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Center Stage for NGF in Peripheral (but Not Central) Sensory Neuron Outgrowth

Nerve growth factor (NGF) has long been known to be required for the survival, axonal growth, and differentiation of cultured sensory and sympathetic neurons. Similarly, data from the NGF and the *trkA/NGF* receptor knockout mice demonstrated a key role for NGF in the survival of nociceptive and sympathetic neurons in vivo. However, because these neurons die without NGF, it has not been possible to study the role of NGF and TrkA signaling in axon growth and differentiation in vivo. To overcome this problem, Snider and colleagues (Patel et al., 2000 [this issue of *Neuron*]) have generated mice that lack not only NGF or Trk but also the proapoptotic BAX protein. The Snider group, in collaboration with Korsmeyer's and Johnson's groups, previously showed that naturally occurring dorsal root ganglia (DRG) and sympathetic neuron cell death is eliminated in mice deficient in BAX, and that these neurons survive indefinitely in culture without trophic factors. The prediction therefore is that the lack of BAX in *NGF/Bax* or *trkA/Bax* double null mice should prevent the sensory neurons deprived of TrkA signaling from undergoing apoptosis. The $NGF^{-/-}/Bax^{-/-}$ and $trkA^{-/-}/Bax^{-/-}$ mice thus provide an ideal system to study the requirements for neurotrophin and TrkA in axon growth and differentiation in the absence of the requirement for these proteins for neuronal survival.

Patel and colleagues (2000) first showed that in the *NGF/Bax* and *trkA/Bax* double null mice, all sensory neurons of the DRG that normally die in the absence of TrkA signaling survived. DRG axons in the double null mice extended centrally into the dorsal roots and established collatorals in the superficial laminae of the spinal cord. In contrast, peripheral sensory innervation and terminal arborization, in particular superficial skin and whisker pad innervation, were absent. Furthermore, the DRG neurons failed to express the nociceptive markers calcitonin gene-related peptide (CGRP) and substance P or the GDNF receptor Ret. This failure to express biochemical markers characteristic of nociceptive neurons appeared to be a direct action of NGF rather than a result of a failure to innervate targets, since cultured DRG explants from $Bax^{-/-}$ mice expressed CGRP in the presence of NGF, even though no target was present. These results demonstrate a requirement for TrkA/NGF signaling for sensory innervation into peripheral but not central targets, and indicate that NGF is necessary for biochemical differentiation of DRG neurons.

The approach of using the double null mice is both clever and elegant, and the data definitively demonstrate a developmentally important role for NGF in regulating sensory axon growth and differentiation, a result that confirms the predictions of numerous earlier in vitro studies. In addition, several fundamental questions about the roles of NGF were addressed for the first time in vivo, including whether NGF may function early to promote axon initiation and elongation or later as a chemoattractant or guidance factor. Snider's, Johnson's and Korsmeyer's groups previously showed that $Bax^{-/-}$ neurons survive in vitro and in vivo in the absence of trophic factor support, although the neurons were smaller and extended only rudimentary projections with short branches (Derkwerth et al., 1996; White et al., 1998; Lentz et al., 1999). Neurotrophin addition to the $Bax^{-/-}$ sensory neurons induced a bipolar phenotype and much more elaborate branching (Lentz et al., 1999). This result suggested that neurotrophins are required for extensive axon branching and guidance but not for neurite initiation or extension. In support of these data, neurotrophins have been shown to function as guidance factors in vitro (Gallo et al., 1997; Ming et al., 1997) and in vivo (O'Conner and Tessier-Lavigne, 1999), and axon extension from peripheral sensory ganglia has been suggested to be neurotrophin independent (e.g., see Lumsden and Davies, 1983; Wang et al, 1999). Patel et al. (2000) found that NGF is in fact required for initial axon elongation, since TrkA-labeled neurons from the $Bax^{-/-}/NGF^{-/-}$ mice were not found in the major nerve trunks of the hindlimb, and axon counts in the cutaneous nerve were markedly reduced. It is possible, however, that the axons could have extended short projections that then retracted during development. Patel et al. (2000) do definitively show that neurotrophin and Trk signaling are required for the maintenance of axon projections and terminal innervation, while strongly suggesting a role in axon elongation.

A key finding of Patel et al. (2000) was the observation that DRG neurons extended central axon projections to the spinal cord in the absence of TrkA signaling. Central projections were grossly normal in double null mice, suggesting that the absence of NGF signaling does not affect the ability of neurons to respond to different guidance and branching cues. Indeed, branching of these central projections may depend upon the Slit family of guidance factors (Wang et al., 1999), which function in an NGF-independent manner. It will also be critical to determine whether correct synaptogenesis occurs or if functional connections were made in the double null mice. In this regard, both NGF and brain-derived neurotrophic factor (BDNF) have been implicated in modulating synaptic density and innervation in vivo (Causing et al., 1997; Davis et al., 1997).

The Patel et al. (2000) study demonstrates a requirement for NGF signaling in sensory growth and differentiation, which prompts an important question about the nature of the intracellular signals used by TrkA to regulate these responses in vivo. This question is not an easy one, since several of the major mediators of NGF signaling may be multifunctional, promoting both survival and axonal growth and differentiation. For example, Ras, MEK/MAP kinase, and SH2-B each regulate NGF-mediated axonal growth and survival (Kaplan and Miller, 2000). Mice whose neurons are deficient in both BAX and Ras or MAP kinase activity will provide a powerful

reagent to elucidate the growth and differentiation functions of the Trk signaling proteins.

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Genetic Evidence for a Nova Regulator of Alternative Splicing in the Brain

How do genetic programs direct the development and physiology of distinct cell types in such complex systems as brain, muscle, liver, and blood? In the brain, regulated alternative splicing is a major mechanism by which neuronal cells acquire specialized molecular structures and regulatory pathways needed to receive and transmit informational signals. New biological functions are generated by subtle alterations in protein structure as a consequence of tissue-specific splicing patterns that occur in an impressive variety of morphological patterns. In contrast to its biological utility, there is a dark side to tissue-specific splicing, since its misregulation is closely associated with some forms of malignant transformation, tumor metastasis, neurodegenerative disease, and myotonic dystrophy (Cooper and Mattox, 1997; Grabowski, 1998).

In the simplest variation, protein isoforms involved in synapse formation, neurotransmitter reception, and ion channel function are synthesized in neurons with an additional (usually small) protein segment not found in other cell types. This means that the corresponding pre-mRNA is spliced in an identical manner in the different cell types, except that a subset of splicing events is activated exclusively in neurons or in a subpopulation of neurons. This suggests that in addition to the general splicing machinery, which is found ubiquitously (small nuclear ribonucleoprotein particles, RNA binding proteins, and related factors), there must be specialized molecular machineries to direct regulation in a corresponding cell type–specific manner. What are these specialized machineries, how do they recognize their specific intron targets, and how do they promote neuron-specific splicing? In the brain as well as in other tissues, splicing regulators with strict, cell-specific expression patterns have been an enigma. Now, a report by Jensen et al. (2000) in this issue of *Neuron* presents genetic evidence for the role of the neuron-specific protein Nova-1 as a tissue-specific splicing regulator in the brain.

Nova-1 and its close relative Nova-2 are RNA binding proteins first identified as autoimmune antigens in patients with certain forms of paraneoplastic neurological disorders (Darnell, 1996). RNA binding proteins are abundant in the cell nucleus where they package nascent transcripts and participate in RNA processing or nuclear export of mRNA, and in the cytoplasm where they regulate mRNA stability, translation efficiency, or mRNA localization (Dreyfuss et al., 1993; Ostareck-Lederer et al., 1998). Biochemical and molecular genetics approaches have shown that a variety of RNA binding proteins of the SR (serine-arginine-rich) and hnRNP (heterogeneous nuclear RNA–associated protein) family can function as splicing regulators for specific sets of alternatively spliced pre-mRNAs. It has been most provocative to note that Nova proteins are found exclusively in neuronal cells of the brain where they are localized to the cell nucleus—hence the prediction that these proteins might be tissue-specific regulators of alternative splicing.

What jobs do neuron-specific RNA binding proteins perform in the nucleus, and how important are these molecules for the survival and/or development of neurons? The study of Jensen et al. (2000) sheds new light on these questions by generating *Nova-1* knockout mice. The *Nova-1* null mice appear normal at birth, but die within about 2 weeks. The hallmark pathological defect is neuronal cell death in precisely the regions of brainstem and spinal cord where Nova-1 is normally expressed. Thus, Nova-1 is important for neuronal cell survival in the postnatal stage of development.

Is the physiological importance of Nova-1 due to it's role as a splicing regulator in brain? The Jensen et al. (2000) study makes a strong case for this by demonstrating significant splicing defects in the brains of *Nova-1* null mice for neuron-specific exons of the inhibitory glycine receptor (GlyRα2) and the γ2 subunit of the $GABA_A$ receptor. There is no indication that general splicing is disrupted, but these particular neuron-specific splicing events are decreased by as much as 3-fold compared to healthy littermates. In several respects the defect in regulation appears to be quite specific, since four additional neuron-specific splicing events tested show no apparent change upon loss of Nova-1 expression. Control experiments that probe in more detail show that regulation of GlyRα2 and γ2 pre-mRNA splicing is not

disrupted in null mice in the area of the forebrain, where Nova-1 is normally absent. In contrast, significant defects are evident in the spinal cord. Thus, to a first approximation, the defects in splicing regulation track with the regions of brain where there is an obvious loss of Nova-1 expression. In wild-type mice, these sensitive splicing events also track with Nova-1 expression during postnatal development.

What explanations might account for the fact that tissue-specific splicing regulation is not completely disrupted in *Nova-1* null mice? This may simply reflect genetic redundancy and the possibility that GlyRα2 and γ2 pre-mRNAs are particularly sensitive Nova-1 targets. The highly related Nova-2 protein is a possible candidate for a compensating function, since it recognizes essentially the same RNA sequence, and because its neuron-specific expression is retained in the *Nova-1* null mice. An alternative interpretation, however, is that the function of Nova-1 is not pivotal, but is rather one of a number of contributing factors required for neuron-specific splicing. Notwithstanding the additional supporting evidence shown in the paper, the possibility that the effects of Nova-1 on splicing in the null mice are indirect cannot be completely excluded at this time.

Is it logical that GlyRα2 and γ2 pre-mRNAs would be splicing regulatory targets based on the known RNA recognition properties of Nova-1? Interestingly, the pyrimidine-rich sequence motif (5′-UCAUPy-3′, where Py = pyrimidine), known to be the preferred Nova-1 target site, is present in multiple copies in GlyRα2 and in γ2 pre-mRNAs within intron flanks of the neuron-specific exons. One wonders if these sequences are absent or less numerous in the brain-specific pre-RNAs that fail to exhibit splicing defects in the *Nova-1* null mice.

How does Nova-1 recognize its specific RNA targets? This central question is addressed in a related study by Lewis et al. (2000), which describes the high-resolution cocrystal structure of a Nova RNA binding domain bound to a high-affinity RNA ligand. The Nova-1 and -2 proteins contain three RNA binding domains of the K homology type (KH), originally identified in the human heterogeneous nuclear ribonucleoprotein K (hnRNP K). By virtue of these new experiments, the interaction of the third KH domain of Nova-2, KH3, with its preferred sequence motif UCAPy, is now presented for our close inspection. The KH3 domain of Nova is both required and sufficient for specific RNA binding. The structure shows that the protein holds the RNA in its grip by forming a molecular vise with the conserved Gly-X-X-Gly motif on one side and the variable loop of the KH domain on the other. In the context of this molecular vise, the RNA sits on an unusual hydrophobic α/β platform, where the protein interacts extensively with the RNA by taking advantage of some cunning molecular mimicry. Sequence-specific RNA recognition is illustrated as the protein engages two of the RNA bases, a uracil and an adenine, with Watson-Crick type hydrogen bonds, while surrounding van der Waals and stacking interactions create an environment that is idiosyncratic for each of these RNA bases. Additional features of the cocrystal structure suggest a model in which Nova recognizes its RNA target as a protein dimer.

How do the structural characteristics of this novel Nova–RNA interaction compare to relevant cocrystal structures in the literature? The most common RNA recognition motif (RRM) is that which is found in hnRNP proteins (also called consensus RNA binding domain, RBD, or RNP domain). In RRM domains, four antiparallel β sheets are arranged to form a platform that contacts RNA directly, with two α helices oriented for support behind the RNA binding platform (Varani and Nagai, 1998). Although α/β RNA binding surfaces have been reported previously, the experiments of Lewis et al. (2000) reveal that the Nova KH3 domain is peculiar in the way it utilizes two α helices and the edge of a β sheet for specific RNA recognition. It is also of interest that the Nova–RNA cocrystal structure provides a framework to think about models for a severe form of fragile X syndrome that is due to a point mutation in a KH domain of FMR1, the fragile X mental retardation gene product.

How does the model that Nova is a splicing regulator fit with existing results found in the published literature? There is clear precedence for RNA binding proteins of the KH family to function as splicing regulators. These include *Drosophila* P element somatic inhibitor, yeast meiosis-specific MER-1 gene product, and human KH-type splicing regulatory protein or KSRP (Wang and Manley, 1997). In addition, splicing factor 1, which is generally required for splicing, contains a KH-type RNA binding domain. A regulatory role, however, is not limited to KH domains, as RRM domain proteins, including alternative splicing factor/splicing factor 2, hnRNP A1, polypyrimidine tract binding protein (also called hnRNP I), and hnRNP H have also been shown to regulate splicing either in biochemical or in transient cotransfection assays. Genetic knockouts that illuminate splicing regulatory factors or pathways have come in the past from *Drosophila*, yeast, and in one case a chicken cell line. With the Jensen et al. (2000) study, which provides the first genetic knockout of any splicing (regulatory) factor in mice, Nova-1 is now added to this growing list of interesting RNA binding proteins. Relevant to the striking changes in brain-specific splicing regulation known to occur during development, it is of further interest that some KH-type RNA binding proteins are connected to signal transduction pathways that operate during development (Vernet and Artzt, 1997).

It is to be hoped that future experiments will address the host of questions arising from the advances of the new studies discussed above. How important are the Nova KH3-specific RNA sequence elements for regulated splicing? How many copies of these elements are needed and in what arrangement or context? Are these sequences sufficient to redirect Nova-1 regulation when they are introduced into new pre-mRNA targets? What RNA or protein sequences do the KH1 and KH2 domains of Nova recognize? By what mechanism does Nova-1 function as a splicing regulator? From previous studies it is known that tissue-specific splicing regulation in general, and neuron-specific splicing in particular, involves a staggering complexity of RNA *cis*-acting elements, both positive and negative, and the *trans*-acting factors to which these sequences bind are numerous. Does Nova-1 promote neuron-specific splicing directly, or does it counteract the effects of a splicing repressor? How are the functions of Nova-1 and -2 interrelated,

and are other protein partners involved? Finally, what RNA binding proteins regulate the neuron-specific pre-mRNAs that are apparently not targets of Nova-1?

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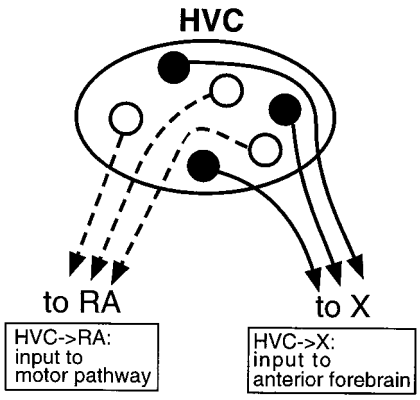
Adult Neurogenesis in Songbirds: A Tale of Two Neurons

Neurogenesis has long been left off the list of tools available to the adult brain for plasticity. Reports of adult neurogenesis are increasing, however, with recent descriptions in macaques (Gould et al., 1999) and humans (Eriksson et al., 1998). In contrast, it has long been known that the avian telencephalon receives new neurons throughout life. Adult neurogenesis has been enthusiastically studied in songbirds, which are especially fascinating given that new neurons are incorporated into circuits important for song behavior (reviewed by Alvarez-Buylla and Kirn, 1997). The high vocal center (HVC), a nucleus critical for song production, has regular neuronal addition in adult zebra finches. How these adults maintain their stereotyped song in the face of ongoing neuron addition is a great mystery and indicates that the incorporation of these new neurons must be tightly regulated. The paper by Scharff et al. (2000) in this issue of *Neuron* steps toward an understanding of the regulation of neuronal addition into a fully mature brain. Also, because of the association between HVC and vocal behavior, this study begins to address whether neuron addition is a source of song plasticity.

The authors examined the incorporation of new neurons into HVC of adult zebra finches. HVC is the origin of two pathways in the song system, a group of nuclei



The Two Pathways of the Song System Originate from HVC

The anterior forebrain pathway receives input from HVC neurons that project to X (closed circles and solid lines); the motor pathway receives input from HVC neurons that project to RA (open circles and dotted lines).

involved in song behavior (see figure). The first, the motor pathway, is essential for normal song production throughout life, and its source lies in the HVC neurons that project to the robust nucleus of the archistriatum (RA). The second, known as the anterior forebrain pathway, is involved in song learning, and it receives input from HVC neurons that project to Area X (X). Although the HVC neurons that project to RA (HVC→RA) and those that project to X (HVC→X) are intermingled within HVC, they are quite distinct from each other in terms of neurogenesis: HVC→RA neurons are primarily born during posthatch development, whereas HVC→X neurons are born during embryogenesis. Furthermore, HVC→RA neurons are replaced throughout adulthood, whereas HVC→X neurons are not. Thus, it would be advantageous to separately manipulate these two types of projection neurons in order to study the regulation of adult neurogenesis in HVC. Scharff et al. (2000) did just that, which let them examine the kind of cell death that leads to addition of new neurons in adult HVC.

To selectively lesion these projection neurons, Scharff et al. (2000) used a targeted photolysis technique (Madison and Macklis, 1993). One group of HVC projection neurons was retrogradely labeled with a compound that, when photoactivated with a laser, resulted in apoptosis. They found that induced cell death actually resulted in increased incorporation of new neurons into HVC. This shows that cell death can trigger neuron addition, which had been previously suggested by an observation that cell death preceded new neuron addition (Kirn et al., 1994). Specifically, Scharff et al. (2000) found that induced death of HVC→RA neurons in adult birds led to an increase in new HVC→RA neurons. In contrast, induced death of HVC→X neurons did not result in increased neuron addition. In juvenile birds, however, this precise lesion resulted in incorporation of new HVC→RA neurons, rather than HVC→X neurons. So, the good news is that new neuron incorporation will follow cell death, but the bad news is that not every kind of cell can be recruited.

The authors also examined the songs of the adult birds that had experienced both death and ensuing addition of

HVC→RA neurons. For some birds, song deterioration occurred during the first week after induced death. This deterioration was then followed by variable recovery to normal song production, during a time concurrent with recruitment of new neurons. How the detailed time courses of cell death and new neuron incorporation compare to that of song deterioration and recovery is unclear; however, this result raises the possibility that new neurons can restore components of song lost after cell death.

The mechanism for this increased and restricted neuronal recruitment in HVC is unknown and may involve control at the levels of neurogenesis, of migration into HVC, or of neuron survival. Particularly, why aren't HVC→X neurons replaced? Are progenitor cells incapable of making this type of neuron, or is the molecular environment in HVC unable to attract or sustain this type of neuron? The developing nervous system has a wealth of mechanisms controlling cell fate determination (McConnell, 1995), and it will be important to compare these to the mechanisms available to adult brains.

The variability in both song deterioration and song recovery is curious: only some of the birds exhibited song degradation, and among those, their songs did not always return to the original song. The authors rule out differences in lesion placement and in song complexity among the birds. They also believe that all birds with induced HVC→RA neuron death experienced similar levels of neuronal recruitment; this suggests that they shared similar levels of cell death. If this is true, it may be that birds differ in the amount of cell death required to cause song deterioration. As for the variable recovery among those birds whose songs did change, it is possible that the morphology and precise connectivity of the new neurons dictate whether song returns to its original state. A clearer picture of the influence of neurogenesis on song behavior will require the ability to prevent neurogenesis once these precise lesions are made.

Although zebra finches do not normally change their songs in adulthood, they can be experimentally induced to exhibit song plasticity. Both deafening (Nordeen and Nordeen, 1992) and altering auditory feedback (Leonardo and Konishi, 1999) can cause gradual song deterioration in adults. Given this behavioral plasticity, it is tempting to attribute a role for the incorporation of new neurons described here. Indeed, a recent study shows that neuronal incorporation into HVC is decreased following deafening (Wang et al., 1999). If neuronal incorporation could be increased in these deafened birds, would this aid song recovery? Similarly, when altered auditory feedback is removed, birds take several months to recover their original songs. Does this song recovery rely on new neuron recruitment? If so, would auditory experience be required to instruct new neurons, perhaps via the stable HVC→X neurons (Nordeen and Nordeen, 1992)? This idea emerges again when considering the effects of X lesions in juvenile birds: these birds' songs become extremely variable and do not recover (Sohrabji et al., 1990; Scharff and Nottebohm, 1991). HVC→X neurons are presumably killed by an X lesion, and so these birds may well have substantial incorporation of new HVC→RA neurons. Their lack of song stabilization could stem in part from the missing HVC→X neurons, which might leave any new neurons without instruction.

This article offers a rare glimpse into the regulation of new cell recruitment into a fully mature brain. Discovering the mechanisms governing the type of neuron added and its placement within the song circuit will provide important information regarding restrictions on neurogenesis in the adult brain. Furthermore, if new neuron recruitment is essential for song recovery, then training a new neuron for its new job is likely to involve a complex interaction of different signals, including molecular cues, contacts with neighboring neurons, and experience. The song system is a promising arena for sorting these questions out, given its provocative mix of neurogenesis and vocal behavior.

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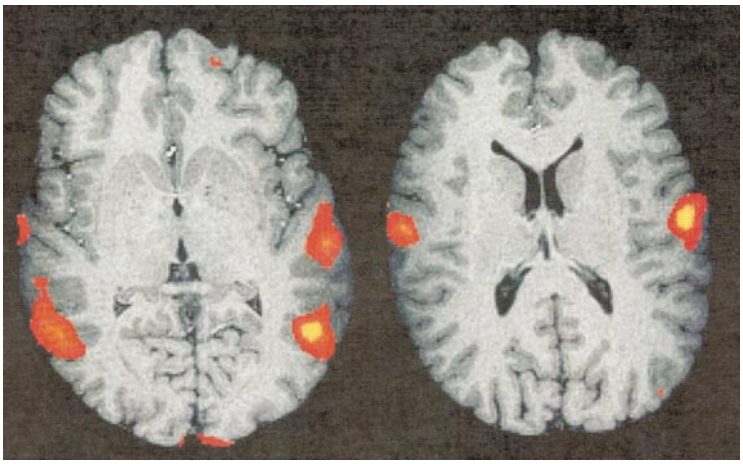
What's the Matter? White Matter?

Although many children acquire reading skills rapidly with some formal training, for others learning to read is among the most difficult challenges they face in the first few years at school. The reasons for these large individual differences in skill acquisition are still obscure, with the relative contribution of biological and environmental factors vigorously debated. As the social and economic consequences of widespread illiteracy can be profound, better understanding of the brain mechanisms responsible for the acquisition of reading skills has become a national priority (Lyon, 1995).

The localization of the neural mechanisms responsible for reading has been an area of active investigation for



The Brain Reading Aloud

Blood oxygenation changes, including in the angular gyrus bilaterally, related to reading. Two axial slices from a single subject are shown (anatomical left is on image right).

over 100 years. Until relatively recently, most of these studies involved careful observation of disordered reading following focal brain injury, with postmortem anatomical localization of the lesion presumed to be responsible for the behavioral deficit. Perhaps the earliest formal structure/function correlation study in man was published by Starr in 1889. On the basis of examining 50 cases of aphasia, Starr hypothesized that the critical lesion responsible for disordered reading comprehension involved the axonal pathways connecting occipital cortex to the left angular gyrus. In 1892, Dejerine proposed a more detailed model of the reading process based on examination of a patient with pure alexia, that is, isolated inability to read. In this account the reading disability was caused by damage to extrastriate visual cortex and its projections to the left angular gyrus, resulting in a disconnection of the word recognition system from its visual input. This notion of white matter damage causing reading disability was elaborated by Geschwind (1965), who further delineated a reading circuit that included posterior temporal cortex (semantic processing), the angular gyrus (phonological processing), and the inferior frontal gyrus (speech). This "disconnection" model predicted that damage to the connections among these regionally specialized processing regions could result in disordered reading.

The advent of structural brain imaging techniques allowed reexamination of these issues in living subjects. Damage involving white matter tracts in left extrastriate cortex was detected using X-ray computed tomography in a series of 16 cases of pure alexia (Damasio and Damasio, 1983). The lesions affected both intrahemispheric and interhemispheric pathways, strengthening the case that pure alexia may result from disconnection of phonological and semantic processing centers from their sensory input. More recently, there have been a large number of investigations utilizing structural magnetic resonance imaging that have largely confirmed the major findings of the earlier studies.

Against this background of structure/function localization derived from the study of *acquired* reading disorders (lesion studies) is the question of the localization of the pathophysiology of *developmental* language disorders, particularly developmental dyslexia. Anatomical studies of postmortem brains of dyslexic individuals have revealed abnormalities in left perisylvian structures (reviewed by Galaburda, 1993). More recent studies involving functional neuroimaging and electrophysiology have suggested that the localization of the pathophysiology in developmental dyslexia might be confined to the junction of temporal and parietal cortex (reviewed by Eden and Zeffiro, 1998). In addition, one group has interpreted functional imaging results collected in a group of dyslexics and controls as being most consistent with a disconnection of posterior and anterior reading areas (Palesu, 1996). These results, and others, have provided strong circumstantial evidence that the core pathophysiology in developmental dyslexia involves posterior perisylvian cortical areas and their connections.

In the context of these previous studies of acquired and developmental reading disorders, it is interesting to note the neuroanatomical localization of the results of a study reported in this issue of *Neuron*. Klingberg et al. (2000) employed diffusion tensor imaging, a novel, noninvasive, structural neuroimaging technique, to examine the relationship between reading skills and the microstructural integrity of cerebral white matter. Their measure of structural integrity, the white matter diffusion anisotropy, was positively correlated with the subject's reading ability in a spatial distribution confined to left posterior perisylvian regions. These results suggest that developmental reading disorder may be due to damage similar to that occurring in cases of acquired reading disorder, that is, damage to the fiber tracts connecting cortical structures responsible for sensory and phonological processing (see figure).

This work is an excellent example of the application of a relatively new technique, diffusion tensor imaging, to the problem of analysis of the pathophysiology of a developmental neurological disorder. As noted by the authors, this is not a functional imaging technique and therefore does not require a high level of subject cooperation (other than remaining still for the duration of the imaging session). As a result, this approach is potentially useful in the study of individuals of widely differing behavioral capacities. This particular application of diffusion tensor imaging represents a significant advance in the study of neurobehavioral disorders. Careful behavioral assessment coupled with noninvasive structural and functional brain imaging is likely to be an effective

strategy to explore the complex mechanisms of reading acquisition and its failure.

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