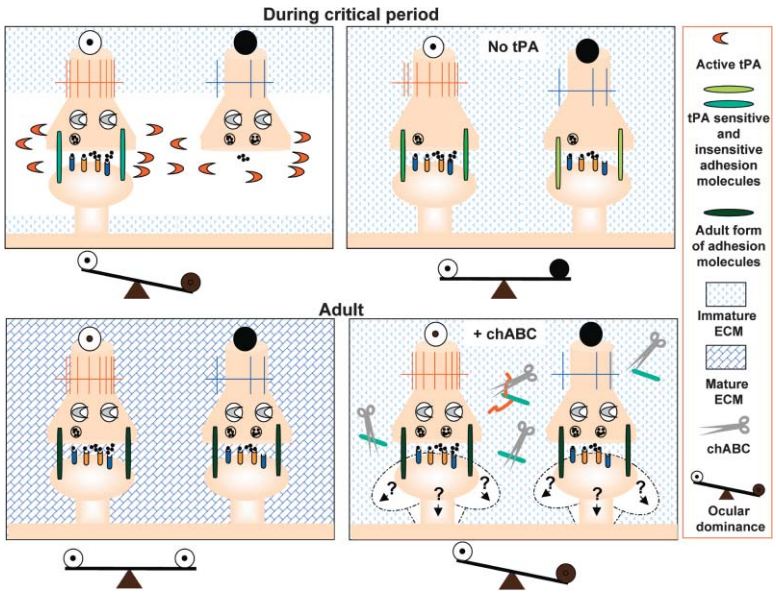

Figure 3. ECM and Experience-Dependent Plasticity: Mechanisms of Action

(Top left) During the critical period, the imbalance in electrical activity caused by MD activates tPA release. It is suggested that activity would endow active synapses with forms of adhesion molecules that are insensitive to tPA, while the adhesion molecules of inactive synapses could be cleaved by tPA, initially causing spine motility and eventually causing spine retraction. As a result, the OD of visual cortical neurons (represented by the schematic scale where the deprived and nondeprived eye are in equilibrium) changes in favor of the nondeprived eye. (Top right) In tPA knockout mice, MD during the critical period still causes a turnover of adhesion molecules from the tPA-insensitive to the tPA-sensitive form at the inactive synapses, but in absence of tPA, no cleaving action takes place and there is no change in spine dynamics and in OD following MD. Changes at the level of synaptic receptor composition could still be present. (Bottom left) In the adult, the ECM is strongly nonpermissive for morphological rearrangements, and tPA is no longer activated following MD. As a result, no change in spine dynamics or in OD are present. (Bottom right) CSPGs are degradated by the exogenous supply of the enzyme chondroitinase ABC (chABC, the scissors); this might reinstate the control of MD on spine dynamics and explain the effectiveness of chABC in reinstating OD plasticity to the adult visual cortex.

change is observed in the monocular portion of the visual cortex because no unbalance of input activity is present in this part of the cortex and tPA is not released.

This model describes the regulation of structural plasticity in the visual cortex during the critical period. In the adult visual cortex, two major differences are apparent: the ECM becomes enriched in components nonpermissive for structural rearrangements such as CSPGs, and tPA is no longer activated by MD (Figure 3, bottom). This could explain the lack of MD effectiveness on OD plasticity in the adult and the dramatic developmental reduction of spine dynamics. Following this logic, the effects of chondroitinase ABC, which reactivates OD plasticity in the adult, could be explained with the removal of the ECM components nonpermissive for spine plasticity and the generation or rearrangement of synaptic connections.

In conclusion, it is now emerging that the intracellular cascades activated in the visual cortex by visual experience could culminate in producing molecules of the ECM that set a fine balance between synapse stabilization, retraction, and growth. In the adult, this delicate balance is disrupted because the ECM becomes strongly inhibitory for structural plasticity and protease release does not take place. The reactivation of OD plasticity in the adult obtained with degradation of CSPGs suggests that this balance might be reactivated when these two obstacles are removed.

Acknowledgments

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