Brain-Watch

What is happening in your brain when you look at a picture or in a rat pup's brain as it suckles? A technique that labels active nerve cells is answering such questions in increasing detail.

BY JULIE ANN MILLER

Put your brain to work. First rest with your eyes closed, then look at diffuse white light and finally at more and more complex scenes. It feels as if your brain is working harder and harder, and indeed it is — as can be seen in a safe, painless technique that is now being used to document and map brain activity. The technique can track increasingly subtle operations, such as hallucinations or the interpretation of sounds, and it can pinpoint the basis of some brain disorders.

The more work done, the more fuel used. That's the principle underlying the general technique. Just as a spy in an industrial nation might determine which factories are operating at top productivity by keeping track of the amount of fuel they consume, scientists can look at cellular uptake of glucose, the predominant fuel of the brain, to see what cells are hard at work.

Louis Sokoloff of the National Institutes of Health devised a gimmick to make possible convenient measurement of a cell's workload. The cells receive glucose doped with a radioactively labeled glucose derivative that they take up but are neither able to use nor to excrete (SN: 11/11/78, p. 324). A clever spy, similarly, might arrange for factories to receive coal mixed with lumps of a material that will not burn. He could then base his analysis on the amount of incombustible material piled up at each factory.

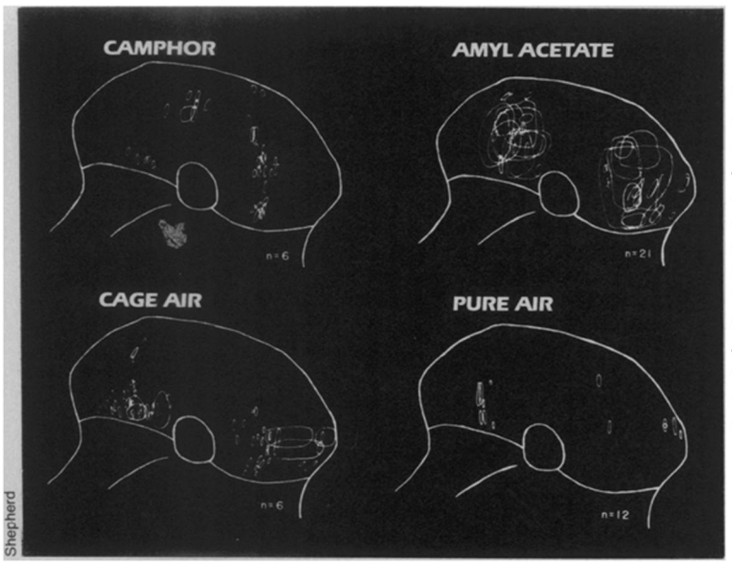
The technique for analyzing brain (and other tissues) is called 2-deoxyglucose, or 2-DG, mapping, after the infiltrating chemical that labels the nerve cells. It allows scientists to see simultaneously all active regions in the brain of an animal exhibiting normal behaviors. Some of the work confirms with dramatic images results worked out with more tedious techniques; for example, the arrangement of brain cells that receive information from each eye (SN: 11/25/80, p. 372).

At the most recent meeting of the Society for Neuroscience, dozens of researchers reported on studies using the 2-DG techniques on a wide variety of subjects — mollusks, primitive fish, birds and numerous mammals, including humans.

A multitude of questions unapproachable with previous methods can now be answered using the 2-DG technique: What areas of the brain develop first? Which age most markedly? What happens in the brain during sleep and hibernation?

The brain's processing of olfactory information is one intriguing area that has been surveyed by the 2-DG technique. "This method is really wonderful," says Gordon Shepherd, a Yale University neuroanatomist. "Because the olfactory system doesn't map the external world, we had no idea what the patterns in the brain would be. Some people thought it wouldn't be a map at all."

The very year that Sokoloff reported the 2-DG technique, work using it began on the olfactory system. Shepherd describes the experimental procedure for an awake, naturally behaving rat injected with 2-DG: "You put the rat on the table, let it sniff the air or a chemical and the olfactory regions show dots of activity [visible after the brain has been sliced and fixed for microscopy]." Shepherd has found a generally reproducible pattern from animal to animal. A given odor — clean air, cage air, camphor or the banana-like smell of amyl acetate — activates cells in a certain region of the olfactory bulb, the brain area that receives input from receptors in the nose. The stronger the odor, the greater the area activated. Different odors have different patterns, although the areas activated can overlap.



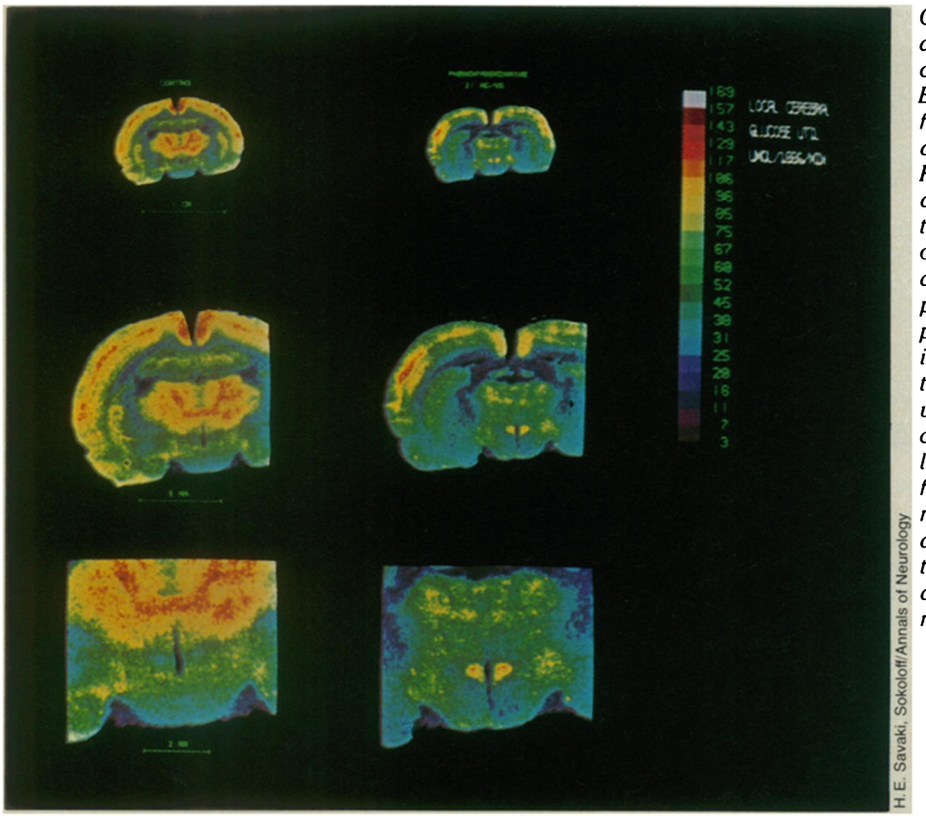
Whiffs of different odors activate brain cells in different patterns in the olfactory processing region. While the patterns are clearly distinct, the areas overlap.

Smelling is crucial to the newborn rat; whiffs of a pheromone are essential for the pup to suckle. And Shepherd and collaborators have found that a small region at the edge of the main olfactory bulb is active when the pup is suckling. To assess the state of young rats' brain development, Charles A. Greer, Shepherd and co-workers exposed rat pups to amyl acetate 12 hours after birth. They observed 2-DG accumulation in the same brain area as in adult rats similarly exposed. The pattern, however, is somewhat different; active spots are fewer, more widely dispersed and less clearly defined. The more distinct adult pattern is observed in rats by the age of 12 to 15 days.

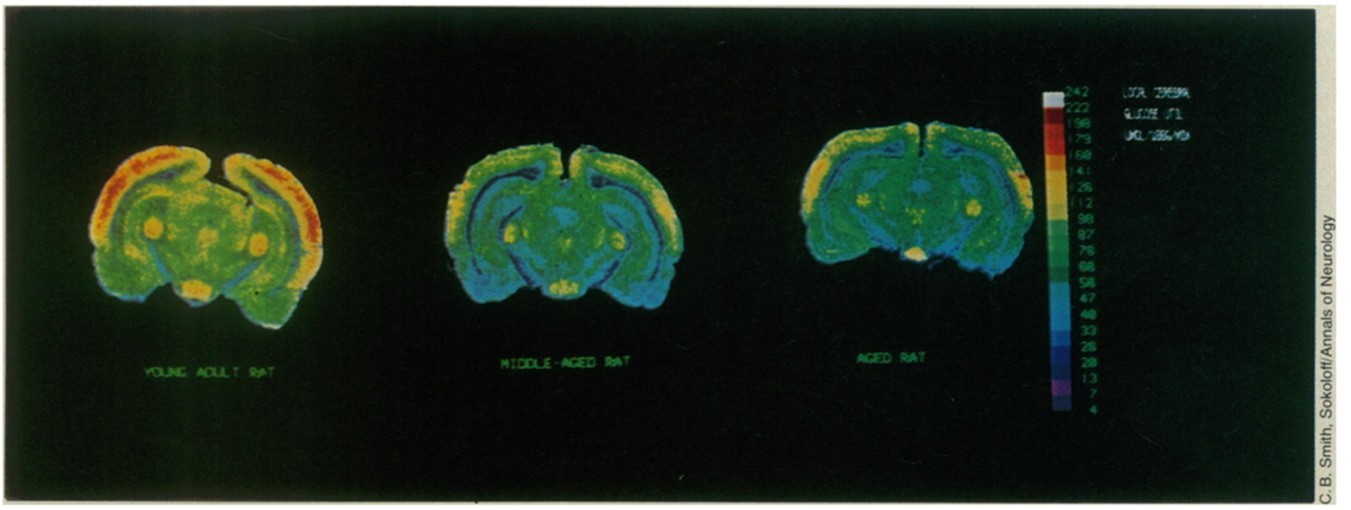
Aging, as well as early development, is being examined with the 2-DG tag. Activity levels in 47 brain structures in young adult, middle aged and elderly rats were compared in a study by Sokoloff, Caroline B. Smith and co-workers. They found that aging is associated with decreased activity in specific brain regions. Some of the decreases appear to reflect decreased sensory input and movement disorders observed in aged rats. The rat limbic system, the brain structures implicated in learning and memory, remain unchanged with age.

Because senility is not generally seen in rats, other researchers have turned to dogs, whose nervous systems deteriorate with age in a manner more suggestive of human senility. Edythe D. London and colleagues at the National Institute on Aging examined 1-year-old and 11-year-old beagles. They report decreased activity in every brain region measured. Some of the larger decrements are related to loss of sensory function; others are observed in the limbic system. The researchers conclude that although there is an overall decrease in glucose use with age in the beagle brain, the regional distribution probably is associated with regional deficits in brain function.

A non-uniform depression of brain activity also has been revealed in hibernation studies using golden-mantled ground squirrels. Thomas S. Kilduff and colleagues at Stanford University and the



Colorful maps that describe the activity of different brain areas are produced by a computerized image processing system. Each color represents a different rate of fuel use; white and reds are the highest and dark blues and purples the lowest. Regions as small as 100 microns can be depicted. The "pseudocolor reconstruction" at left shows a section of the brain of a normal, conscious rat (left column) and of a rat treated with the drug phenoxybenzamine, which affects blood pressure (right column). Resolution of the images increases from the top images to the bottom. The small structures activated in the drug-treated animal are called the paraventricular nuclei. (Below) Reconstructions of brain sections from young adult, 4 to 6 month (left), middle-aged, 14 to 16 month (center) and aged, 26 to 36 month, rats show a reduction in activity with age. The drop is most dramatic between the young and middle-aged animals.

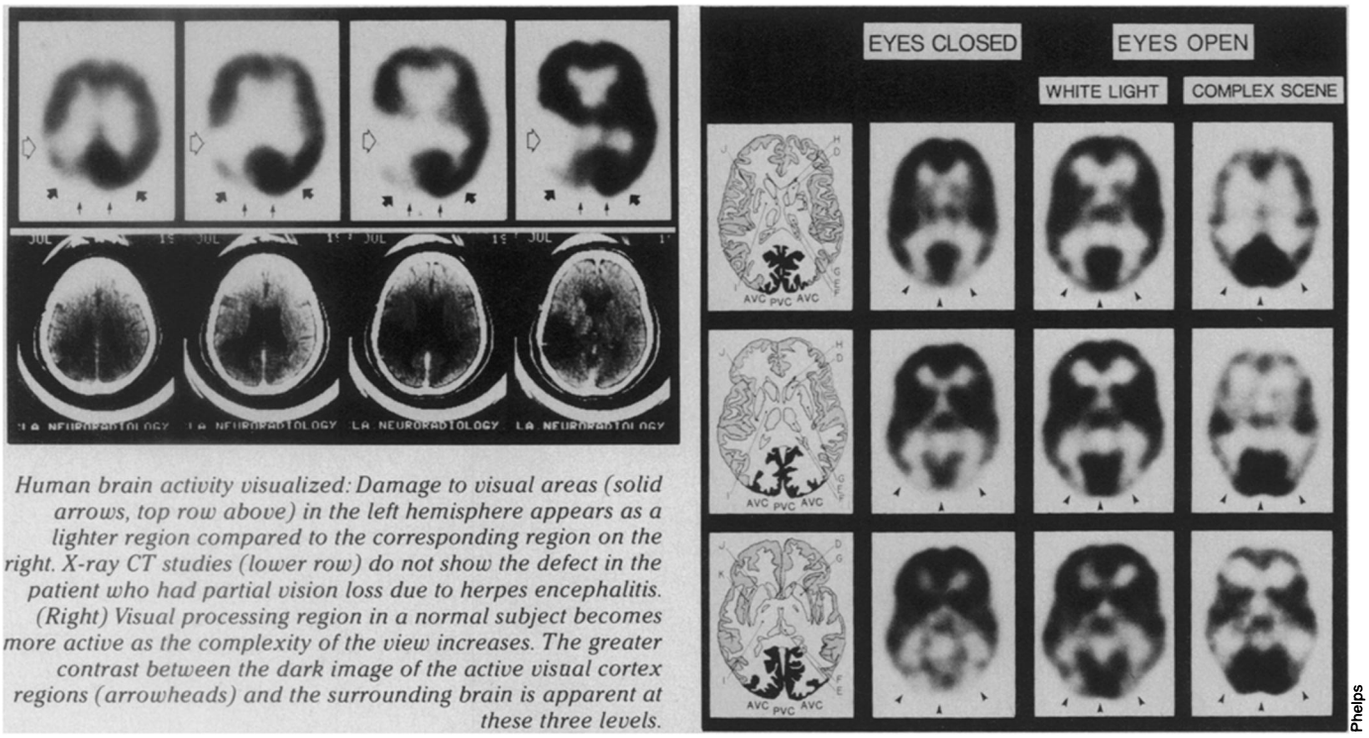


University of California Medical Center in San Diego see decreases in activity in almost all of the 83 neural structures they have observed. In general, areas the most active normally showed the greatest drops. Several sensory and proprioceptive areas showed only small changes during hibernation, as if the animal keeps some of its senses on the alert. Brain activity during arousal suggests that the mamillary bodies, brain structures that receive and relay olfactory impulses, play a special role in the wake-up process. Similar work is underway in other laboratories on rats and cats to map out brain activity during sleep.

As originally described, the 2-DG technique only could distinguish brain structures, it could not detect individual active nerve cells. But now modifications of the technique allow observation of single cells. Terrence J. Sejnowski of Harvard Medical School and Stephen C. Reingold of Princeton University, working on clusters of nerve cells in the mollusk *Limax maximus*, report that using tritium instead of carbon-14 as the radioactive tag and dehydrating the preparation before freezing it allows resolution not only of single cells but of their nuclei and cytoplasm. Other researchers have identified single neurons in goldfish retinas and in mouse cells growing in culture, and Shepherd's team is beginning to label individual cells in the rat olfactory system.

A considerable amount of work in animals is being done modeling diseases of human concern and investigating therapeutic treatments. For instance, the 2-DG technique is being used to examine brain activity in rats with spontaneous hypertension and, in other experiments, in rats receiving lithium and other drugs. But while such experiments can provide valuable information, the power of the 2-DG technique is that observations also can be made directly in humans.

Examining the activity of the living human brain still takes an expensive, heroic effort. But more than 30 medical research centers in North America and Europe now have such programs (compared to three centers just three years ago [SN: 11/18/78, p. 340]) and the growth is expected to continue, says Michael E. Phelps of the University of California at Los Angeles School of Medicine. The technique is an extension of X-ray computerized tomography (CT or CAT scans), in which 3-dimensional computer-gener-



Human brain activity visualized: Damage to visual areas (solid arrows, top row above) in the left hemisphere appears as a lighter region compared to the corresponding region on the right. X-ray CT studies (lower row) do not show the defect in the patient who had partial vision loss due to herpes encephalitis. (Right) Visual processing region in a normal subject becomes more active as the complexity of the view increases. The greater contrast between the dark image of the active visual cortex regions (arrowheads) and the surrounding brain is apparent at these three levels.

ated images allow examination of internal anatomy.

To view the biochemical process within the brain, instead of just brain structure, an adaptation of the 2-DG method is used. The chemical is tagged not with carbon-14 or tritium, but with the isotope fluorine-18 in the place of one of the hydrogen atoms. Fluorine-18 is a short-lived radioactive element that emits positrons, positively charged electrons, that give off gamma radiation when they combine with electrons. The gamma radiation is detected outside the body by a scanner, and computer techniques form cross-sectional images of the isotope's distribution in the brain. The resolution in the human studies is not as great as in animal studies, because tomography cannot pick up the details available from actual slices of animals' brains.

Describing the normal functioning of the brain is one task for researchers. Much of the work with Positron Emission Tomography (PET) has focused on brain processing of visual and auditory information. The results show that viewing more and more complex scenes increases the amount of activity in the vision-processing regions of the brain. Subjects looking out the window at a park used about 50 percent more fuel in the associative visual cortex than they did when they had their eyes closed. The increase in metabolic response over the eyes closed level was ten times more for the park scene than for diffuse white light and twice the difference for a simple alternating checkerboard pattern.

Even thinking about a visual image makes the brain perform work in the visual areas, as do visual hallucinations. The highest activity level so far observed in the visual processing areas occurred during a seizure in which a patient, who has a brain tumor, hallucinated white, flashing numerals moving across his lower left visual field.

A surprising dichotomy showed up among subjects who were asked to report whether two groups of tones were different or similar. In some subjects the right hemisphere of the brain was more active; in others it was the left hemisphere that was put to work. The difference turned out to be in the way the subjects processed the auditory information. Those who tried to remember a melody used the right hemisphere; those who mentally plotted the notes on a music staff used the left. In the brains of persons who pictured the musical notes, activity was observed in the visual as well as the auditory processing areas.

Insight into human diseases is the other mandate to the PET workers. The studies are already providing powerful new methods of diagnosis. Since June 1979, all patients at the UCLA Medical Center who are admitted for brain surgery to control epilepsy receive a PET scan to determine the location of the foci, or origin, of their seizures. A surprising finding, now thoroughly confirmed, was that the focal areas, although extremely hyperactive during a seizure, display unusually low activity between seizures.

New diagnostic methods for other diseases may also result from PET. Until now, for instance, there has been no way to diagnose the rare genetic disease Huntington's chorea before the appearance of its debilitating symptoms — movement disorders, intellectual deterioration and psychosis. Phelps and colleagues David E. Kuhl and Charles Markham have found a marked change in brain activity in all the Huntington's chorea patients they have examined. They reported a lack of activity in two brain structures, the caudate and the putamen. The scientists also have observed abnormally low levels of activity in those structures before the onset of disease symptoms in subjects who have a family history of the disease. PET thus may provide a unique opportunity for scientists to investigate subtle changes in brain function early in the disease's development.

Similarly, early senility, or Alzheimer's dementia, is being examined with the PET technique. Frank Benson, Kuhl and Phelps report "exciting results in terms of the functional transitions of the normal brain to decreased capacity to perform mental functions." They find no deficiencies in the activity of the visual and auditory areas. But the metabolism is low in the frontal lobe, the area involved in such complex processes as reasoning.

Phelps likes to show an image from an X-ray CT scan alongside a totally dark square labeled PET. He asks the audience to make a diagnosis. Someone eventually shouts the correct answer, "The patient is dead." Phelps explains, "X-ray CT still looks normal after the patient has been dead five months." He admits, "Of course, the clinical interview would pick that up rather quickly."

Still, the example is effective in emphasizing that the 2-DG techniques provide reflections of a living, functioning brain. In animals it provides the opportunity to trace information processing in finer and finer detail. And with positron emission tomography it allows solid investigation of those mental processes that are uniquely human. □