Million-Cell Memories

Surprisingly large portions of the brain may participate in a simple memory, thus challenging the notion that memory 'traces' are stored in crucial chains of brain cells

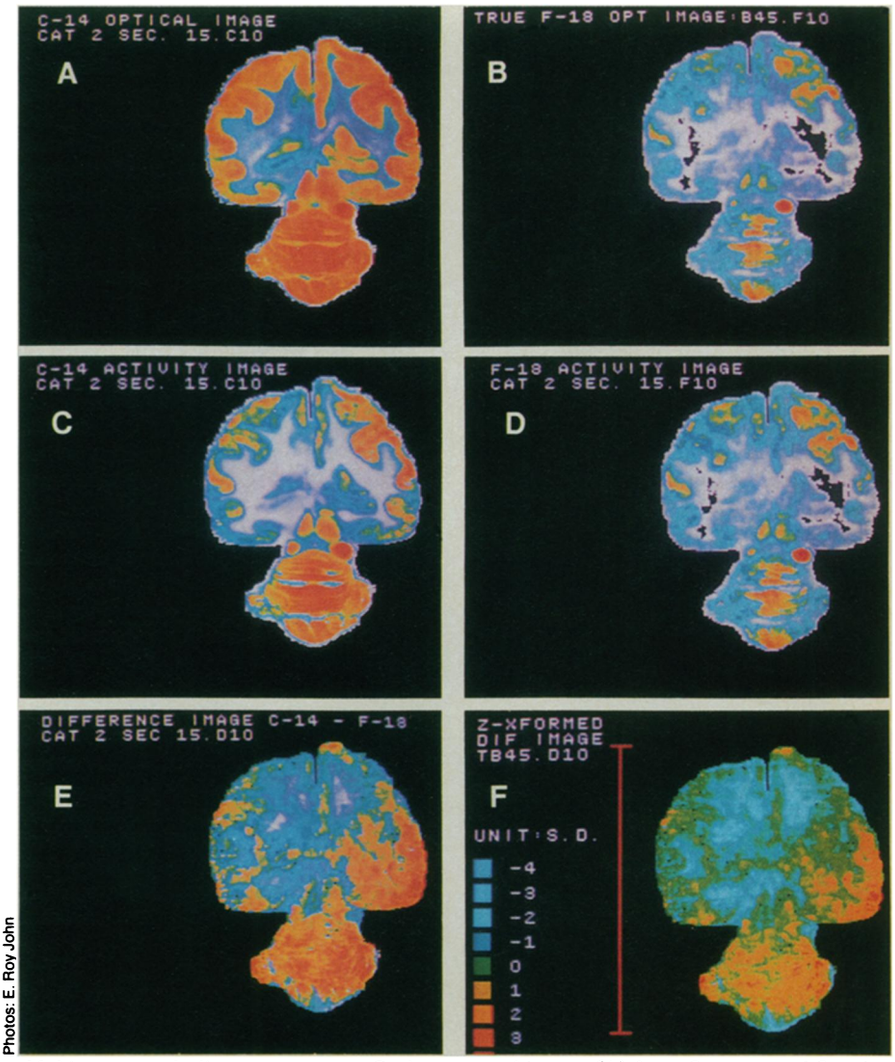
By BRUCE BOWER

A cat peers at two doors and decides that only the door marked with two concentric circles can be nudged aside to obtain a bowl of food. As the cat remembers what it has learned from prior conditioning trials, millions of widely distributed brain cells called neurons begin to work together in complex ways. The animal's memory for the simple response is stoked by as-yet-unrecognized properties of that enormous system of cooperative neurons.

This, at any rate, is the view expressed by a team of scientists in the Sept. 12 SCIENCE. Their contention, which challenges a key aspect of conventional memory theory, stems from radioactively labeled maps of neuron activity throughout the brains of two cats that underwent simple learning trials.

"I thought we'd find maybe 20,000 to 40,000 cells involved in the learned memory," says physiological psychologist E. Roy John of New York University, who developed the color-coded brain images with Yong-Nian Tang and A. Bertrand Brill of Brookhaven National Laboratory in Upton, N.Y., Ronald Young of the University of West Indies in Kingston, Jamaica, and Kenji Ono of Nagasaki (Japan) University. "The shock was that it was so easy to see widespread metabolic change," continues John. "The number of brain cells [between 5 million and 100 million] involved in the memory for a simple learned discrimination made up about one-tenth of the whole brain."

Cell cooperation on such a vast scale calls into question the assumption of some researchers that each memory is localized in a discrete set or "trace circuit" of neurons. A trace is a chain of neurons linked by synapses, where electrical impulses and chemical transmitters jump from one cell to another. Traces for different types of memo-



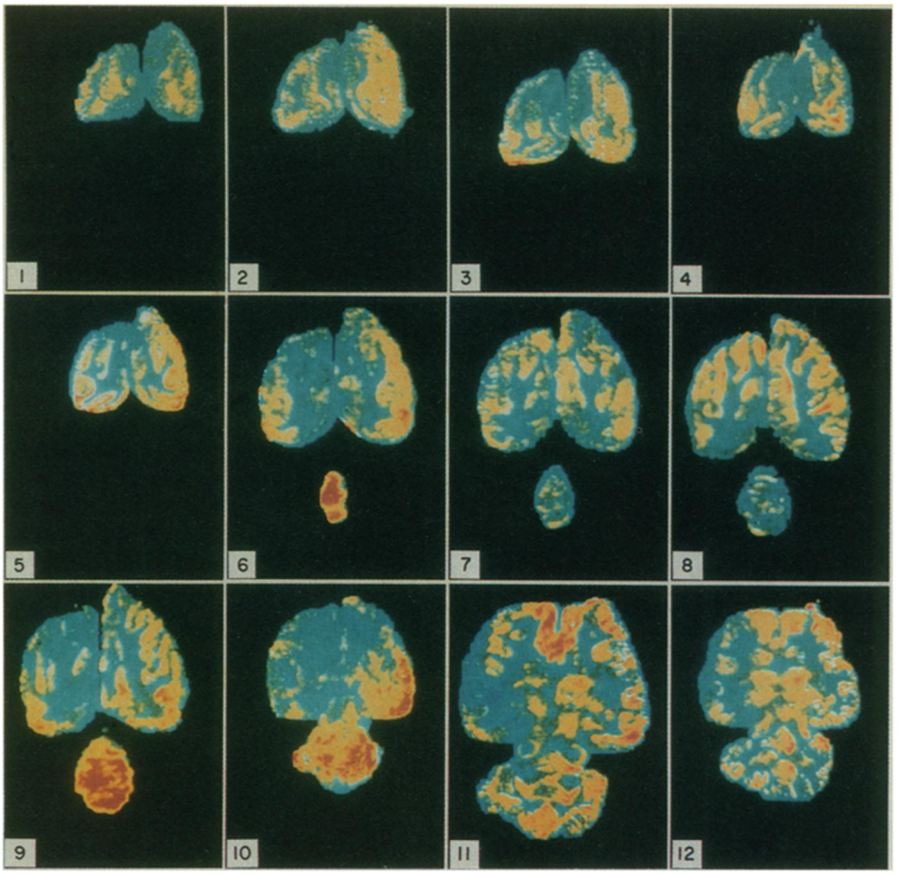
Photos: E. Roy John

Making a metabolic memory map: Carbon-tagged image (A) from first test and fluorine image (B) from second session are corrected for exposure times and decay in (C) and (D). Fluorine image is subtracted from carbon image (E) and corrected for metabolic "noise" (F). This final image, also shown on the cover, shows greater metabolic activity (orange and red areas) predominantly on the right side of the brain, the only side exposed to a previously learned visual cue.

ries have been hypothesized to lie in specific brain regions far more limited than the expanse of activity picked up by John and his colleagues. For example, it has been reported that calcium floods into receptor sites on rats' hippocampal cells after electrical stimulation similar to that occurring during learning; continued stimulation, say researchers, permanently changes the cells and creates new neuronal connections in the hippocampus. They suggest that this process is involved in storing memories for facts.

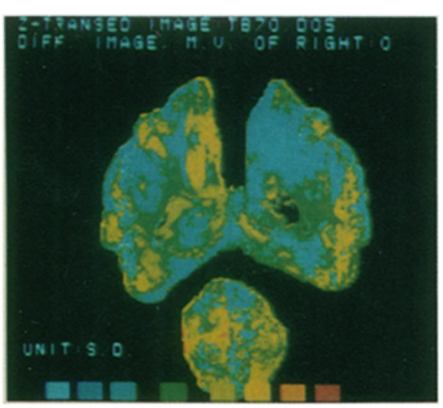
But there is a problem with assuming that each memory for a fact, skill or whatever is tucked into its own exclusive circuit of neurons, says John. If as many as 100 million brain cells were involved in one, and only one, feline memory for a simple task, there would not be enough neurons to handle an entire catalog of memories. If each remembrance were stored in a "megatrace" made up of millions of neurons, asserts John, "the brain would burn out after learning a few things."

Thus, he sees the focus on memory traces as misguided. An alternative proposal that has guided John's work and the efforts of a number of other investigators over the past 50 years is that memory — the processing and reclaiming of experience over short and long stretches of time — is somehow widely distributed in the brain, with some neurons participating in more than one memory.



Metabolic maps from 12 brain slices of cat with right hemisphere exposed to cue. Glucose use is significantly higher on right side of most images (yellow, orange and red areas) relative to average glucose use throughout the whole brain.

John says there is plenty of evidence indicating that large portions of gray matter participate in the same learned responses. For example, researchers have found widespread electrical changes in the brain after learning and the absence of memory loss despite critically located brain lesions in animals and humans. To get a better idea of how many neurons "turn on" when a cat's memory is activated, John and his colleagues devised a way to compare metabolic activity in the animal's brain during exposure to novel and previously learned visual cues.



Metabolic memory image from brain slice of cat with left hemisphere exposed to learned cue. Yellow and orange areas indicate marked activity increases.

First, cuts were made in the brains of two cats to separate the left and right hemispheres. The split-brain cats were trained to discriminate between transparent green figures on adjacent doors; two concentric circles on a door meant it could be pushed open to obtain food, while a door bearing a star was locked. When an opaque contact lens was placed over one eye, thus blocking visual information to its corresponding hemisphere, and a green lens was placed on the other eye, the cats still performed the task correctly, indicating that each hemisphere had a memory for the discrimination. When fitted with an opaque lens and a red lens, the cats displayed no memory for the cues' meanings.

Before glucose metabolism was tracked, a green contact lens was placed over one eye (the right eye for one cat, the left for the other) and a red lens over the other eye. New cues were then presented. Each door bore a transparent red triangle in addition to the green circles or star. With the colored contact lenses in place, one hemisphere was exposed to the learned cues and the other side received only novel information. As soon as the lenses were inserted, the cats were injected with a glucose analog, 2-fluorodeoxyglucose, labeled with radioactive carbon isotopes. Discrimination trials followed over the next hour.

After a rest interval, a second trial series began. This time, both doors had a green triangle and a red triangle. The red and green contact lenses remained in place, and no learned information was available to either hemisphere. At the start of this series, the cats received an injection of the analog labeled with radioactive fluorine. In this way, distinctive "tags" identified metabolic activity during the two trial periods.

After completing the tasks, the cats were killed. Microscopic brain slices were covered with a film that picks up the emission of the radioactive tracers. The researchers used computer-generated images of the slices to statistically analyze glucose use in the two trials as picked up by the dual markers. First, they produced a fluorine image for each section, representing neural activity during the second trial, since radioactive fluorine decays much faster than carbon. A day later, they developed first-trial images using the carbon tracer. In each image, the degree of glucose uptake was depicted with various colors.

One side of each cat's brain served as a control for estimating metabolic changes in the two trials due to influences having nothing to do with memory, such as blood pressure, heart rate, arousal, motivation, movement and smells picked up from the experimenter and the laboratory. The

A little 'whispering' among cells

There are two ways to look at communication within and among cells, says medical physiologist W. Ross Adey of the Jerry L. Pettis Memorial Veterans Hospital in Loma Linda, Calif. The traditional view among biologists is that a large burst of energy is needed to overcome the randomly arranged energy within a cell and convey a message to the cell's interior. The result: Ions burst across the cell membrane, bearing signals and boosting cell activity.

But another view is gaining prominence, notes Adey, and it is not far removed from E. Roy John's theory of a charge distribution affecting millions of neurons during memory retrieval. Adey says evidence from his and other laboratories shows that "the cell surface, or membrane, is where the action is. It amplifies weak electromagnetic activity occurring between many types of cells and gives specificity to cell function."

A synthesis of research conducted over the past several decades, says Adey, reveals the following picture, which he describes more fully in the January/February 1986 THE SCIENCES. Cell membranes contain protein strands that are activated by packets of electromagnetic energy, perhaps generated by another cell or at the membrane by a chemical reaction. The weak signal embodied in the electromagnetic energy rapidly spreads across the cell surface, is amplified and passes across the membrane to the cell's interior. It is not clear what form the signal takes at that point, but it appears to have two main effects. It can alter fibers carrying messages from the cell membrane to the interior, and it can alter the activity of key enzyme systems that regulate growth, metabolism and communication between cells. There are indications, says Adey, that tiny packets of vibrational and electrical energy travel as solitons, or solitary waves, conveying energy or signals within and among cells.

A thorough understanding of how electromagnetic "whispering" among cells is amplified into louder "talking" will shake up assumptions about all sorts of cell processes, says Adey, including those involved in memory. *—B. Bower*

other side was used to map metabolic changes related to seeing the learned cues, as well as changes caused by the other factors.

Images from the control side of the brain, which received only novel information, provided data on normal variations in glucose uptake and were used in estimating normal uptake variations on the experimental side. Fluorine-tagged images from each brain section were then subtracted from corresponding carbon-labeled images on the computer. The resulting image reflects random glucose changes or "noise" on one side and the activation of a memory plus random noise on the other side. The researchers readjusted this image to show which metabolic changes on the experimental side strayed significantly from average deviations on the control side.

They found that, depending on the statistical probablities used, between 5 million and 100 million neurons underwent significantly increased metabolic activity related solely to viewing the learned cues. Although these cells were widespread, activity was most intense at several cortical folds, a section of the brainstem related to vision, the hippocampus and the connecting lobe of the cerebellum.

The bottom line, says John, is that no neuron or circuit of neurons can evaluate the enormous amount of activity involved in remembering a simple visual discrimination. "There must be some kind of cooperative process among huge ensembles of neurons," he says. The nature of that process is unknown.

Other memory researchers see promise as well as problems in the work of John and his colleagues.

"There's no question that they're getting at the metabolic background for whatever is happening during a learned discrimination," says physiologist Walter Freeman of the University of California at Berkeley. "The findings work against the notion that only one part of the brain is goosed when a memory is stored."

Measures of glucose use, adds Freeman, do not reveal actual electrical, magnetic or chemical changes occurring milliseconds after exposure to a learned cue. But the notion of a widely distributed memory system, he says, takes into account the fact that messages in the central nervous system do not travel a straight path; each neuron typically receives information from many surrounding neurons and transmits messages to many other neurons, including some or all of those from which it receives input.

"Although the neurons [identified by John and his colleagues] are widely distributed, they don't appear to be diffusely distributed," says Mortimer Mishkin of the National Institute of Mental Health. Mishkin has reported that two brain structures, the hippocampus and amygdala, exert powerful influences on visual memory in monkeys (SN: 12/10/83, p.378). A closer look at the tremendous amount of data generated from the split-brain images, he notes, might pinpoint critical brain areas involved in memory for the cues.

"It's hard to reach conclusions based on two cats," says Daniel Alkon of the Marine Biological Laboratory in Woods Hole, Mass. "And no one, including John, has distinguished neurons that actually store information from those that are merely involved in its retrieval." Alkon, who has identified lasting membrane changes in specific neurons of sea snails that have undergone learning trials (SN: 1/29/83, p.74), notes that the use of dual radioactive tracers in the same animal, known as "double labeling," shows a lot of potential for studying memory.

But Bernard Agranoff of the University of Michigan at Ann Arbor, who helped develop the double label method nearly 10 years ago, says the statistics used to isolate the metabolic effects of memory from the two tracer images are flawed. He and his co-workers plan to study memory in rabbits once these technical problems are worked out. Until then, holds Agranoff, "I'm not convinced that [John and his colleagues] are showing the metabolic background for a memory."

John, however, says the findings are compatible with his theory that one memory is activated by millions of neurons, with each of those neurons also participating in other memories. He contends that syncopated patterns of electrical charge fluctuations — which can actually be heard if electrodes inserted into neurons are hooked up to a loudspeaker — affect the membranes of widely distributed neurons involved in a specific remembrance. In his scheme, the distribution of electrical charge interacts with cells representing a specific memory, and more and more of those cells are recruited into the firing pattern of the "neural ensemble." As resonance builds up in response to this process, the membranes of participating cells are depolarized and ions are allowed to pass into and out of neurons. This theory is spelled out in more detail in *Foundations of Cognitive Processes* (Robert W. Thatcher and E. Roy John, Lawrence Erlbaum Associates, 1977).

"The important thing is the charge distribution in the neural ensemble," says John. "No single neuron or neural network in the ensemble can detect what must be a cooperative process among millions of cells."

Memory investigators have not, for the most part, embraced John's proposal. Experimental evidence of "cooperative neural ensembles" orchestrating memories is lacking, they say.

"There's been little search for cooperative processes involved in memory," responds John, "so it's not remarkable that little evidence has been found." □