Cortical development: **With an eye on neurotrophins**
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Recent observations suggest that neurotrophins are involved in activity-dependent plasticity of the developing cerebral cortex. What molecular mechanisms underlie activity-dependent competition between axons for trophic factors?

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Sensory stimulation profoundly influences the formation of appropriate connections in the developing cerebral cortex. The most compelling evidence for this came from the pioneering work of David Hubel and Torsten Wiesel [1], who first described the effects of monocular deprivation on the organization of 'ocular dominance' columns in the developing visual cortex (Fig. 1). (Ocular dominance columns are alternating regions of the primary visual cortex that receive input preferentially from one or the other eye.) The formation of ocular dominance columns, and their reorganization following monocular deprivation, can be prevented by blocking neuronal activity. These observations led to the hypothesis that neuronal-activity-dependent mechanisms underlie the development and plasticity of geniculocortical connections (reviewed in [2]). There has been intense interest in identifying such mechanisms, as they are likely to be involved both in the formation of appropriate cortical connections during development and in the adaptive responses of the brain such as learning. A number of recent observations suggest that neurotrophins, identified initially as regulators of neuronal differentiation and survival, mediate important aspects of activity-dependent cortical plasticity.

The notion that trophic factors are involved in sculpting patterns of connections during development is itself not new. The idea is that ingrowing axons compete for limited amounts of target-derived trophic factors, and that those axons which do not successfully compete are eliminated by cell death. It is also thought that the more active inputs might have a competitive advantage, and that such mechanisms may underlie the shift in ocular dominance seen after monocular deprivation. Despite the simplicity of this model, direct supporting evidence has been difficult to come by. The nature of the trophic factors in the visual cortex for which ingrowing thalamic axons compete has not been clear, and the mechanism by which neuronal activity might confer a competitive advantage is not known. In the past few years, however, a connection has been made between neuronal activity and the action of neuronal growth factors which may well turn out to be central to mechanisms of activity-dependent plasticity.

Neurotrophins are regulated by neuronal activity
Neurotrophins are a family of neuronal growth factors that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), NT4/5 and NT6 (reviewed in [3]). These factors have been most extensively characterized with regard to their role in neuronal differentiation and survival. The possibility of a link between neurotrophins and neuronal activity first became apparent with the discovery that there was a marked increase in the expression of NGF and BDNF in the hippocampus and cortex following experimentally induced seizures (reviewed in [4]). Subsequent experiments on primary cultures of hippocampal and cortical neurons indicated that excitatory and inhibitory neurotransmitters had opposing effects on the expression of BDNF, and that induction of BDNF by excitatory amino-acid stimulation required calcium influx *via* either glutamate receptors of the N-methyl-D-aspartic acid (NMDA) receptor class or voltage-sensitive calcium channels (VSCCs).

Figure 1

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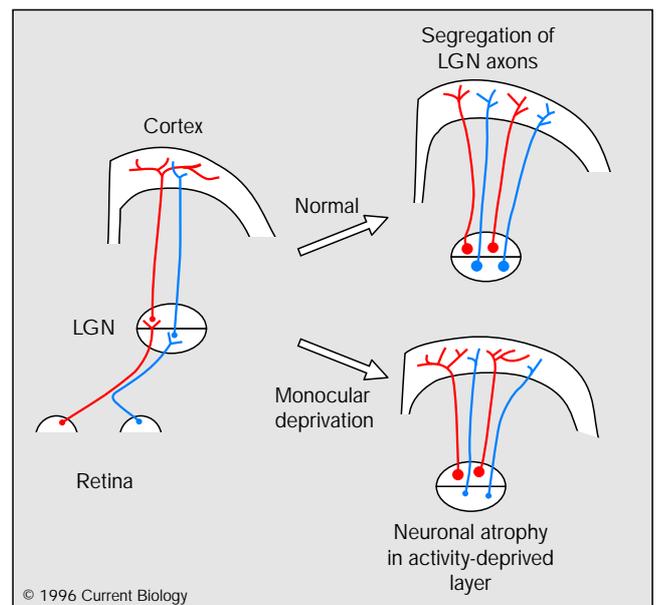
Diagrammatic representation of the development of ocular dominance columns and their reorganization following monocular deprivation.

The first evidence that the activity-dependent regulation of trophic factors is of physiologic consequence came from experiments exploring the mechanisms of activity-dependent survival of cortical neurons *in vitro* [5]. In this cellular model, VSCC activation led to an increase in the survival of cortical neurons in culture and an increase in BDNF expression, but this survival could be completely prevented by neutralizing BDNF antibodies. These experiments therefore suggested that one mechanism of activity-dependent cell survival involved the regulation of BDNF expression by calcium channel activation.

Neurotrophins can influence the development of thalamocortical connections

The discovery that neuronal activity can regulate the expression of neurotrophins, together with the known growth-promoting effects of neurotrophins in the peripheral nervous system, suggested that neurotrophins participate in the activity-dependent rearrangement of thalamocortical axons. Some of the first evidence that neurotrophins may influence visual cortical plasticity came from the work of Lamberto Maffei and his colleagues [6], who demonstrated that the physiological shift in ocular dominance distribution could be prevented by infusion of NGF into the cortex during the critical period. The mechanism of this effect, however, is unlikely to involve a direct action on thalamic neurons, as they do not express high-affinity NGF receptors (Trk), and instead is more likely to be mediated by the effects of NGF on the basal forebrain cholinergic projection.

Neurons in the lateral geniculate nucleus (LGN) do express receptors for the neurotrophins BDNF (Trk-B), NT-3 (Trk-C) and NT-4/5 (Trk-B), and evidence that these factors can influence patterns of connectivity in the cortex has come from Carla Shatz's laboratory. Shatz and colleagues reported last year that infusion of BDNF or NT-4/5 (but not NGF or NT-3) during the critical period could prevent the formation of ocular dominance columns in the cat visual cortex [7]. This finding was of importance, not only because it indicated that neurotrophins can influence the patterning of projections within the cortex, but also because it suggested that thalamic axons normally compete for limiting amounts of trophic factors. These experiments did not, however, address the role of neurotrophins in activity-dependent plasticity, as the observed effects could also be explained by a growth-promoting action of BDNF or NT-4/5 on thalamic axons within layer 4, independent of neuronal activity.

The involvement of neurotrophins in activity-dependent events has recently been tested more directly by examining the influence of exogenously applied neurotrophins on the development of LGN neurons in monocularly deprived ferrets [8]. As in the cat, monocular deprivation in the ferret causes not only a redistribution of geniculocortical axons within layer 4, but also leads to an atrophy of the LGN cell bodies that receive input from the deprived eye. To test whether neurotrophins could overcome such atrophy, Larry Katz and his colleagues [8] injected fluorescent microspheres coated with neurotrophins into the visual cortex of ferrets at the onset of monocular deprivation. These microspheres were taken up locally by the LGN axon terminals and transported back to the cell bodies, allowing for unambiguous detection of LGN cells exposed to the neurotrophin-coated beads. An analysis of such retrogradely labelled LGN neurons indicated that, while most neurotrophins had no detectable effect, NT-4/5 was effective in preventing, to a large extent, the atrophy of LGN neurons. These observations suggest that LGN neurons may normally compete for cortex-derived NT-4/5, and that the atrophy of LGN neurons following monocular deprivation may indeed be due to a competitive disadvantage that the silent inputs have in accessing or responding to NT-4/5.

Although these are exciting observations, suggesting that competition for BDNF or NT-4/5 may be involved in activity-dependent plasticity in the developing visual cortex and providing some of the first direct evidence linking neurotrophins to plasticity in the cortex, there are still many outstanding issues that need to be resolved. For example, it is implicitly assumed in these studies that the neurotrophins are acting directly on thalamic axon terminals. This may turn out to be the case, but it should be noted that, at present, there is no evidence to favour that interpretation over the possibility that the neurotrophins act on cortical neurons which in turn influence the development of thalamic axons or cell bodies. This possibility is particularly important to consider, as it has recently been reported that neurotrophins, including NT-4/5 and BDNF, can have marked effects on the morphology of cortical neurons [9]. These effects include an increase in dendritic complexity and dendritic spine density, which could provide additional synaptic sites for thalamic inputs and thereby lead to decreased competition among thalamic axons. To rule out such indirect effects of neurotrophins on the development of thalamic neurons, it will be important to demonstrate direct trophic effects of these factors on thalamic projection neurons.

Even if it is convincingly demonstrated that neurotrophins can act directly on thalamic axons to regulate their growth and survival, it needs to be determined whether BDNF and/or NT-4/5 are indeed the endogenous factors that participate in activity-dependent plasticity in the cortex. With the availability of mice that carry targeted disruptions of the BDNF and/or NT-4/5 genes, experimental evidence that addresses this issue should be forthcoming. In addition, it should be possible to inhibit the action of endogenous neurotrophins (perhaps by using neutralizing

antibodies) in cats or ferrets, to evaluate their effects on thalamocortical development and plasticity.

Finally, the electrophysiologic consequences of modulating neurotrophins on ocular dominance plasticity in the cortex need to be examined. It will be particularly important to examine the acute effects of neurotrophins on cortical electrophysiology, in the light of recent reports that synaptic physiology may be modulated by the brief application of neurotrophic factors [10–12].

Mechanisms of activity-dependent competition for trophic factors

If thalamic axons compete for cortex-derived BDNF or NT-4/5 during development, then how does neuronal activity confer a competitive advantage? One can imagine a number of molecular mechanisms by which neuronal activity might influence how well a neuron competes for a target-derived trophic factor. For example, neuronal activity might modulate the binding and uptake of neurotrophins by their receptors, active inputs may induce the localized release of neurotrophins at synaptic sites, or the ability of an axonal input to respond to a target-derived trophic factor may be modulated by neuronal activity.

One mechanism by which the activity of a neuron may influence its trophic response is by the activity-dependent regulation of neurotrophin receptor expression. In support of this possibility, it has been shown that depolarization leads to an increase in Trk receptor expression in the sympathoadrenal cell line MAH, and this level of induction appears to be sufficient to confer an NGF response on the cells [13]. It is not yet known whether stimulation of the visual pathways influences neurotrophin receptor expression in the LGN, which would have immediate implications for the mechanisms of activity-dependent competition for trophic factors.

A recent set of experiments on the survival of retinal ganglion cells (RGCs) *in vitro* suggests an alternative mechanism. Barbara Barres and her colleagues [14] have used purified cultures of RGCs to examine their trophic requirements, and report that although various growth factors, including BDNF, insulin-like growth factor 1 (IGF1) and ciliary neurotrophic factor (CNTF), show limited trophic action on RGCs, their influence is enhanced several-fold when the cells are simultaneously treated with forskolin or potassium chloride. The increase in trophic effects of a purified growth factor caused by these agents appears to be mediated by an increase in intracellular cAMP, as they are blocked by inhibitors of the cAMP-dependent protein kinase. Similarly, in PC12 cells, the ability of NGF to elicit neurite outgrowth is enhanced by simultaneous depolarization of the cells with increased extracellular potassium chloride (A.G. and M.E. Greenberg, unpublished observations).

This enhancement does not involve a change in the levels of Trk receptor expression. Thus, depolarizing stimuli appear to enhance the response of certain neuronal populations to neurotrophic stimulation.

If such observations were to be generalized to geniculocortical development, one would predict that the response of thalamic inputs to target-derived factors such as BDNF and NT-4/5 may be modulated by their levels of activity. Together with the evidence that neural activity can regulate the expression of BDNF, one can postulate a mechanism for activity-dependent competition in the developing visual cortex (Fig. 2). In this model, the activity of thalamocortical afferents regulates the expression of BDNF (and perhaps NT-4/5) in the cortex. (There is evidence that visual stimulation can regulate BDNF expression in the adult cortex [15], but the effects of visual activity of BDNF expression during the critical period have not been reported.) The BDNF thus produced and released locally may then act to ensure survival of the post-synaptic cell, and would also be available to thalamic axons for uptake. The efficacy with which the thalamic axons respond to the neurotrophins may in turn be regulated by their levels of activity, thereby conferring a growth advantage to the active inputs.

Figure 2
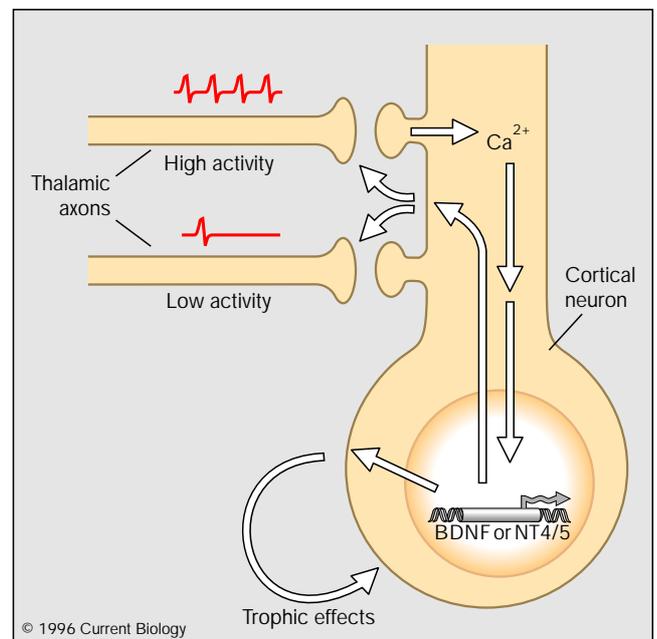
A possible mechanism by which neurotrophins mediate activity-dependent plasticity in the visual system. In this model, the production of neurotrophins by the post-synaptic cell is regulated by trans-synaptic activity, and the response of a thalamic axon to the target-derived trophic factors is proportional to its level of activity.

Although elements of such a model may well be correct, the mechanisms of cortical plasticity are unlikely to be this simple. For example, just as evidence has been accumulating that neuronal activity can influence the expression of neurotrophins, it is becoming increasingly clear that neurotrophins can modulate synaptic transmission [10–12]. Such a role for neurotrophins complicates the interpretation of the neurotrophin infusion experiments [7,8]. Although it is easier to interpret the results of those experiments as reflecting the direct effects of neurotrophins on the growth and survival of thalamic axons, the factors may act in part by altering levels of activity in the cortex. If so, are BDNF and NT-4/5 influencing thalamocortical development primarily by modulating neuronal activity (which, by definition, regulates activity-dependent plasticity)?

Finally, one must be cautious not to over-interpret the role of activity-dependent competition for trophic factors in cortical plasticity, as it does not explain certain central observations regarding synaptic plasticity in the developing cortex. The behaviour of thalamocortical connections appears to follow the Hebbian principle that correlated activity between the pre- and post-synaptic cells leads to synaptic strengthening. A striking demonstration of this principle came from experiments in Mike Stryker's laboratory [16], which showed that pharmacologically suppressing the activity of post-synaptic cortical neurons strengthens inputs from the deprived eye. In this experiment, infusion of the gamma aminobutyric acid (GABA) agonist, muscimol, led to the effective strengthening of connections driven by the deprived eye and a relative weakening of open eye inputs.

If active inputs have a competitive advantage in responding to target-derived trophic factors, why would the inputs driven by the open eye lose out to those from the deprived eye in the experimental paradigm used by Stryker and colleagues [16]? One could think of ways of elaborating on the model to incorporate this observation, perhaps by drawing a distinction between synaptic plasticity and anatomical plasticity. But such model-building is likely to be much more fruitful once we have experimental evidence that addresses some of the many unresolved issues regarding the involvement of neurotrophins in thalamocortical development. Are we really on the verge of formulating a molecular framework for activity-dependent cortical plasticity? With all the excitement that the recent observations have generated, we can hope that the necessary evidence will not be long in coming.

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