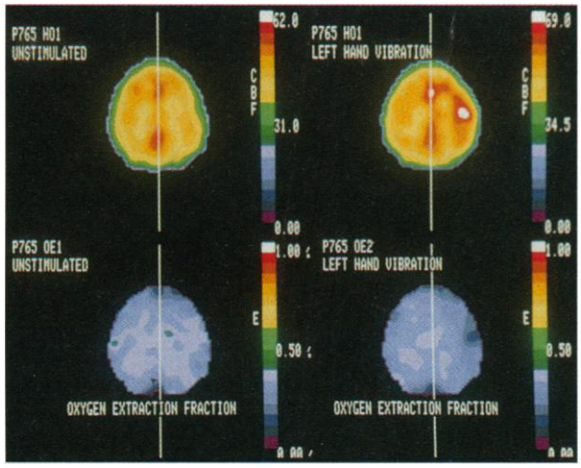
# What Makes Brain Neurons Run?

Neuroscientists disagree over the way brain metabolism creates the signals used to produce high-tech images of activated brain areas. At the debate's center: How much oxygen do active neurons need?

Open virtually any modern neuroscience text, and you are likely to find striking color images of the working human brain. Recent imaging methods that highlight areas of brain activity in vivid hues have revolutionized the field, helping researchers map the brain regions involved in functions ranging from sensation and movement to language and memory. Given the methods' widespread use, you might expect that neuroscientists know exactly how neural activity produces these images. Surprisingly, however, that's far from the case. Instead, that topic is at the heart of a decade-old debate still raging at scientific meetings and in the published literature.

The problem is that the most commonly used brain-imaging methods—positron emission tomography (PET) and functional magnetic resonance imaging (fMRI)—don't record the activity of brain neurons directly. Instead, they measure surrogates for neural activity: blood flow in the case of PET, blood oxygenation in fMRI. Prominent neuroscientists disagree over how those indicators actually relate to brain activity, and the field as a whole is searching for experiments that will settle the debate. "It is very important to understand what we are looking at [with] these nice color-coded pictures," says neuroscientist Per Roland, who does brain imaging at Sweden's Karolinska Institute.




**Bombshell.** These PET images from the mid-1980s showed that as blood flow *(top)* increases with brain activation, oxygen extraction from the blood *(bottom)* drops, indicating little or no increase in oxygen use.

PETER FOX AND MARCUS RAICHLE/*PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES* 83, 1140 (1986)

The implications of this conflict aren't merely academic. Without a full grasp of how the PET and fMRI signals relate to neural activity, neurobiologists worry that they may misinterpret brain images, perhaps missing active brain areas or assigning activity to a larger or different area than is actually activated by a particular stimulus or mental task. Moreover, a better understanding of the brain metabolism that underlies the images we see would help neurologists treat patients with strokes or other damage that affects brain blood flow.

Some recovered stroke patients, for example, never regain the ability to increase blood flow to active brain areas, and researchers don't know exactly what that means for the health of those areas. "You wonder about circumstances in which there is impairment in vasculature," says PET imaging pioneer Marcus Raichle of Washington University in St. Louis. "What is the brain being deprived of?" An answer to that question might help design drugs to improve brain function in stroke patients.

The main issue in this debate concerns whether activated brain neurons need more oxygen than those at rest. Until 12 years ago, this issue was not controversial: Neuroscientists knew that brain tissue is absolutely dependent on oxygen for survival; they also assumed that active neurons use more energy than those at rest and so must consume more oxygen. But their assumption remained untested because oxygen consumption can't be measured directly in living brains, and the indirect methods are arduous.

In 1985, Raichle and his then-postdoc Peter Fox took on that arduous task and shocked the neuroscience world with their answer: By their calculations, active brain areas don't consume significantly more oxygen than areas at rest. Based on that finding, Fox and Raichle proposed that neural activity produces only a modest boost in a brain area's energy demand, and that demand is met by anaerobic glycolysis—the metabolism of glucose to lactate, which doesn't use oxygen. While their idea at first met with disbelief, it gained wide acceptance over the next decade as evidence grew to support it.

Now, however, many researchers believe the evidence is shifting back the other way, thanks to more sensitive techniques that show an initial oxygen uptake as brain areas become active. But Raichle and Fox are not backing off, and—given all the pieces still missing from the picture of brain metabolism—the controversy seems far from over.

### Metabolic origins

The origins of PET imaging date back to work begun in the 1950s by Louis Sokoloff and Walter Freygang, working with Seymour Kety at the National Institute of Mental Health. By then, thanks to earlier work by Kety and his colleagues, researchers knew that the brain has few energy stores and gets most of its energy by oxidizing glucose to carbon dioxide and water. But Kety's earlier work said nothing about what was going on specifically in activated brain areas. To begin addressing this issue, Sokoloff and Freygang used radioactive inert gases to show that blood flow increases to activated areas of cats' brains. Then, in the 1970s, Sokoloff and his colleagues, using an artificial form of glucose that is taken up by cells but only partly metabolized, showed that glucose use surges in activated areas as well.

Those findings became the basis of human-brain imaging with PET, developed by a number of groups in the 1970s. PET subjects are injected with chemicals, such as water or glucose, labeled with isotopes that emit positrons, which annihilate with electrons to produce gamma rays. A PET scanner detects the gamma rays throughout the brain, and a computer then uses this information to produce images that show changes in blood flow, glucose use, or other functions in specific parts of the brain. PET provided no direct way, however, to measure one key factor in metabolism: oxygen consumption. Based on Kety and Sokoloff's work, researchers simply assumed that oxygen use would mirror the increase in blood flow.

Fox and Raichle were uncomfortable with that assumption. So, they used a method developed by Mark Mintun, in Raichle's lab, to calculate oxygen consumption from three separate measurements made with PET: blood flow, oxygen concentration in the blood, and the fraction of that oxygen that is extracted by the tissue. At the June 1985 meeting of the International Society for Cerebral Blood Flow and Metabolism in Ronneby, Sweden,

Raichle and Fox dropped their bombshell. They had compared blood flow to oxygen consumption in the brains of subjects whose hands were stimulated with a vibrator to activate sensory brain areas. As expected, PET images showed blood flow in the areas jumping by roughly half. But the researchers found only a small, statistically insignificant rise in oxygen use (about 5%) in those areas. That report "stopped the proceedings," Fox recalls, and the debate that ensued "was an utter madhouse."

Raichle and Fox's result—combined with their subsequent finding that while oxygen consumption shows little increase, glucose use goes up an equal amount to blood flow—triggered an uproar because it suggested that working brain areas use glucose anaerobically, as muscles do when they run out of oxygen. That idea was "anathema" to brain physiologists, says brain-imaging researcher Richard Frackowiak of University College, London, because it flew in the face of the well-accepted view that brain metabolism is totally dependent on oxygen supply.

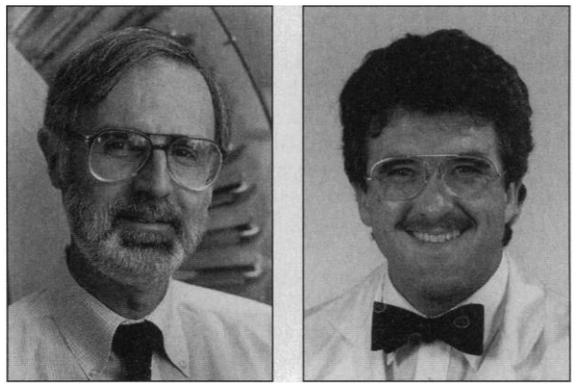
Fox's explanation for this surprising result was that even resting brain neurons need so much energy to maintain the electrical potential of their copious and leaky membranes that their oxidative enzymes are already working at full throttle—an idea, he notes, that is supported by calculations made in the 1980s by Dutch biochemist Cees Van den Berg of the University of Groningen. If so, then any extra energy needed when the brain area becomes active would have to come from anaerobic glycolysis of glucose, Fox argues.

That explanation spurred several labs to search activated brain areas for increases in lactate, the end product of the anaerobic glycolysis pathway. James Prichard, Robert Shulman, and their colleagues at Yale University were the first to find it, reporting their results in 1991. "In a sense, that was partial support for the nonoxidative glycolysis hypothesis," says Shulman. He notes, however, that the lactate peak they saw seemed too small and transient to account for the large increase in anaerobic glucose breakdown predicted by Fox and Raichle's measurements of glucose use. Nevertheless, other labs also found lactate in activated brain areas, and many researchers took that as support for Fox and Raichle's view.

Still, not everyone's experiments pointed in that direction. Several other groups also had been doing the technically difficult measurements required to compute oxygen consumption. In 1987, Roland's team at the Karolinska Institute, using a variation on the technique employed by Fox and Raichle, measured an 18% increase in oxygen consumption in activated brain areas.

Roland says Fox and Raichle's results may have been thrown off because they used water labeled with oxygen-15 to measure blood flow. Roland, who uses butanol labeled with oxygen-15, says that water can skew the calculations, diminishing the final value for oxygen consumption. "He is absolutely correct technically," says Raichle, "but I think it is not a big enough effect to make the difference" between his and Roland's data.

Albert Gjedde, of the Aarhus University Hospital in Denmark, and his team repeated the Fox and Raichle experiment with a slightly different method for calculating oxygen use and found—as Fox and Raichle had—an insignificant increase in such use. But when they employed a more complex visual image as the stimulus, he says, "we found a very substantial increase in oxygen consumption." Based on that result, Gjedde proposed that the ability of brain neurons to increase their oxygen use may vary, depending on the type of task a neuron performs.



**Iconoclasts.** Peter Fox *(right)* and Marcus Raichle suggested that brain neurons work anaerobically.

On top of that collection of results came, in the spring of 1992, what most people saw as the most persuasive support for Raichle and Fox's hypothesis: the emergence of a new brain imaging technique, fMRI. Its very existence depended on the imbalance between blood flow and oxygen demand that Fox and Raichle had reported.

Researchers had used MRI for years to map brain structures, based on the different magnetic properties of the organ's tissues. But in 1990, biophysicist Seiji Ogawa at AT&T Bell Laboratories in Murray Hill, New Jersey, proposed that MRI also could be used to follow blood-oxygenation changes in living brains. He based his proposal on a discovery made decades earlier by biochemist Linus Pauling: that when the blood pigment hemoglobin loses its oxygen, it becomes paramagnetic, which means it should interfere with the magnetic field during MRI, creating a dip in the magnetic resonance signals emitted by water protons. Ogawa manipulated the blood oxygenation of rats and detected a change in the MRI signal, which he dubbed the blood oxygen level–dependent (BOLD) effect.

If Raichle and Fox were right in their claim, Ogawa figured that the surge of oxygenated arterial blood to activated brain areas—without a matching rise in oxygen use—would cause a positive BOLD effect. He teamed up with MRI specialist Kamil Ugurbil and his colleagues at the University of Minnesota, Minneapolis, to test this idea in human subjects looking at visual images. The researchers found the predicted surge in MRI signal in the activated brain areas. A team led by Tom Brady and Bruce Rosen at Massachusetts General Hospital in Boston was working on a similar track; when the groups published their findings in 1992, fMRI was born.

Many neuroscientists took the positive sign of the BOLD signal in fMRI as support for the idea that activated brain areas had very little, if any, demand for extra oxygen to meet their energy needs. "Initially, people didn't quite know what to do with [the anaerobic hypothesis]," says neuroscientist Costantino Iadecola, of the University of Minnesota. "But functional MRI really supported the validity of Fox and Raichle's observation."

There was another possibility, however: The blood flow to the active brain areas might have increased so much that it produced an excess of oxygen, even in the face of stepped-up oxygen use by neurons. "If Raichle and Fox are correct, there should be a BOLD effect, but there could be a BOLD effect even if they are wrong," says Ugurbil.

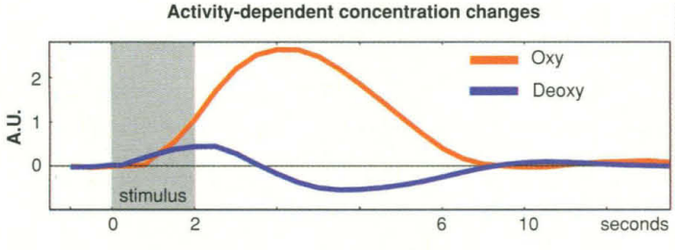
### New insights

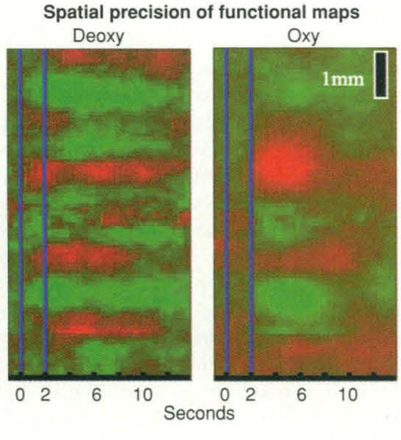
Despite this uncertainty, the weight of the fMRI findings kept the balance of opinion on Raichle and Fox's side—until recently. Now, different sorts of evidence have begun to shift that balance toward the aerobic point of view once again. The first nudge came in 1995. Shulman and his colleagues observed an increase in the BOLD signal in the sensory area of rats' brains when they stimulated the animals' forepaws with an electric shock. But when the researchers calculated oxygen use (employing nuclear magnetic resonance spectra to follow the metabolism of carbon-13–labeled glucose in active brain areas), they found evidence of "a very large increase in oxygen consumption," says Shulman.

Researchers in the field say that finding alone didn't turn the tide of opinion because, like Raichle and Fox's, it was indirect and depended on calculations and assumptions that could be challenged. Then, last April, Amiram Grinvald and graduate student Dov Malonek at the Weizmann Institute of Science in Rehovot, Israel, published more evidence.

Grinvald and Malonek used a method developed over the past decade by Grinvald and his colleagues at IBM, Rockefeller University in New York City, and later at the Weizmann Institute. The two researchers shone light through holes in the skulls of anesthetized cats and analyzed the spectrum of light reflected off the cats' brains. Because the oxygenated and deoxygenated forms of hemoglobin are different colors, the spectra of light reflected from the two forms are different. This allowed Grinvald and Malonek to measure changes in blood oxygenation as the brain area was activated.

They saw a rise in deoxyhemoglobin in the cat's visual cortex within 200 to 400 milliseconds after an image was presented to the cats' retinas. But, within 3 seconds, that effect was swamped by a rise in oxyhemoglobin, caused by a wave of extra blood flow. This surge, they observed, kicks in 2 seconds after stimulation and occurs over a larger area than the region where they saw the increase in deoxyhemoglobin. But their glimpse of an early dip in oxygenation before the surge in blood flow provides direct evidence, says Grinvald, of "delivery to the tissue of oxygen that had been bound to hemoglobin." One reason the dip had not been observed in fMRI is that most fMRI images are collected over several seconds and thus would miss such a brief effect.




**The dip.** Grinvald and Malonek see an initial rise in deoxyhemoglobin (top graph and red areas in left panel) in activated ocular dominance columns in the cat's visual system, before a less spatially precise surge in oxygenated blood causes oxyhemoglobin (top graph and red areas in right panel) to rise.

Ugurbil, with then-postdoc Ravi Menon, learned of the dip in the early 1990s from work by Grinvald's team and by Juergen Henning at Freiberg University in Germany. He began tooling up to use fMRI for a look at that early deoxyhemoglobin increase. After some technical refinements by team member Xiaoping Hu to improve the sensitivity of its fMRI techniques, the Minnesota team tested human subjects with visual stimuli and saw the oxygenation dip in the subjects' visual cortex—with the same timing Grinvald and Malonek had seen. Their findings are in press at the journal *Magnetic Resonance in Medicine*. A group at the University of Alabama, led by Don Twieg, also has seen the effect. Those findings, along with Grinvald's work, are "consistent with an early deoxyhemoglobin increase," says Ugurbil, "and this suggests a relatively significant increase in oxygen consumption," which is masked when the extra blood rushes in.

These recent results have persuaded many observers that brain activity is indeed aerobic, says Frackowiak, of London's University College: "There still is a controversy over the amount that oxygen utilization goes up, but the thinking of most of the community has shifted" toward an aerobic model of brain activity.

### The mystery of the extra glucose

Despite what many interpret as the growing evidence that active brain areas do increase their oxygen use, Raichle and Fox are standing firm. Before the issue is resolved, they say, a nagging question must be answered: What becomes of all the extra glucose consumed when a brain area is activated? As Raichle puts it, nobody doubts that glucose consumption rises as much as blood flow in activated brain areas, and that oxygen use—by anyone's measure—lags behind. And that, he says, argues strongly that there must be anaerobic metabolism of glucose in active brain areas.

Neuroscientist Pierre Magistretti, of the University of Lausanne in Switzerland, has proposed an explanation for the excess glucose consumption. He suggests that non-neuronal brain cells called astroglia metabolize the excess glucose to lactate, which they then pass on to neurons as an energy source. That would explain the transient appearance of lactate in activated brain areas, says Magistretti, whose team has shown that both glia and neurons have the right enzymes and lactate transporters to form the basis for such a lactate shunt. But Fox counters that if such an explanation were true, oxygen use would eventually have to rise to balance glucose consumption, because the neurons would require oxygen to process the lactate. And that, he says, is not what most researchers observe.

As the controversy boils on, researchers who use imaging techniques puzzle over what the various findings may mean to the interpretation of their images. If Aarhus's Gjedde is right, for example, and some brain areas increase their oxygen use more than others do upon activation, neurons with high oxygen consumption might produce less of a BOLD signal, and fMRI imagers could miss their activity. And Grinvald and Malonek's finding, that blood flow increases over a broader area than does electrical activity, could render the BOLD signal spatially inaccurate at high resolution. The fMRI technique hasn't yet reached a level of resolution where that is an issue, but Ugurbil says it could become one as his group and others push to higher resolution.

Meanwhile, researchers on both sides of the debate are searching for the definitive experiment to resolve the issue. Some would like to see further exploration of Magistretti's model, while Fox says he is designing better experiments in which to look for the prolonged lactate production he believes to exist in active brain areas. Ugurbil notes that researchers at various imaging centers are gearing up to explore the early oxygen dip that Grinvald's group has found, as well as to search for new ways of using fMRI to measure oxygen consumption. And Raichle wonders what need the excess blood flow is serving, if it's not answering a call for oxygen.

"What this imaging business has done is to bring to light information about the [brain] and its relationship to its blood vessels and metabolism that we clearly don't understand," says Raichle. Karolinska's Roland cautions those caught up in the debate not to decide what the answers should be before all the results are in. "We are here to find out what is actually happening," he says. "Then, we can philosophize about whether it makes sense or not."

**–Marcia Barinaga**


Additional Reading

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