pikachurin is critical for the normal development of the ribbon synapse, the challenge now is to determine the precise mechanism by which pikachurin orchestrates the apposition of photoreceptor and bipolar cell termini. It also remains to be determined whether pikachurin/dystroglycan binding is necessary for pikachurin function and whether pikachurin function is affected in human muscular dystrophies. Biochemical and ultrastructural analyses of patient tissue or more readily available tissue from mouse models of muscular dystrophy are necessary to address some of these questions. With its intriguing parallels to the deficits seen in muscular dystrophies, pikachurin provides a useful tool for probing the defects associated with mutations of dystrophin or abnormal dystroglycan processing.

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Fresh air is good for nerves: hypoxia disturbs axon guidance

Chun-Liang Pan & Gian Garriga

The transcription factor hypoxia-inducible factor 1 (HIF-1) triggers multiple cellular responses to cope with hypoxia. A study in this issue suggests that elevated HIF-1 also causes axon guidance defects under hypoxic conditions.

Oxygen is critical for the function and survival of all eukaryotic cells; consequently, cells have developed various protective mechanisms for coping with hypoxia. Neurons are the cells most sensitive to hypoxic insults and hypoxia causes a number of human neurological diseases, such as perinatal brain injury, ischemic stroke and hypoxic encephalopathy as a result of circulatory collapse. Conserved HIF-1, a transcription factor of the basic helix-loop-helix family, protects cells from hypoxia by regulating a wide variety of target genes to increase anaerobic metabolism or enhance vascularization of hypoxic tissues[1]. Previous studies had shown that HIF-1 is required for neuronal survival in hypoxic conditions, and HIF-1–deficient mice show severe nervous system anomalies[2]. In this issue, however, Pocock and Hobert[3] add a surprising twist to the story by showing that increased HIF-1 levels are responsible for hypoxia-related axon defects in the nematode *C. elegans*.

Under physiological normoxic conditions, the oxygen-dependent prolyl 4-hydroxylase EGL-9 hydroxylates a specific proline residue of HIF-1. The modified proline is recognized by the E3 ubiquitin ligase von Hippel-Lindau protein 1 (VHL-1), and the ubiquitinated HIF-1 is then targeted for proteasomal degradation[1]. Hypoxia inhibits the initial hydroxylation of HIF-1, allowing it to accumulate, which in turn activates various anti-hypoxic molecular pathways.

Pocock and Hobert[3], however, found that hypoxia caused cell migration and axon-guidance defects in specific types of neurons of *C. elegans*. The authors focused on two classes of neurons: the HSN motor neurons and the PVQ interneurons. When reproductive young *C. elegans* hermaphrodites were subjected to hypoxia, substantial defects in HSN neuronal migration and PVQ axon guidance, which are normally completed during embryogenesis before the animals hatch, were found in the progeny that they produced. Moreover, extension of the HSN axons, which occurs post-embryonically, was also affected. These defects were completely suppressed by the loss of HIF-1, suggesting that HIF-1–regulated genes are responsible for the defects induced by hypoxia. This conclusion was supported by two additional observations. First, loss-of-function mutations in the genes *egl-9* and *vhl-1* caused cell migration and axon guidance defects that were similar to those induced by hypoxia, and these defects were also suppressed by a *hif-1* mutation. Second, expression of a stable form of HIF-1 that cannot be hydroxylated by EGL-9 also caused similar axon defects. Expression of this stable HIF-1 in midline motor neurons, which serve as guideposts for the axons of the HSN neurons, induced the HSN axon defects, but expression of HIF-1 in the HSN neurons themselves did not. These results indicate that increased levels of HIF-1 induce axon defects in a non–cell-autonomous fashion.

What are the intracellular signals that stabilize HIF-1 in hypoxic cells? In vertebrates, reactive oxygen species (ROS) produced by mitochondria stabilize HIF-1 under hypoxic conditions[4]. Mutations in several *C. elegans* superoxide dismutases and catalases that remove ROS also induced axon defects that looked similar to those caused by hypoxia. These defects were also suppressed by the loss of HIF-1. Insulin signaling is known to increase ROS levels. Thus, consistent with the hypothesis that ROS can mediate the effects of hypoxia on axon guidance, the loss of DAF-2, the *C. elegans* insulin/IGF receptor, suppressed hypoxia-induced axon defects. Loss of DAF-2 failed to suppress the axon defects caused by expression of stable HIF-1, indicating that *daf-2* acts upstream of *hif-1*.

What are the downstream targets of HIF-1 that affect axon guidance in hypoxia? Answers to this question may provide insights to the mechanisms of axon guidance in hypoxic situations and might also shed light on the design of potential treatments for human neurological disorders caused by hypoxia. To address this question, Pocock and Hobert[3] took advantage of work done by other labs using microarray analysis to identify potential HIF-1 targets. One HIF-1 target is *vab-1*, which encodes the sole *C. elegans* Eph receptor tyrosine kinase[5]. Eph receptors and their ephrin ligands regulate axon guidance in metozoans[6].

Pocock and Hobert[3] confirmed that *vab-1* transcription was increased under hypoxic conditions and in *vhl-1* mutant animals.

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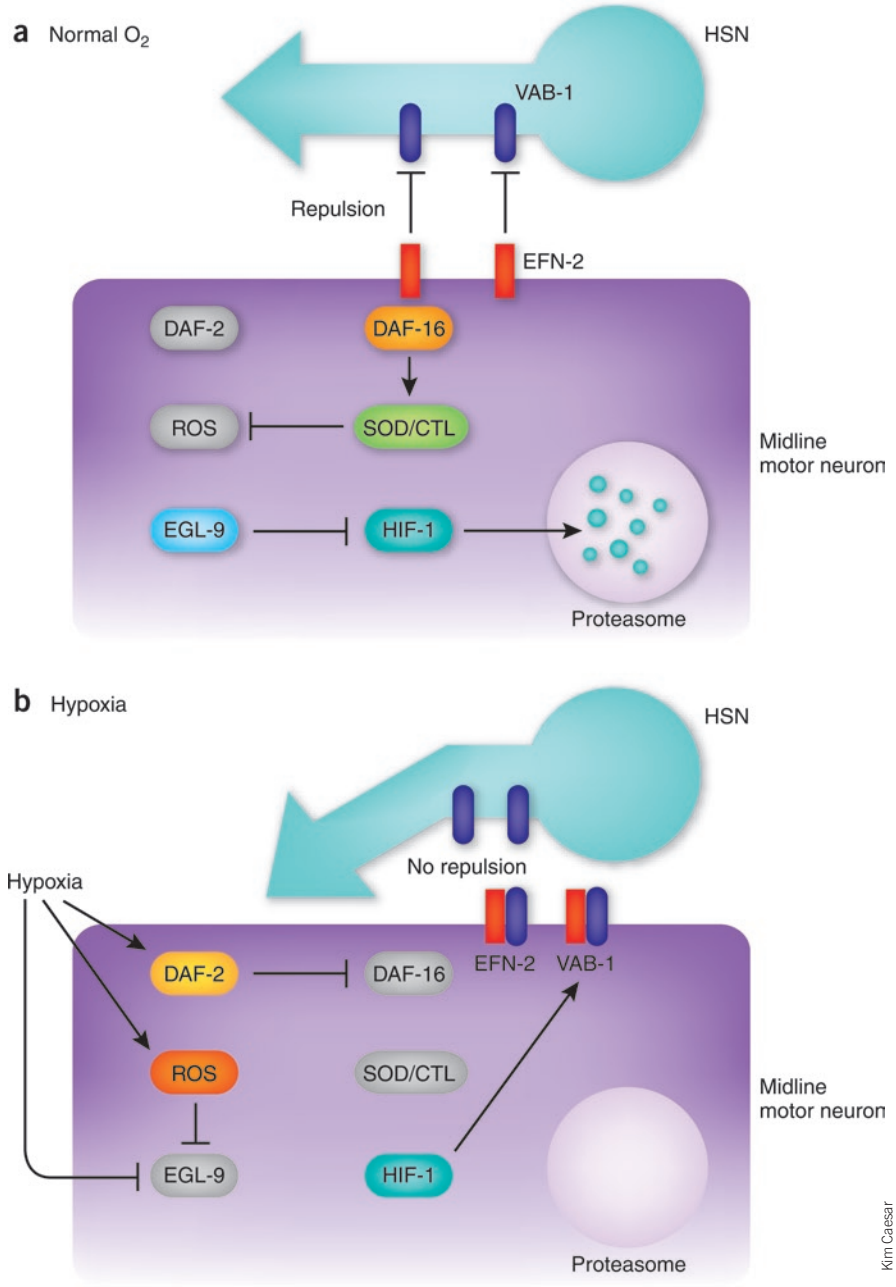

Figure 1 Model for the effects of hypoxia on axon guidance[3]. Components that are inactive are in gray. (**a**) At normal oxygen levels, HIF-1 is targeted for proteasomal degradation by hydroxylation and ubiquitination, mediated by the EGL-9 and VHL-1, respectively. (**b**) Under hypoxic conditions, the activity of EGL-9 is suppressed. Hypoxia also increases the level of ROS by acting through the DAF-2/IGF receptor pathway to downregulate superoxide dismutase (SOD) and catalase (CTL), which normally remove ROS. Together with the reduction in EGL-9 activity, these changes cause accumulation of HIF-1, which in turn upregulates VAB-1/Eph-receptor levels in the guidepost motor neurons. Excess VAB-1 in these neurons interferes with EFN-2/ephrin ligands in the same cell, resulting in the disruption of normal repulsion between the guidepost neurons and the HSN axons.

They found that the axon defects of the PVQ interneurons induced either by hypoxia, a *vhl-1* mutation or expression of stable HIF-1 were all suppressed by a *vab-1* mutation. Strengthening the hypothesis that *vab-1* is the target of *hif-1* in hypoxic axon defects, expression of excess VAB-1 in neurons caused axon defects that were indistinguishable from those observed in hypoxic animals. The effects of excess VAB-1 depended on the *C. elegans* ephrin EFN-2. Similar to the effects of expressing stable HIF-1, HSN axon defects were induced when excess VAB-1 was expressed in the guidepost motor neurons, but not in the HSN neurons themselves.

The finding that increased HIF-1 levels are responsible for hypoxia-induced axon defects seems to be counterintuitive, as the primary role of HIF-1 is the coordination of multiple protective mechanisms against hypoxia. It seems equally odd that HIF-1 should increase the level of the VAB-1 receptor, which disrupts normal axon projections. The authors, however, also found that VAB-1 is required for embryonic viability under hypoxic conditions, providing an explanation as to why HIF-1 increases expression of a target gene that alters nervous system development. The Pocock and Hobert model for HIF-1–induced axon defects is presented in **Figure 1**.

The report by Pocock and Hobert[3] reveals an important mechanism for hypoxia-induced axon defects that could have important implications for understanding the mechanisms of developmental nervous system diseases caused by hypoxia, such as perinatal brain injuries. Notably, their findings are consistent with previous work indicating that HIF-1 might adversely affect the nervous system[1,7]. For example, when neurons and astrocytes are cultured together, removal of HIF-1 function from neurons increases hypoxia-induced neuronal death, but removal of HIF-1 function from astrocytes alleviates neuronal death[7]. Some of the deleterious effects of astrocytic HIF-1 activity on neuronal survival might be mediated by inducible nitric oxide synthase. Nitric oxide is a potent vasodilator and thus serves as a protective mechanism for neuronal hypoxia resulting from compromised blood flow. Paradoxically, some of the neuronal death observed in cerebral ischemia could be a side effect of increased HIF-1 activity in non-neuronal cells.

Although increased expression of the VAB-1 Eph receptor by HIF-1 is critical for the embryos to survive hypoxic conditions, excess VAB-1 causes axon guidance defects. Along similar lines, Eph receptor levels increase in astrocytes at the injury site in a mammalian model of traumatic spinal cord injury[8,9]. Eph receptors normally mediate axon repulsion during development and blockade of Eph receptors can partially restore locomotor function in adult animals with spinal cord injuries[9], indicating that these repulsive receptors may prevent regenerating axons from connecting to their targets. Upregulation of HIF-1 has also been reported in spinal cord injury, probably in response to local hypoxia caused by microvascular injury and compression[10]. On the basis of the current report, it is tantalizing to speculate that HIF-1 could be responsible for the undesired reappearance of some guidance receptors, which in the adult nervous system

constitute a barrier for axon regeneration after ischemia or injury. These new results also suggest that this undesired VAB-1 activity functions in the guidepost cells and the tissues that growing axons traverse, which is quite reminiscent of the astrocytic expression of Eph receptors in the injured mammalian spinal cord. In theory, selective removal of this repulsive activity might help to restore axonal connection in nervous system injuries caused by hypoxic insults.

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Mapping the microcircuitry of attention

John H Reynolds

A study uses electrophysiological recordings from primary visual cortex of the monkey to demonstrate that the effects of attention are modulated by task difficulty and that two different neuronal populations mediate this effect.

A major goal of neuroscience is to understand cognitive functions in terms of their underlying neural circuitry—to link the mental level of description used in cognitive science with the physiological and anatomical levels that are the province of neurobiology. In this issue of *Nature Neuroscience*, Chen *et al.*[1] take a substantial step toward such mechanistic understanding of an important cognitive function, selective attention. Although spatial attention has been shown to modulate responses of cells in the primary visual cortex (V1), it is unclear how task difficulty affects this modulation. Moreover, are different cell populations affected uniformly by attention or not? Answers to these questions are important for building realistic models of how this information is coded in V1 and modulated by attentional state. On page 974, Chen *et al.*[1] take an important first step in this direction. By recording neuronal responses in the primary visual cortex of monkeys performing an attention-demanding task, the authors show distinct roles for two major types of neurons in selecting task-relevant stimuli from among task-irrelevant distracters.

In the experiment, monkeys had to attend to a stimulus to detect a change in its color. The color change could be easy or hard to detect, thus varying the attentional effort required to perform the task. Furthermore, attention was either directed to a stimulus appearing in the receptive field of the neuron under study or to one of several stimuli that appeared simultaneously around the receptive field. When attention was directed into the receptive field, neuronal responses typically grew stronger with increased attentional effort. However, when attention was directed to one of the stimuli outside of the receptive field, firing rates typically diminished with increased effort. That is, increasing attentional effort appeared to enhance neuronal responses at the focus of attention while suppressing responses outside of the focus of attention. This suggests that attention may modulate the neural circuitry that gives rise to the center-surround organization of the receptive field in V1. This study also found evidence that enhancement and suppression are, to some extent, mediated by distinct groups of neurons. Neurons that showed the strongest response suppression with attention outside of the receptive field tended to show the weakest response enhancement with attention to the center. Neurons that showed the strongest response enhancement showed no response suppression.

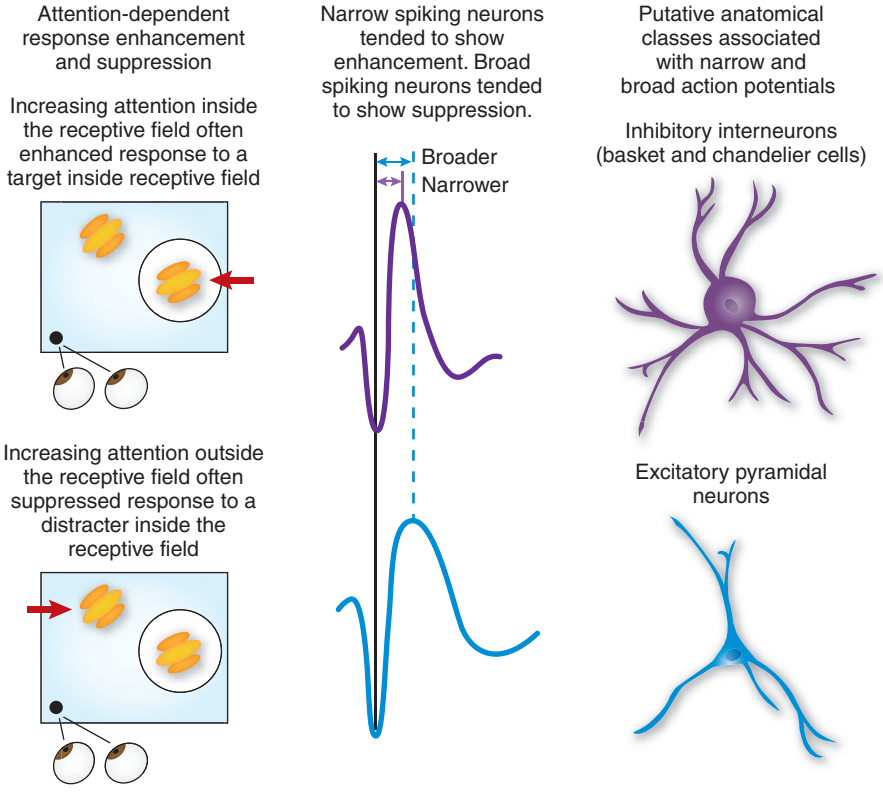

Figure 1 Attention enhanced responses at the attended location and suppressed responses to nearby distracters (red arrows indicate focus of attention). These two types of attentional modulation were associated with different classes of neurons.

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