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Neurotrophins and Neuronal Plasticity

Hans Thoenen

There is increasing evidence that neurotrophins (NTs) are involved in processes of neuronal plasticity besides their well-established actions in regulating the survival, differentiation, and maintenance of functions of specific populations of neurons. Nerve growth factor, brain-derived neurotrophic factor, NT-4/5, and corresponding antibodies dramatically modify the development of the visual cortex. Although the neuronal elements involved have not yet been identified, complementary studies of other systems have demonstrated that NT synthesis is rapidly regulated by neuronal activity and that NTs are released in an activity-dependent manner from neuronal dendrites. These data, together with the observation that NTs enhance transmitter release from neurons that express the corresponding signal-transducing Trk receptors, suggest a role for NTs as selective retrograde messengers that regulate synaptic efficacy.

Neurotrophic factors have been considered predominantly with respect to their functions in regulating the survival and differentiation of selective populations of neurons during embryonic development and the maintenance of specific functions of those neurons in adulthood (*1*). The spectrum of the biological actions of neurotrophins (NTs) [the general term coined for members of the nerve growth factor (NGF) gene family] is determined by the site and extent of their expression (*2*) and of the expression of the corresponding receptors (*1*, *3*) (Fig. 1). The delicate equilibrium between the availability of NTs and the survival and maintenance of specific populations of neurons becomes impressively apparent in NT knockout mice (*4*–*7*). For instance, in mice in which the gene encoding NT-3 has been targeted by homologous recombination, the inactivation of even one allele of the NT-3 gene reduces NT-3 mRNA amounts by about half and results in a massive reduction of cutaneous mechanoreceptors and of the corresponding end organs, the Merkel cells (*8*). Accordingly, in the peripheral sensory and sympathetic nervous systems, specific populations of neurons are supported by specific NTs, and the inactivation of the genes encoding them results in serious characteristic defects of the sensory and sympathetic nervous systems (*4*–*7*). In contrast, in the central nervous system (CNS) there is much overlap in the trophic support of individual neurons (*9*, *10*). The disruption of the expression of an individual factor by gene targeting does not result in changes as dramatic as those in the periphery (*4*–*7*). Therefore, the results of NT gene targeting experiments in the CNS may, at first sight, appear disappointing, showing at best a reduced expression of choline acetyltransferase (*4*) or of selective neuropeptides and calcium-binding proteins (*6*), which are known to be regulated by different NTs (*6*, *9*, *11*, *12*). However, such gene targeting experiments offer the possibility of identifying subtle NT effects that refine neuronal functions, such as activity-dependent neuronal plasticity. "Neuronal plasticity" is used to describe a great variety of changes in neuronal structure and function, but its use here is confined exclusively to activity-dependent, prolonged functional changes, accompanied by corresponding biochemical and possibly morphological alterations. Indeed, there is increasing evidence that NTs are involved in specific aspects of neuronal plasticity. This evidence originates from observations made in complex integrated systems in vivo, in particular of the influence of prolonged administration of NTs and of corresponding antibodies (Abs) on the development of the rat and cat visual cortex, the most extensively studied and well-understood area of the mammalian cortex (*13*). Although these observations did not reveal the underlying mechanisms, including the identification of individual neurons involved in NT-mediated plasticity, they nevertheless made NTs attractive candidates for the performance of essential roles in the development and activity-dependent modification of neuronal circuits. However, increasingly detailed analyses of the activity-dependent regulation of NT synthesis (*9*), the mechanism and site of NT release from neurons (*14*, *15*), and the presynaptic modulation by NTs of the release of classical transmitters (*15*–*19*) have recently appeared, which are promising with regard to development of a molecular understanding of the more complex integrated systems of established physiological relevance.

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Activity-Dependent Regulation of NT Synthesis in the CNS

In contrast to the peripheral nervous system, in the CNS NTs are synthesized predominantly by neurons under physiological conditions (*2*, *9*) and, at least in the case of brain-derived neurotrophic factor (BDNF) and NGF, the amounts of these NT mRNAs are regulated by neuronal activity (*9*, *20*) in addition to hormonal influences (*9*, *11*, *21*). Combinations of in vitro and in vivo analyses, performed mostly in the rat hippocampus and cerebral cortex, have demonstrated that the activity-dependent regulation is mediated by classical neurotransmitters. Up-regulation is effected by glutamate via *N*-methyl-D-aspar-

tate (NMDA) and non-NMDA receptors and, with different relative prevalence during development, also by acetylcholine via muscarinic receptors (*9, 22*). Down-regulation is mediated predominantly by γ-aminobutyric acid (GABA) via $GABA_A$ receptors (*9, 22, 23*). More important, it has become apparent that this activity-dependent regulation not only functions under extreme experimental conditions, such as initiation of seizures (*20, 24*), but that these regulatory mechanisms also are involved in the maintenance of normal physiological amounts of NGF and BDNF (*22*). However, neither NT-3 nor NT-4/5 is directly regulated by neuronal activity (*9*). Moreover, the synthesis of NTs, in particular BDNF, is regulated by physiological stimuli such as visual input (*25*). Specifically, blockade of sensory input to the visual cortex by intraocular injection of tetrodotoxin or by dark-rearing results in a rapid down-regulation of BDNF mRNA (*25*), and exposure of dark-reared animals to light rapidly restores the layer-specific BDNF mRNA amounts in the rat visual cortex (*25*). Because dark-rearing dramatically influences the functional development of the visual cortex (*26*), it is of particular interest that, when newborn rats are raised in the dark, the developmental increase of BDNF in the visual cortex is reduced, indicating that visual input is essential for the developmental regulation of BDNF in the visual system (*25*). Further examples of highly specific regulation of NT synthesis by physiological or subtle experimental stimuli are the up-regulation of BDNF in the paraventricular nucleus by osmotic stress (*27*) and by stimuli designed to initiate long-term potentiation (LTP) (*28*).

Activity-Dependent Secretion of NTs and Sites of Release

In the context of the evaluation of the potential functions of NTs in neuronal plasticity, it is important to characterize NT secretion from neurons. In the periphery, NGF is synthesized in a great variety of nonneuronal cells (*1, 2, 29*). The regulation of synthesis and release is independent of neuronal input (*30*). NGF is secreted according to the constitutive calcium-independent pathway (*31*). In the CNS, as first demonstrated for NGF (*14, 15*) and recently also for BDNF (*32*), NTs are secreted by neurons in both constitutive and activity-dependent pathways. In hippocampal slices and primary cultures of hippocampal neurons, the activity-dependent secretion of NTs initiated by high potassium or by veratridine, glutamate, or carbachol depends on extracellular sodium but is independent of extracellular calcium (*14, 15, 32*). However, it does depend on intact intracellular calcium stores, and their depletion by thapsigargin or blockade by dantrolene results in a drastic reduction of activity-dependent NT release, which is also blocked by the high-affinity calcium chelator 1,2-bis(2-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, tetra(acetoxymethyl)-ester (BAPTA-AM), which penetrates the plasma membrane (*14, 32*) and neutralizes the action of calcium released from intracellular calcium stores. Thus, the activity-mediated sodium-dependent secretion of BDNF and NGF is unusual and shows characteristics that are distinctly different from those of the regulated secretion of neurotransmitters and neuropeptides (*33*) and the activity-dependent secretion of other proteins, such as acetylcholinesterase (*34*).

Immunohistochemical localization of NGF by confocal microscopy has demonstrated that NGF is not only localized in the perikaryon but also in all neuronal processes (*15*). The pattern of distribution is compatible with storage of NGF in endoplasmic reticulum–like compartments. However, the resolution attainable by light microscopy is unsatisfactory, and ultrastructural analysis remains mandatory in order to delineate the relation between the storage compartments from which NTs are released and the internal (intact) calcium stores that are necessary for activity-dependent NT release (*14, 15, 32*).

After more precise information on the ultrastructural localization of NTs is established, further analyses will examine their possible co-localization with other well-characterized secretory molecules, such as secretogranin-II. Abs to secretogranin-II stain not only large dense-core vesicles but also dendrites (which do not contain large dense-core vesicles) of hippocampal neurons (*35*). Moreover, in spite of the unconventional characteristics of the activity-dependent NT release mechanism, it nevertheless seems to share mechanisms with well-established, conventional regulated secretion (*33*). For instance, in preliminary experiments it has been demonstrated that neuronal NGF secretion is stimulated by cyclic adenosine 3′,5′-monophosphate (cAMP) derivatives, which penetrate the cell membrane (*36*). Conversely, cAMP antagonists interfere with activity-dependent release, and an involvement of synaptobrevin or synaptobrevin-like molecules (*37*) can be deduced from the observation that tetanus toxin also blocks the activity-dependent secretion of NGF (*36*).

It recently has become possible to identify the sites of activity-dependent NGF release (*15*). A high-affinity monoclonal antibody (mAb) that exclusively recognizes the nondenatured, biologically active NGF (*14, 15*) was used to reveal the sites of release after depolarization of cultured hippocampal neurons by high potassium or by glutamate (Fig. 2). Given that BDNF shows the same secretion characteristic (*32*) as NGF, it is expected that the sites of release are also very similar.

Enhanced Release of Classical Neurotransmitters by NTs

In order to more precisely understand the function of NTs in neuronal plasticity, a comprehensive evaluation of the effects of neuronally released NTs is mandatory. In this context, discussion of the different mechanisms involved in prolonged facilitation of neurotransmission (that is, LTP) (*28*) is of particular interest. The existence and identity of the putative "retrograde factor" or factors released as a result of postsynaptic activation are a matter of controversy (*38*). The different NTs have specific pat-

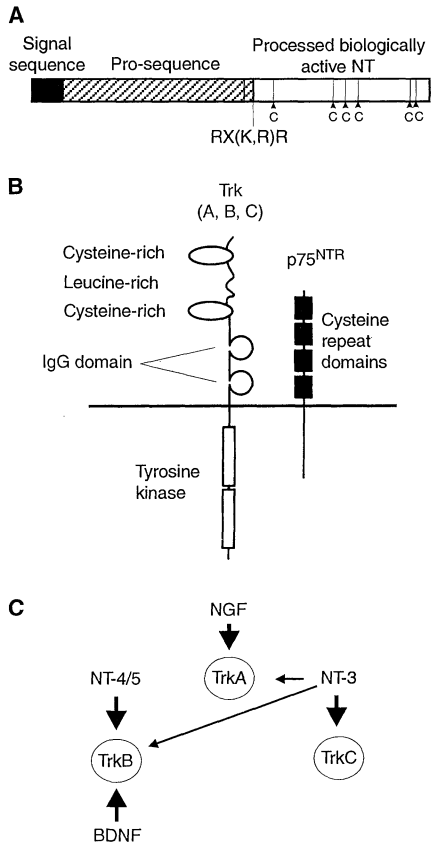

Fig. 1. NTs and their receptors. All the members of the NGF gene family (NTs) are synthesized as precursors (**A**) and are processed at classical dibasic cleavage sites into mature biologically active NTs that contain about 50% conserved domains. Those domains are mainly arranged around the strictly conserved cysteine (C) residues, which form three disulfide bridges that are essential for the three-dimensional structure and biological activity of NTs (*1*). K, lysine; R, arginine. The NT effects are mediated via Trk (A, B, or C) tyrosine kinase receptors and by $p75^{NTR}$ (**B**). The individual NTs (NGF, BDNF, NT-3, and NT-4/5) are assigned to individual Trk receptors (**C**). All NTs have the same affinity to $p75^{NTR}$, which enhances the affinity and increases the specificity of the individual NTs to their Trk receptors (*3*). The significance of the Trk-independent signal transduction via $p75^{NTR}$ is a matter of controversy and intense research (*3*).

terns of expression, as do the corresponding Trk receptors (*2*, *3*), and therefore NTs cannot be the retrograde messengers that enhance synaptic transmission in general, as is postulated for NO, CO, and arachidonic acid (*28*, *38*). However, NTs might have a more limited and specific function as retrograde modulators. Indeed, in co-cultures of embryonic *Xenopus* spinal neurons with myotubes, the administration of BDNF and NT-3, but not NGF, initiates an increase in spontaneous synaptic currents, reflecting an enhanced acetylcholine release from presynaptic terminals (*16*). That NTs have similar effects in the CNS can be deduced from observations that, in synaptosomal preparations of the rat hippocampus, NTs enhance the release of acetylcholine and glutamate (*17*), and that BDNF and NT-3 enhance spontaneous synaptic activity in primary cultures of hippocampal neurons (*18*) and also enhance the synaptic transmission evolving from the stimulation of the Schaffer collaterals in hippocampal slices (*19*). Injection of NTs into the hippocampus of behaving rats immediately initiated characteristic electrical activities resulting from neurotransmitter release (*15*, *39*). The electrical activity started at the injection site and propagated to the contralateral hippocampus and then to both cortices (*39*). The effects initiated by NGF were predominantly cholinergic in nature and could be blocked by muscarinic receptor antagonists. For BDNF, the glutamatergic effects dominated and could be blocked by corresponding antagonists (*39*). All of these observations indicate an enhancement of transmitter release from presynaptic nerve terminals that presumably express the appropriate Trk receptors. As is consistent with this interpretation, all of these effects can be blocked by K252A, a tyrosine-kinase inhibitor with a preference, although not absolute specificity, for Trk receptor tyrosine kinases (*40*). The proposal that transmitter release is facilitated by NTs via presynaptic Trk receptors is further supported by the observation that NTs initiate an immediate increase in intracellular calcium concentrations (*15*, *41*, *42*), an increase that is dependent on extracellular calcium (*42*), which is a prerequisite for activity-dependent release of classical neurotransmitters (*33*). Interestingly, hippocampal neurons, kept in culture for several weeks, exhibit spontaneous oscillation of intracellular calcium concentrations in their dendritic processes. These periodic calcium transients are acutely enhanced by BDNF administration (Fig. 2).

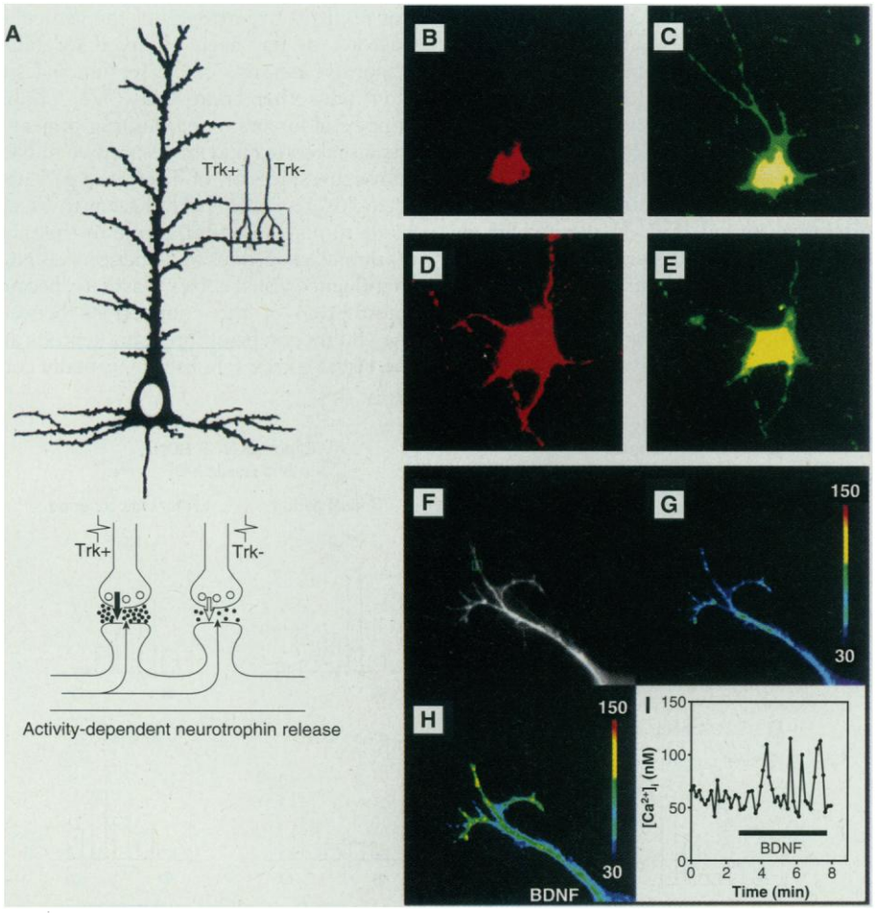

Fig. 2. Activity-dependent release of NTs from dendrites and their function as retrograde messengers. (**A**) Schematic presentation of NT-synthesizing neuron receiving input from a neuron that expresses Trk receptors at its synaptic endings (Trk+) and a neuron that does not (Trk−). Activity-dependent release of NT from the dendrite has a positive feedback effect on the Trk+ nerve terminal, enhancing the efficacy of this synapse (solid arrow, lower panel). (**B** to **E**) Constitutive and activity-dependent release of NGF from hippocampal neurons. Constitutive release, which can be blocked by lowering the temperature below 15°C (*14*, *15*), becomes apparent predominantly at the surface of the perikaryon (B). In contrast, glutamate-evoked NGF release occurs throughout the processes, including dendrites (D). All experiments were performed in hippocampal cultures that were previously transfected with an NGF construct containing a reporter gene [chloramphenicol acetyltransferase (CAT)] in order to identify the transfected neurons. The experimental procedures were performed as described (*14*, *15*). NGF at the cell surface was visualized in native unpermeabilized cells (*15*). After photographs were taken [(B) and (D)] the same preparation was permeabilized and the CAT reporter gene was identified [(C) and (E)]. (F) to (I) show enhancement of spontaneous fluctuations of calcium transients in a dendrite by BDNF in 3-week-old cultures of hippocampal neurons. These neurons, forming an extensive network of synapse-forming neuronal processes, were loaded with fura-2 (*15*, *41*). The changes in internal calcium concentrations $[Ca^{2+}]_i$ are indicated in nanomolar concentrations represented by a color scale [(G) and (H)] ranging from 30 to 150 nM. (**F**) Fluorescent picture of a fura-2–loaded neuron; the point of observation is boxed. (**G**) Calcium transients in the absence of BDNF. (**H**) Calcium transients in the presence of BDNF. (**I**) Time course of oscillating calcium transients in the boxed domain of the dendrite in the absence and presence of BDNF.

Involvement of NTs in Neuronal Plasticity in Organotypic Integrated Systems in Vitro

The NT-mediated enhanced release of conventional transmitters from neurons expressing the appropriate Trk receptors strongly suggests a function of NTs as retrograde messengers, enhancing synaptic efficacy in selective neuronal systems. Indeed, in hippocampal slices, exogenous administration of NTs resulted in enhanced synaptic efficacy in the CA3-CA1 system (*19*). Local administration of BDNF resulted in enhanced CA1 field potentials, induced by stimulation of Schaffer collaterals (*19*). Conversely, recent experiments have demonstrated that in BDNF knockout mice, the LTP in the same system is drastically reduced (*43*). The frequency with which a successful LTP response could be elicited was reduced from 90% in controls to 30% in BDNF-deficient animals. This LTP impairment was not restricted to homozygous mutant mice but was also present in heterozygotes (*43*), in which the hippocampal

BDNF mRNA amounts were reduced to about 50%, demonstrating that gene dosage–dependent reductions of NTs may result in distinct functional impairments in the CNS as well. This impairment of LTP became apparent while the basic pharmacological parameters of neuronal transmission remained unchanged (*43*). The specificity of the function of BDNF in the modulation of LTP is further supported by the recent observation that adenovirus-mediated BDNF gene transfer into CA1 neurons of hippocampal slices of BDNF knockout mice restored LTP in this system (*44*).

Involvement of NTs in Neuronal Plasticity of the Visual Cortex in Vivo

The information collected from the experimental systems summarized above is compatible with the interpretation that NTs may be essential for neuronal plasticity. In spite of gaps in the detailed understanding of the molecular mechanisms, information that is already available may contribute to understanding of the spectacular changes that result from the prolonged administration of NTs to the visual cortex of rats (*13, 45*) and cats (*46–48*) at different developmental stages. Connectivity in the visual cortex can be manipulated by change in the pattern of visual input—for instance, by monocular deprivation during restricted periods of development (*49*). Monocular deprivation renders neurons of the visual cortex, most of which are normally binocular, nonresponsive to stimuli presented to the deprived eye (ocular dominance shift) (Fig. 3). The expansion of the connections contributed by the nondeprived eye depends critically on the activation of neurons via NMDA receptors (*50*), and it also is modulated by cholinergic, serotoninergic, and adrenergic mechanisms (*51*). Maffei and coworkers (*45*) demonstrated that in rats the intraventricular injection of NGF during the critical period of ocular dominance plasticity, when cortical neurons are sensitive to manipulations of visual experience, prevented the effect of monocular deprivation (Fig. 3). One possible interpretation is that NGF affects the development of cortical neurons by interfering with normal synaptic transmission. However, because other visual cortical functions such as visual acuity and orientational selectivity (*52*) developed normally in these animals, it seems more likely that NGF precociously stabilizes the neuronal connections that are responsible for the binocular response. Recently, Cabelli and co-workers reported that in developing kittens the local infusion of BDNF and NT-4/5 (but not NT-3 and NGF) prevented the formation of ocular dominance columns (*47*). Whether this process is due to a sprouting of geniculocortical afferents or to a blockade of normal development is not yet known.

The dramatic effects of NTs on the visual system are of particular interest, as this system can be manipulated experimentally in a very precise manner (*53*) by changes in the visual input (*49, 53*). Although the observations made do not permit an understanding of the underlying mechanisms, the physiological relevance is supported by recent reports from the laboratories of Maffei and Shatz that blockade of endogenous NTs by mAbs to NGF or by TrkB immunoglobulin G (IgG) fusion proteins (which block the effects of BDNF and NT-4/5) had dramatic effects on the development of the visual cortex (*54, 55*). The implantation of hybridoma cells secreting blocking Abs to NGF into the lateral ventricle of rats in the postcritical period (Fig. 3) resulted in an extension of the time period during which the binocularity of visual cortical neurons can still be influenced by monocular deprivation (*54*). This situation is a mirror image of the effects of the intraventricular administration of NGF during the critical period (*45*) (Fig. 3). Moreover, the neutralization of endogenous NGF by mAbs also resulted in a reduction in the size of the neuronal cell bodies of the lateral geniculate body that project to the visual cortex (*54*). However, this seems to be an indirect effect, because these neurons do not express TrkA receptors and, accordingly, labeled NGF injected into the rat visual cortex is not retrogradely transported to the cell bodies in the lateral geniculate body (*56*). Moreover, the prolonged administration of NGF did not result in hypertrophy of the geniculate neurons, as has been observed for NGF-responsive neurons in the septum and striatum and other brain areas (*57*). Cholinergic basal forebrain neurons that project to the visual cortex and are responsive to NGF [through expression of TrkA and p75 neurotrophin receptor (NTR)] seem to be unlikely to play an essential role in the modulation of exogenous and endogenous NGF to influence visual cortex plasticity, because interruption of the connections between the cholinergic basal forebrain neurons and the visual cortex (abolishing virtually com-

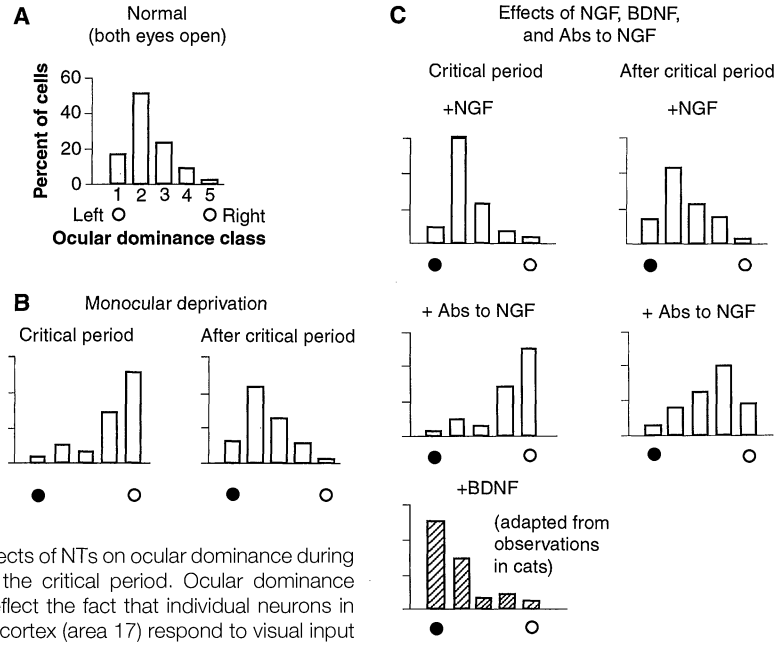

Fig. 3. Effects of NTs on ocular dominance during and after the critical period. Ocular dominance classes reflect the fact that individual neurons in the visual cortex (area 17) respond to visual input from the left and right eyes in a different manner (*49, 64*). Class 1 and 5 neurons respond exclusively to visual input from the left (class 1) or right (class 5) eye. Class 2 and 4 neurons respond to visual input from both left and right eyes, but with a preference for the left (class 2) or right (class 4) eye. Neurons of class 3 respond strictly binocularly—that is, they respond equally to visual input from right and left eyes (*64*). Besides the classification from 1 to 5, which has been chosen for reasons of simplicity, there are also more subtle classifications from 1 to 7 (*49*). (**A**) During the critical period [in rats from about postnatal day (P) 15 to 45 and in cats from about P28 to P72], the responsiveness of these neurons can be shifted so that the response is turned toward the open eye, and this shift in responsiveness remains fixed after reopening of the eye after the critical period. (**B**) Data analyzing the effects of NGF and Abs to NGF on the changes in ocular dominance caused by monocular deprivation of rats are adapted from references (*45, 54*). (**C**) The side of eye closure is indicated by filled circles. Because no data on the effects of BDNF in rats are available, data obtained in cats (*48*) were adapted to those in rats, with the assumption that BDNF would have similar effects in rats. This seems to be justified, because the effects of NGF in cats appear to correspond to those in rats, at least with respect to visual acuity (*46*).

pletely the retrograde labeling of septal cholinergic neurons from the visual cortex) did not mimic the effects of Abs to NGF on the morphology of the lateral geniculate neurons (*13*). However, it should be borne in mind that in cats the extensive cytotoxic destruction of basal forebrain neurons, resulting in a massive decrease of choline acetyltransferase activity in the visual cortex, had no effect on ocular dominance plasticity, whereas blockade of muscarinic receptors prevented ocular dominance changes (*51*), indicating that, under these experimental conditions, ocular dominance plasticity is only abolished if the cholinergic block is complete.

The local administration of BDNF to the visual cortex of kittens during the critical period prevented the formation of ocular dominance columns (*47*) and produced a paradoxical shift of the responsiveness of cat visual cortical neurons toward the deprived eye (*48*) (Fig. 3). The blockade of the formation of ocular dominance columns by BDNF infusion after monocular deprivation is thought to result from interference with the normal activity-dependent competition for BDNF by neurons (*53*) that project from the lateral geniculate nucleus to spiny neurons in layer IV. Unexpectedly, in the rat visual cortex, layer IV neurons contain particularly small amounts of BDNF mRNA and do not exhibit light-induced regulation of BDNF mRNA synthesis (*25*). Activity-dependent competition includes a variety of possible mechanisms, such as activity-dependent local release, activity-dependent regulation of synthesis, or activity-dependent uptake of BDNF. However, in the periphery, no enhancement of the retrograde transport of NGF in sympathetic neurons could be demonstrated by an augmented neuronal activity (*58*). The preventive effects of local infusion of BDNF and NT-4/5 (*47*), as well as the paradoxical shift in the cat visual cortex after monocular deprivation (*48*), alternatively may result from an activation of GABA-containing interneurons in the visual cortex. In rodents, cortical GABA-containing neurons express TrkB receptors, and the observed effects could be due to augmented GABA synthesis, together with a sprouting of these interneurons and an enhanced release of GABA, in analogy to the enhancement of neurotransmitter release from cholinergic and glutamatergic neurons (*15–18, 39*) by NTs acting via the corresponding Trk receptors. This interpretation is supported by the fact that the infusion of BDNF into the visual cortex of monocularly deprived kittens had a similar effect, as had been shown in previous experiments to be caused by the infusion of the $GABA_A$ receptor agonist muscimol (*59*).

Conclusions and Perspectives

The evidence for the involvement of NTs in neuronal plasticity evolves from observations made in complex integrated neuronal systems in vivo and from detailed analyses in a variety of in vitro systems. The local administration of NTs (*13, 45, 47, 48*) and molecules that neutralize the action of NTs (*13, 54, 55*) (blocking Abs or Trk-IgG fusion proteins) to the visual cortex of rats or cats changes the structural and functional development of the visual cortex in a dramatic manner. Most important, the continuous release of blocking Abs to NGF from implanted hybridoma cells not only antagonizes the effects of exogenous NGF but also dramatically influences the normal development of ocular dominance in a manner that is a mirror image of that of exogenously administered NGF (Fig. 3). Moreover, the development of ocular dominance columns in kittens by means of monocular deprivation is not only prevented by the local infusion of BDNF and NT-4/5 (*47*), but also by TrkB-IgG, which blocks the effect of BDNF and NT-4/5 (*55*). Thus, the effects of NTs do not only represent pharmacological actions, but clearly indicate that NTs play an essential physiological role in the activity-dependent development of the visual cortex. A major deficit in the analysis of NT function in visual cortex plasticity is the lack of precise information about which neurons are involved in the synthesis of NTs and which are the neuronal elements that respond to exogenous and (more importantly) endogenous NTs.

NGF and BDNF mRNAs are regulated in a highly specific manner by physiological stimuli in different areas of the CNS (*9, 22, 25, 27, 28*). In contrast to the situation in the periphery, they are not only released constitutively (*31*) but also by means of an unconventional activity-dependent mechanism (*14, 15, 32*). The sites of activity-dependent release include dendrites (*15*). More detailed information on the precise ultrastructural localization of the storage sites of NTs and their relation to calcium stores, which have to be intact to mediate the sodium-dependent release of NTs, is needed. It will also be essential to evaluate whether localized release of NTs from restricted domains of dendrites or even individual spines is possible.

In addition to the localization of NTs and their receptors in integrated structures of interest, such as the visual cortex and the hippocampus, "second generation" knockout procedures seem promising. They should permit restricted inactivation of NTs and their receptors in subpopulations of neurons (*60*), for example, by using the different BDNF promoters (*61*) and the choline acetyltransferase promoter (*62*) for the transgenic expression of a specific recombinase as a prerequisite for tissue-specific gene inactivation (*60*). Moreover, preliminary experiments with gene transfer by adenoviruses (*44*) look promising for the controlled overexpression of NTs in integrated systems in vitro (primary cultures and slice preparations) and in vivo (*63*), thus opening up the possibility of locally interfering with the function of NTs by the use of antisense constructs or local expression of Abs to NT and Trk-IgG molecules. Thus, the transgenic approach, which so far has been restricted to mice, can be complemented by viral-mediated gene transfer in other species, not only for the local overexpression of NTs or inhibition of their synthesis by antisense constructs but also by the expression of blocking Abs directed against NTs or their receptors.

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RESEARCH ARTICLE

Biostratigraphic and Geochronologic Constraints on Early Animal Evolution

John P. Grotzinger, Samuel A. Bowring, Beverly Z. Saylor, Alan J. Kaufman

Two distinct evolutionary pulses, represented by the Vendian Ediacaran fauna and Cambrian small shelly faunas, are generally thought to characterize the emergence of macroscopic animals at the end of Precambrian time. Biostratigraphic and uranium-lead zircon age data from Namibia indicate that most globally distributed Ediacaran fossils are no older than 549 million years old and some are as young as 543 million years old, essentially coincident with the Precambrian-Cambrian boundary. These data suggest that the most diverse assemblages of Ediacaran animals existed within 6 million years of the Precambrian-Cambrian boundary and that simple discoid animals may have appeared at least 50 million years earlier.

Early animal evolution is widely thought to have occurred in two discrete steps, set apart by tens of millions of years. Evolutionary models have had to explain an initial episode at the end of Precambrian time (Vendian Period) in which simple, mostly soft-bodied, cnidarian-grade organisms, bilaterians, and problematica (collectively referred to as the Ediacaran fauna) first appeared, followed by a second phase early in the Cambrian Period in which small shelly invertebrates (*1*) and complex trace fossils appeared in the Nemakit-Daldyn stage and rapidly diversified during the subsequent Tommotian, Adtabanian, and Botomian stages (*2–8*). Because the affinities of the earlier, Ediacaran fauna are still debated (compare *5*, *9*, *10*), the apparent wide separation in time of these two evolutionary pulses has been used to support phylogenetic arguments that these creatures are not simple precursors to later forms, but instead

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