NEUROBIOLOGY

New Imaging Methods Provide A Better View Into the Brain

In addition to providing new insights into brain function, improved imaging methods are revolutionizing other scientific fields as well. The Special News Report that begins on page 1981 and selected research Reports provide a look at some of these recent developments.

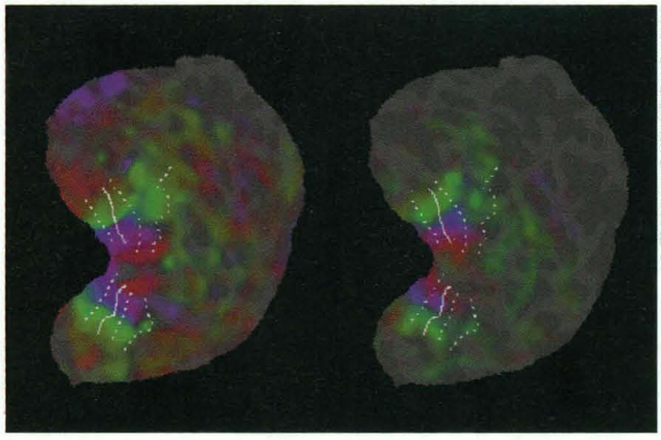
For a field that didn't even exist 20 years ago, human brain imaging has developed at a mind-boggling pace. Thanks to one advance after another, neurobiologists can peer into the living human brain and produce pictures that shed new light on brain functions ranging from the processing of sensory information to higher level thinking tasks. But breathtaking as the developments have been, improvements already under way will soon give imagers new perspectives on how the brain goes about its business.

Most of these advances are based on functional magnetic resonance imaging (fMRI), a technique that spots the increases in the blood oxygenation that reflect a boost in blood flow to active brain areas. Because of advantages—including greater speed and higher resolution—in recent years, fMRI has largely eclipsed positron emission tomography (PET), the method that got the imaging field rolling nearly 20 years ago. Now, researchers are devising a whole new wave of modifications, several of which were showcased at a recent conference on brain imaging,* that will allow fMRI to be used to even better advantage.

Some modifications will permit more sophisticated experimental designs that link brain images more closely to a subject's perceptions and behavior. In addition, increased magnet strengths will give even greater spatial resolution of activated brain areas. By combining fMRI with other techniques, researchers are now able to answer previously unaddressable questions about the timing with which brain areas are activated. Those answers will yield insights into how information moves through the brain.

As these advances invigorate the field, a next wave is waiting in the wings: methods that may image neural activity directly by following the flux of sodium ions, or by measuring the scattering of light by brain tissue. These newest directions are as yet unproven, but in a field where methods go from inconceivable to commonplace in a couple of years, researchers have learned never to say never. "I hesitate to say [any technique] isn't going anywhere, because I could be writing a grant to try to buy the equipment in 2 years," jokes cognitive neuroscientist George Mangun of the University of California (UC), Davis.

Mangun learned that lesson, he says, from the fast ascent of fMRI. When he heard of an early form of the technique in 1990, Mangun recalls, he thought the technique was "interesting ... but [would] never go anywhere." Within a year, additional advances had paved the way for it to become the mainstay of the field.



R. TOOTELL AND NOUCHINE HADJIKHANI

Bigger is bolder. fMRI at 3 T *(left)* shows subtle activations in the visual cortex that are less obvious at 1.5 T *(right)*.

But even as fMRI was catapulting to its position of prominence, researchers using the technique unwittingly handicapped themselves with old habits carried over from PET imaging that have prevented them from taking full advantage of fMRI. PET, which uses radioactive tracers to detect the increased blood flow to activated brain regions, is slow, taking up to a minute to gather the data for a brain image. As a result, neuroscientists using the method do "block trials," in which the subject performs a string of similar short tasks, causing the brain to repeat the same mental process while the data are gathered. Researchers continued to use block trials with fMRI, although that technique takes only 2 seconds to collect an image. "We always assumed if we only looked at one trial, the signal would be so small we wouldn't be able to see it," says neuroimager Randy Buckner of Harvard Medical School in Boston.

That assumption evaporated in 1995, when Robert Savoy and his colleagues at Harvard Medical School reported that fMRI could detect brain activations in response to a visual stimulus lasting only 30 milliseconds. The next year, Buckner and his colleagues did a similar experiment with a cognitive task. They used the word-stem completion test, in which subjects are given three-letter parts of words and asked to complete the word. A single word-stem completion, the researchers found, activated brain areas nearly identical to those activated by a block trial.

Thus was born a new method, "event-related" fMRI, in which researchers collect brain image data from individual trials, which they can then sort and pool as they wish. It opens up many avenues for cognitive experiments, says Buckner. For example, some tests don't work well in block trials, because they involve an element of surprise.

Studies with electroencephalograms (EEGs), which record electrical activity inside the brain, have demonstrated that if you show a person a series of pictures, say, of geometric shapes, and then throw in something different, like a picture of an animal, the oddball picture produces a bigger neural response than the others. Neuroimagers would like to know which brain areas react to the surprise, but they can't find out from a block experiment, says Buckner, because "if you do that kind of surprise three or four times, [the response] goes away." With event-related fMRI, researchers can mix "surprise" trials with other types of trials, and then afterward pool the data from the surprise trials to analyze together.

Research groups have leaped to use the new approach, not only to identify brain areas that react to unusual events, but also to relate brain activity directly to subjects' responses or perceptions which can only be determined once the experimental trial is over. For example, the technique allows researchers to sort brain images based on whether a subject got the right or wrong answer in a test, and see how the brain activation differed. Because of its ability to address some of these important questions with imaging for the first time, the technique "is catching on incredibly rapidly,"

* "Neuroimaging of Human Brain Function," The Arnold and Mabel Beckman Center of the National Academy of Sciences, Irvine, California, 29–31 May.

says Buckner.

It is likely to catch on even faster, thanks to some recent troubleshooting done by Buckner and his Harvard colleague Anders Dale. The problem they addressed is this: The fMRI response to a single trial takes more than 10 seconds to run its course, so it seemed that individual trials would have to be separated by 16 seconds or so to be sure the response to one trial was finished before the next one was presented. That is not only time-consuming, but could also alter the results. "Sixteen seconds is a long time to do nothing," says Buckner. "People have more time to work on the problem, more time to prepare." Dale and Buckner have a paper in press in *Human Brain Mapping* showing that trials can be presented as fast as every 2 seconds, and an algorithm can then be used to extract the overlapping brain activation data associated with each trial.

Is bigger better?

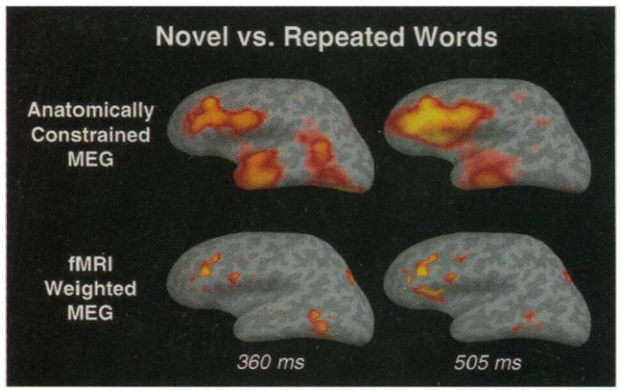
Advances like event-related fMRI have opened up countless questions for cognitive neuroscientists to address. Most can be tackled using standard fMRI machines, which have magnetic field strengths of 1.5 or 3 teslas (T) and can distinguish activated brain areas separated by as little as half a centimeter. But "there will be a time when we will definitely have to look beyond" that resolution, says brain imager Kamil Ugurbil of the University of Minnesota, Minneapolis. Ugurbil, who feels that time is rapidly approaching, is leading the charge to higher magnetic fields. The imaging center at Minnesota has a 4-T MRI machine, one of only a handful in the world. With those machines, researchers have revealed multiple strengths of higher fields.

At higher fields, Ugurbil says, event-related fMRI can be developed to its full potential, producing robust images from single trials, and reducing the need for researchers to sort and pool their results. What's more, researchers have already seen two brain features with 4-T machines that have eluded those using lesser magnetic fields. One, the so-called early oxygenation dip, is an apparent drop in blood oxygenation in active brain areas before the rise in blood flow (*Science*, 11 April, p. 196). Researchers using 4 T have also seen ocular dominance columns—columns of neurons in the visual cortex with diameters on the order of 1 millimeter—which respond selectively to images from one eye or the other. All these effects are sure to be even clearer at fields higher than 4 T, Ugurbil says.

Indeed, neuroimager Roger Tootell, of Harvard Medical School in Boston, calls Ugurbil's sighting of ocular dominance columns a "watershed" in brain imaging. Imagers now focus on activity in whole brain areas, but the opportunity to see the activity of individual columns within those areas promises "a quantum jump in insight," Tootell says. "There are columns all over the brain, and we don't know what they do."

Ugurbil's team hopes to pursue both the oxygenation dip and column resolution with a 7-T human imaging machine they are due to receive in December. It will be the first of its kind in the world. But no human has ever been exposed to a 7-T field, and safety tests on animals, already begun by Ugurbil's group, will be needed before that can be done.

Even if those colossal fields are deemed safe, neuroimager Marcus Raichle of Washington University in St. Louis warns that bigger is not better for everyone, because the bigger machines require an engineering team devoted to tinkering and tuning constantly. "You become hostage to the equipment if you're not careful," he says, likening a high-field magnet to a "Ferrari that needs a $5000 tune-up every year" and isn't really suited to just going for a ride. "A well-equipped, well-running 1.5-T machine, with ... people who know how to ask the questions, is an enormously powerful piece of equipment," Raichle argues. "There is a tremendous amount of neurobiology that can and will be done on such machines." Nevertheless, he says, someone, preferably with Ugurbil's level of experience, needs to be developing higher field machines to pave the way for a time when the biological questions demand the next wave in resolution. "We may, 5 years from now, say, 'Gosh, we all have to be at 5 T.' "



A. DALE, E. HALGREN, AND J. LEWINE

In focus. Combining fMRI with MEG provides a sharp image of brain activity caused by viewing a novel word for each time examined.

A timely union

One trick not even the biggest MRI machine can presently pull off on its own is following precisely when brain areas become active during a cognitive process. That's because the neurons themselves respond within 10 milliseconds of a triggering stimulus, while the blood-flow changes measured by fMRI or PET take several seconds to develop. This limitation has been a great frustration for neuroimagers. "Timing is everything in the brain," says UC Davis's Mangun. Without timing information, researchers can only guess about how different brain areas build on each other's work as they perform a task.

To remedy this problem, Mangun's group and others have recently arranged a marriage of convenience between fMRI and PET imaging techniques and a pair of brain-recording methods whose forte is timing: EEG, which measures the electrical fields produced by brain neuron activity, and magnetoencephalography (MEG), which measures neurally generated magnetic fields. Both methods can take readings at more than 100 points on the scalp and can track how neural activity changes with time along the surface of the head. But they have a big weakness: They can't pinpoint the source of the electromagnetic signal.

Mathematical equations can point to brain areas where the activity might be, but the equations yield multiple solutions, with no way to tell which one is right. But "if you can calculate a [candidate] position, and then show that neuroimaging shows that there are active cells in that particular place, then that increases your confidence that you've got it right," says EEG researcher Steven Hillyard of UC San Diego.

Hans-Jochen Heinze at Otto von Guericke University in Magdeburg, Germany, along with Mangun and Hillyard, did just that with a cognitive task in 1994. They presented subjects with pairs of symbols in both their right and left visual fields and directed their attention to either the right or left field by asking them to judge whether the symbols appearing there were the same or different. Earlier work in Hillyard's lab had shown that the EEG wave evoked by the symbols differs, depending on whether the subject is paying attention to them or not: A bump in the wave beginning about 80 milliseconds after the symbols were flashed, known as the P1 component, gets bigger when the subject pays attention.

To find the source of the activity that creates P1, the Heinze team had the subjects do the task once while the researchers took EEG recordings, and again in the PET scanner. The PET data showed two areas in the so-called "extrastriate" portion of the visual cortex that could be the source of P1, and the team then returned to the model to see whether these spots would work as possible sources that would explain the EEG data.

Those sites, says Mangun, explained the data "very, very well." Mangun has since shown that making the perceptual task easier selectively reduces both P1 and the attention-associated extrastriate activation seen in PET, further support that the two techniques are measuring the same brain function.

That experiment showed that imaging and electromagnetic techniques can work together, says Harvard's Dale. But the math used by the Heinze team could consider only two or three simultaneously active brain areas as possible sources of the EEG signal. And while that was fine in the case they had chosen, Dale points out that in most cognitive processes, many brain areas are activated. Dale is one of several researchers deriving a new generation of mathematical models that can pose thousands of sites of brain activity as potential sources and contain other improvements as well.

Like the model used by the Heinze group, Dale's model begins with electromagnetic data recorded on the scalp and predicts which configuration of active areas in the brain could best explain that activity. But instead of relying just on EEG recordings, it can use MEG and EEG data taken simultaneously. And while older methods model the brain as a sphere inside the skull, Dale's limits the potential sources of activity to the cerebral cortex. Moreover, because each brain is unique in how its cortex is folded, Dale uses a structural MR image to tailor the calculations to the individual brain.

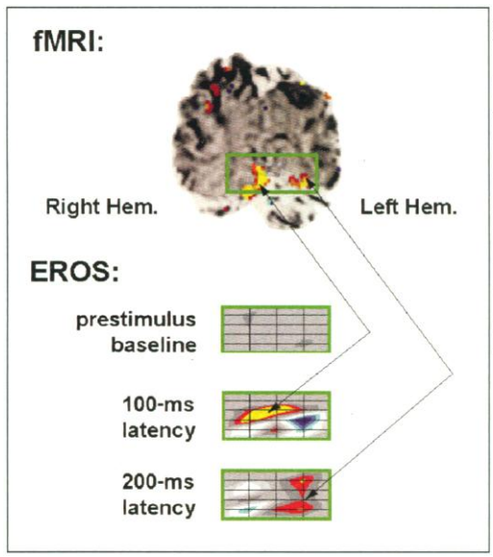
The result is a localized, though fuzzy, estimate of combined activity in the brain that could produce the EEG and MEG signals at any point in time. Dale then takes fMRI data on brain activity during an identical experimental trial and uses those data to "weight" the solutions by having the equations favor areas shown to be active by the fMRI. The end result is a set of crisp images with the spatial resolution of fMRI that show changes in brain activity on a time scale of tens of milliseconds. "You can make a movie animating this," Dale says.

Dale, Tootell, and Jack Belliveau, also of Harvard, have validated the technique by using it to look at the timing of the brain's response to a moving image, and Dale and Eric Halgren of UC Los Angeles have studied the time course with which the brain responds to novel versus repeated words. "It is an important wedding of techniques," says Washington University's Raichle and is likely to become a staple of the field for researchers who want to know the pathways information takes in the course of a thinking process.

Future frontiers

Millisecond movies of neural activity using fMRI, EEG, and MEG might seem visionary enough, but some in the field think such wonders will someday be possible with a single technique—either a new form of fMRI or a much less expensive alternative: imaging with ordinary light beams.

Keith Thulborn's team at the University of Pittsburgh Medical Center is working to devise a way to get images with real-time resolution information directly from fMRI, by measuring changes in the sodium magnetic resonance signal. "Sodium imaging may be a very direct way of looking at neuronal activity," says Thulborn, because sodium ions flow into neurons when they fire.



G. GRATTON, M. FABIANI, P. CORBALLIS, AND J. HIRSCH

The timing factor. fMRI shows two activated visual areas; EROS shows that activity in the primary visual cortex (100 msec) precedes that in the extrastriate cortex (200 msec).

The passage of ions into the neurons changes sodium's magnetic resonance properties in a way that should be detectable by MRI, Thulborn says.

The imaging center at Pittsburgh already uses sodium imaging clinically to assess brain damage in patients with strokes, epilepsy, and tumors. Because the sodium signals are weak, it takes 10 minutes to create a reliable three-dimensional image, says Thulborn. But because MR images are built up from many individual snapshots, Thulborn says it would be possible to construct images that capture the immediate neural response by taking repeated snapshots timed at a very short interval after a repeated stimulus. Thulborn and a team of engineers and physicists have been working for 6 years to improve the MRI machine's ability to detect sodium. Their work has reduced the detection time from 45 minutes to 10, while increasing spatial resolution an order of magnitude, and they plan to test the experimental approach on a 3-T machine within the next few months.

Still other researchers are hoping to image neural activity directly without the $1-million-per-tesla price tag of fMRI. Their preferred medium: light. Studies in living brain slices have shown that the light-scattering properties of neurons change when they become active. Cognitive neuroscientists Gabriele Gratton and Monica Fabiani of the University of Missouri, Columbia, lead one of several labs trying to take advantage of that property by using near-infrared light from a fiber-optic source to image activity changes in living human brains. Their system, which they call EROS, for event-related optical signals, has a bargain-basement cost of less than $50,000.

When a fiber-optic source placed on the scalp shines light into the head, the light penetrates the skull and is scattered by brain tissues before some of it reemerges. EROS uses light sensors placed on the scalp just centimeters from the source to measure the time the light takes to emerge. Because that time is influenced by light scattering, which in turn is affected by neural activity, the system can detect changes induced by an experimental task. And it does it with a temporal resolution similar to that of an EEG. EROS can also locate the source of the scattering changes, based on detector placement and timing of the light's emergence, with spatial resolution of less than a centimeter. Using EROS, Gratton repeated the experiment by which Heinze, Mangun, and Hillyard first showed the power of combining PET with EEG. EROS produced the same results, localizing the effects of attention to the extrastriate cortex.

One limitation of EROS is that the light can only penetrate several centimeters into the head, and so the technique is unable to register activity from deep brain areas. Indeed, some researchers worry that it will not reliably image parts of the cortex that are buried in folds. "If it is limited to the superficial cortex, it will never replace fMRI," says cognitive neuroscientist Steven Luck, of the University of Iowa, Iowa City. But Gratton and Fabiani say they have already imaged cortical areas deep in a fold and have ideas about how to reach even deeper regions.

"My eye is on optical techniques in terms of the next wave," says neuroimager Bruce Rosen, of the magnetic resonance imaging center at Harvard Medical School. "In 10 years, I wonder if we will all be doing optical imaging and throwing away our magnets." While most brain imagers might think that unlikely, this is a field that has learned never to say never.

–Marcia Barinaga