

Neurotrophins and Dementia— Keeping in Touch

Down's syndrome patients develop dementia similar to Alzheimer's disease and show elevated levels of amyloid precursor protein in brain. Two papers in this issue of *Neuron* show that reduced retrograde transport or signaling of the neurotrophins NGF or BDNF, respectively, may account for the neuronal pathology in mouse models of Down's syndrome.

Single hepatocytes can process metabolites, and individual red blood cells can carry oxygen, but individual neurons are useless when left alone. The brain is a network of neurons, and individual neurons only work when they are in touch with other neurons through synaptic contacts. Connections between neurons are not random but are constantly remodeled and tuned to optimally reflect the external or internal environment of the organism. The first hints about how this optimization happens were revealed over half a century ago, when Hamburger and Levi-Montalcini found that peripheral sensory neurons were initially produced in excess during development (Huang and Reichardt, 2001). When their axons reached the target tissue, the majority of neurons died, and only those that had established a functional contact with the target remained. Levi-Montalcini correctly predicted that the remaining neurons survived because they had access to a diffusible factor released from the target cells in limiting amounts. Subsequent work led to the identification of this factor, nerve growth factor (NGF), and the other members of the neurotrophin family, brain-derived neurotrophic factor (BDNF) and neurotrophins 3 and 4 (Huang and Reichardt, 2001). It should be noted that the physiological role of neurotrophins is not just to keep neurons alive but also to select-out among the excessively produced competing neurons those that have made an optimal connection with the target tissue. In the brain, the innervating axon can greatly increase the release of neurotrophins from the target neurons by actively stimulating them. This ensures that the established neuronal connections and networks that they form are synaptically active. Maintenance of connections within functional neuronal networks in the adult brain requires continuous access to the trophic factor that is released by target neurons in an activity-dependently manner (Huang and Reichardt, 2001). Thus, one critical task for the individual neuron is to keep in touch with the network.

Failures in the structure or function of neuronal networks critical for cognitive processes may lead to dementia. Many patients with Down's syndrome (DS, trisomy 21, Ts21) develop dementia, which shares features with early-onset AD. At least partially, the increased risk of dementia is accounted for by the increased gene dosage of the amyloid precursor protein (App) gene, which is located in the Ts21 region. In this is-

sue of *Neuron*, two groups have investigated the role of neurotrophins in the pathophysiology of DS by analyzing mice with triplications in mouse chromosome 16 (Ts16), which carries genes homologous to those in human chromosome 21. These studies shed light not only on the role of neurotrophins in DS, but on the general neurobiology of dementia.

Neurotrophins act by binding to their receptors, either Trk family receptor tyrosine kinases or the p75 neurotrophin receptor (Huang and Reichardt, 2001). BDNF receptor TrkB is alternatively spliced into a kinase-containing isoform or a truncated, dominant-negative isoform TrkB.T1. The expression of different TrkB splice variants is associated with cognitive capacity: while overexpression of the kinase-containing form enhances memory and learning in transgenic mice (Koponen et al., 2004), overexpression of the TrkB.T1 isoform in adult neurons impairs long-term memory (Saarelainen et al., 2000). Receptor-bound neurotrophins get internalized into presynaptic terminals and are retrogradely transported to neuronal soma. Retrograde transport of target-derived growth factors is a critical process whereby the metabolic machinery of the cell soma is kept informed about the status of target innervation. The cessation of flow of the target-derived factors indicates that the neuron no longer has a functional contact with the target.

Salehi, Mobley, and coworkers (Salehi et al., 2006) have examined mouse models with a segmental trisomy 16 and analyze the retrograde transport of NGF within the basal forebrain cholinergic neurons (BFCNs), which are known to degenerate early in AD and which can be rescued by NGF (Cooper et al., 2001). They demonstrate that in Ts65Dn mice, retrograde transport of NGF is greatly diminished and this effect correlates with the reduction in number and size of BFCNs. They then provide several lines of evidence that suggest that the decreased retrograde transport of NGF is produced by the increased expression levels of App. First, they show that NGF transport is much less affected in another DS model, where App levels are not increased, and that these mice show no degeneration of the BFCNs. Second, when they cross Ts65 mice with heterozygous App null mice, resulting in a mouse with normal App levels in the Ts65 background—NGF transport is greatly improved and the degeneration of the BFCNs is prevented. Third, they examined the retrograde transport of NGF in the BFCNs in mice that overexpress either the wild-type human App or App with mutations found in early-onset AD patients and observed decreased NGF transport in these mice. Interestingly, reduced NGF transport was not correlated with an increase in A β fragment, but it did correlate with an increase in the C-terminal fragments of App. The authors went on to provide evidence that NGF and App are at least partially colocalized in early endosomes, which are considered the transport compartment, and that App-containing early endosomes are abnormally large in the BFCNs of the Ts65Dn mice. Importantly, the decreased retrograde transport of NGF was not produced by a general

dysfunction of the retrograde transport process or defects in NGF binding or internalization, indicating that the pathology may be confined to the transport of those vesicles where NGF and APP are colocalized. Taken together, the results of Salehi et al. suggest a model in which increased App levels in the early endosomes of DS and AD model mice impairs the retrograde transport of NGF and thereby brings about cholinergic neurodegeneration.

In the second paper, Dorsey, Tessarollo, and co-workers examine another DS model, trisomy 16 (Ts16) mice, which die before birth (Dorsey et al., 2006). The authors build on their previous observation that cortical neurons derived from Ts16 mice show reduced survival in culture and cannot be rescued by the addition of BDNF (Dorsey et al., 2002). Furthermore, Ts16 neurons display increased levels of the dominant-negative TrkB.T1 isoform of the BDNF receptor (Dorsey et al., 2002). In the current paper, the authors test the hypothesis that the increased expression of the TrkB.T1 causes the excess neuronal death in these mice. The authors reduced the level of TrkB.T1 in Ts16 mice by intercrossing the Ts16 mice and mice heterozygous for a targeted deletion of the T1 isoform, thus generating mice with normal TrkB.T1 levels on the Ts16 background. Remarkably, correction of the abnormal T1 levels rescues the cortical neurons, restores the resting intracellular calcium levels in these neurons, and normalizes their responsiveness to BDNF. Importantly, reduction of the TrkB.T1 to normal levels also reduces the increased apoptosis in the cortex of Ts16 mouse embryos *in vivo* to the level of euploid control mice. These data are consistent with a model where increased expression of TrkB.T1 in Ts16, through a dominant-negative action or other mechanisms, prevents neurons from taking advantage of the trophic support provided by BDNF, which leads to degeneration and death of cortical neurons. Although the authors do not comment on other features of the Ts16/Ts16^{+/-} mouse phenotype, their data suggest that normalization of the expression of a single gene can restore a normal neuronal phenotype in the embryonic brain, which is remarkable given that Ts16 produces a triplication of hundreds of genes.

Alzheimer's disease (AD) is characterized by neuritic plaques, neurofibrillary tangles, and loss of neurons, especially of BFCNs. However, early signs of AD include loss of synapses and compromised axonal transport (Stokin et al., 2005), while neuronal death occurs only later. Interestingly, increased content of the TrkB.T1 and decreased catalytic TrkB has been observed in AD patients (Ferrer et al., 1999), which suggest that, similarly to the Ts16 mouse, neurons in AD cannot take full advantage of BDNF. Furthermore, the bulk of available evidence suggests that brain BDNF content is diminished in cortex and hippocampus of AD patients. In contrast, cortical levels of NGF are normal or elevated in most studies, while decreased levels have been reported in BFCNs (Scott et al., 1995), which is consistent with failed retrograde transport of NGF in AD.

Although retrograde transport of NGF may fail in both DS and AD, the underlying mechanisms are likely to be different. Namely, App overproduction is not

common in AD, but A β accumulation results from abnormal processing of App or impaired A β degradation. Nevertheless, the results of Salehi and coworkers emphasize axonal transport as an Achilles' heel of neurons: inadequate retrograde transport blocks the access of the cell soma to the trophic factor provided by the target even if the target was functionally innervated, which in turn may lead to degeneration. BFCNs can be kept alive in Ts65Dn mice by injecting NGF into the brain (Cooper et al., 2001), but this treatment does not replace the critical role of the target-derived factor, which is the maintenance of functional innervation. Indeed, providing neurons with the trophic factor from a source other than the target may actually reduce the tendency of the remaining neurons to maintain or regain their target innervation. Thus, neuronal death in AD may be a consequence of a disconnection between neuronal soma and target rather than its cause.

The common message of both of these papers is that the inhibition of signaling or transport of a single neurotrophic factor may be partially responsible for the neuronal pathology observed in DS mouse models, and the same mechanisms may be affected also in AD. Several questions remain open, however, and will no doubt be subjects of further studies. Is the expression of TrkB.T1 also increased in Ts65Dn mice, which survive to adulthood? Would intercrossing of Ts65Dn mice with the TrkB.T1 heterozygous mice influence the survival of BFCNs and the behavioral phenotype of these mice? Since the TrkB gene is not located in mouse chromosome 16, the mechanism that leads to increased TrkB.T1 expression in the brain of Ts16 mice remains unclear but may involve the regulation of alternative splicing of the TrkB gene. Better understanding of the splicing process might reveal mechanisms that could become targets for novel drug therapy. Furthermore, many issues in the role of App in NFG transport need further clarification. Do the C-terminal fragments of App resulting from α versus β secretase cleavage equally interfere with NGF transport? If not, it may be possible to rescue the transport defect in DS by pharmacologically influencing the cleavage. Do App and NGF interact in the early endosomes, and what is the mechanism? Is TrkB also retrogradely transported in the same endosomes, and might TrkB.T1 contribute to the phenotype of these mice? Finally, designing and analyzing new animal models that address the role of TrkB.T1 in AD may further facilitate our understanding of the role of neurotrophin signaling in neurodegenerative diseases.

These two papers, together with other recent observations (Capsoni et al., 2000), indicate a revived interest in the role of neurotrophic factors in dementia. Although the results of these studies are still far from suggesting any new therapeutic strategies for DS, it might be possible that drugs influencing App processing could in the future help to restore the retrograde transport of NGF and thereby cognitive symptoms in DS. In any case, the papers provide interesting insights into the pathophysiology of DS and underline the notion that the primary aim of treatment of neurodegenerative disorders is not only to keep neurons alive but also to keep them connected.

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Rustling Synaptic Vesicle Cargo after Exocytosis

In this issue of *Neuron*, Voglmaier et al. provide new evidence that the retrieval of synaptic vesicle transporters after exocytosis proceeds along at least two different endocytic pathways. This work provides new insight into the mechanisms of sorting synaptic vesicle cargo at the cell surface.

The most abundant excitatory neurotransmitter in the central nervous system is glutamate, which is packaged into synaptic vesicles via a family of vesicular glutamate transporters (VGLUTs). Three highly homologous VGLUT genes in the mammalian CNS have been identified, each with very similar biochemical transport properties. Although the similarity in these proteins suggests redun-

dancy in function, the intriguing spatial distribution of these transporters may indicate unique roles for each transporter. Several groups have demonstrated that VGLUT1 and -2 show nonoverlapping, complementary expression in the adult mouse brain (reviewed in Takamori, 2006). Previous work by the research groups of Robert Edwards and Roger Nicoll shed light on the unique roles of VGLUTs by demonstrating that during development different isoforms of VGLUTs may be expressed within an individual cell, sorted into separate vesicle populations, and released under different phases of exocytosis (Fremeau et al., 2004).

Voglmaier et al. (2006) (this issue of *Neuron*) continue to explore the functional differences between different VGLUT isoforms. The authors, along with two other groups (De Gois et al., 2006; Vinatier et al., 2006), identified an interaction between endophilin and a polyproline (PP)-rich sequence at the C terminus of VGLUT1. Endophilin is known to be involved in synaptic vesicle recycling from the plasma membrane (Guichet et al., 2002). To examine the influence that endophilin has on VGLUT1 endocytosis, Voglmaier et al. fused the pH-sensitive form of GFP, pHluorin, to the luminal region of VGLUT1 and expressed it in hippocampal cultures. Since the lumen of the synaptic vesicle is acidic and pHluorin is essentially nonfluorescent at low pH, the pHluorin-tagged constructs brighten upon exocytosis and dim after endocytosis when the vesicle reacidifies (Miesenböck et al., 1998). VGLUT-pHluorin and Δ PP VGLUT1-pHluorin (without the PP sequence that binds endophilin) show similar levels of exo- and endocytosis at low frequency and relatively short duration stimulations. However, when stimulated at higher frequencies and for longer durations, more of the Δ PP VGLUT1 mutant becomes stranded on the cell surface than wild-type VGLUT1. At the cessation of stimulation, endocytosis of both constructs proceeds rapidly at similar rates. Using pharmacological tools to rule out effects on exocytosis and/or vesicle acidification, the authors demonstrated that the differences in fluorescent signal observed with the various VGLUT1 constructs were due to alterations in endocytosis. Moreover, by removing the domains of endophilin and VGLUT1 that interact with each other and replacing them with exogenous interacting domains, the authors were able to reconstitute wild-type rates of endocytosis during prolonged stimulation. Taken together these results strongly suggest that the interaction between endophilin and VGLUT1 is important for the retrieval of vesicular proteins (at least a subset), but only during prolonged high-frequency trains of action potentials, and that the endophilin-VGLUT1 interaction is not crucial for poststimulus retrieval.

Although not a major point in the paper, the authors also show that VGLUT1-pHluorin's fluorescent signal decayed faster than the widely used SV snare VAMP-pHluorin. If the reported difference in decay rates following exocytosis reflects endocytosis (rather than lateral diffusion of the protein or some other factor), then this result suggests distinct routes of internalization for the different proteins. Since VAMP and VGLUT1 are eventually repackaged at the correct stoichiometry into new SVs, but internalized via different routes, cargo mixing and sorting must occur at later steps.