antigenicity (most/least; $t = 3.87$), but at a lower level than both mobility ($t = 5.84$) and low packing density ($t = 4.66$) (*1*).

In sum, while subjectively appealing as a reflection of the tautology that antibodies must have access to an antigenic site in order to bind, the approach and algorithm applied by Novotny *et al.* here and elsewhere (*2, 3*) do not satisfy the requirements of (i) compatible methodology; (ii) consistent, objective, and accurate results, and (iii) defined criteria for correlation. In contrast, our statistically significant correlation of mobility with the most frequently recognized sites suggests structural shifts in the antigen upon antibody binding. This prediction has since been confirmed in the crystallographic structure of the neuraminidase-antibody complex (*5*). Decoding the structural basis for macromolecular recognition will benefit from multiple approaches, but will require both imagination and rigor.

Note added in proof: Monoclonal antibodies raised against both the mobile C-helix region of myohemerythrin and the whole protein recognize the region 79 to 84 (*6*), which is a large probe accessibility minimum (Fig. 1).

H. MARIO GEYSEN
STUART J. RODDA
TOM J. MASON
Department of Molecular Immunology, Commonwealth Serum Laboratories, Parkville, Victoria 3052, Australia
JOHN A. TAINER
HANNAH ALEXANDER
ELIZABETH D. GETZOFF
RICHARD A. LERNER
Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037

REFERENCES

1. H. M. Geysen *et al.*, *Science* **235**, 1184 (1987); E. D. Getzoff *et al.*, *ibid.* p. 1191.
2. J. Novotny *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 226 (1986).
3. J. Novotny and E. Haber, *Biochemistry* **25**, 6748 (1986); ———, M. Handschumacher, R. E. Bruccoleri, *Immunol. Today* **8**, 26 (1987).
4. S. Sheriff, W. A. Hendrickson, R. E. Stenkamp, L. C. Sieker, L. H. Jensen, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1104 (1985).
5. P. M. Colman *et al.*, *Nature (London)* **236**, 358 (1987).
6. T. M. Fieser, J. A. Tainer, H. M. Geysen, R. A. Houghten, R. A. Lerner, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8568 (1987).

8 June 1987; accepted 23 July 1987

Do 15 Million Cat Neurons Mediate the Memory of a Circle and a Star?

E. R. John *et al.* (*1*) write that about 15 million (*2*) neurons increase activity when the memory of a circle or a star is activated in the cat brain. We believe that ambiguities inherent in John *et al.*'s experimental procedure do not justify this conclusion.

1) Three split-brain cats were trained to go down a runway and push through one of two doors labeled with a white geometric figure (two concentric circles) for a food reward. The door bearing the negative cue (a star) was locked, and self-correction was not permitted. Training sessions consisted of 40 trials, at 1-minute intervals, held at the same time each day. After the end of each training session, the animals had free access to food in their home cage until evening. Upon reaching criterion (90% correct), after a training period of unknown length, each cat was trained for another 6 weeks—or a minimum of 1680 additional trials. We are not told anything about response latencies or about the nature of "incorrect" responses (were they incorrect choices or a refusal to choose?).

2) The white geometric figures were then replaced by identical green symbols. The cat saw the world through only one eye, which was covered with a green transparent contact lenses. After an unspecified "brief initial period of hesitation" each cat once more performed for food at criterion levels. By using various combinations of opaque and colored lens, the authors showed that each hemisphere in these split-brain cats could perform the visual discrimination and presumably guide the motor response.

3) After the first label, [^{14}C]2DG, was injected into a paw, the green contact lens was placed over one eye and a red transparent contact lens over the other eye. Each door was then labeled with a transparent red triangle in addition to the transparent green circles or star. At this point input about the learned cues was delivered only to the hemisphere that saw through the green contact lens. The other, reference, hemisphere saw only the red triangle on each door and thus was denied a discriminant cue.

4) After the injection of the second (control) label, [^{18}F]2DG, into the other paw, the same lenses remained in place, but the symbols on the doors were changed. The card on each door bore both a green and a red triangle, that is, no discriminant cues were offered to either eye.

The effect of the learned cues on neural activity was obtained by subtracting the brain activity of labeled 2DG during the control tests ([^{18}F]2DG) from that in the tests ([^{14}C]2DG) during which one hemisphere received familiar discriminant cues and the other hemisphere received unfamiliar nondiscriminant cues. The difference images of the two hemispheres were then compared. The authors conclude that areas which showed hemisphere asymmetry in their metabolic maps were involved in the storage or processing of the memories of the discriminant cues.

John *et al.* appear to be saying that the paradigm described above has eliminated all information not specific to the learned task, and, therefore, that their difference images characterize the brain components active during retrieval of the "circles and star" memory. However, they (*1*) present no evidence that all or any of the 15 million neurons that show excess activity were involved in the storage of the discriminant cue memory. Part of the increase in neural activity could be due to general processes of memory retrieval. For example, circuits in visual cortex could recognize a particular visual input as familiar, and this could then trigger a memory search to uncover any other memories associated with the familiar input. Much of the observed increase in neural activity could be related to this search function, which tells us little about the anatomical representation of an engram.

Equally basic, during the first [^{14}C]2DG test the hemisphere that looks through the green contact lens sees a double circle on one door and a star on the other one. Both clues are familiar, and as discriminanda, have a symbolic meaning about the presence or absence of food. The hemisphere that looks through the red lens sees unfamiliar cues—the triangles (at least they are unfamiliar the first time they are seen). In the second [^{18}F]2DG test, the situation is more complex; the triangles are then novel—on first exposure—for the green lens hemisphere, and familiar for the red lens hemisphere, which has seen them during the first experimental condition. During the first experimental condition the cat obtained food by pushing doors labeled with red triangles, so that triangles may not be a neutral cue to the red lens hemisphere. Thus, it cannot be argued that during the second test the two hemispheres are operating under "informationally symmetric conditions." Differences in the activity of the green lens hemisphere during the [^{14}C]2DG and [^{18}F]2DG tests could be related to the presence or absence of discriminant cues on which to base a visual choice or to the presence or absence of familiar cues, or both.

It is not clear how the cats performed during the two experimental sessions. If there was a difference in motivation, for whatever reason (unfamiliar cues, number of trials, decreasing hunger, physical uneasiness after injection of 2DG into the second paw, and boredom) during the second test, the cats might have shown differences in attentiveness, response latency, and motor activity. Surely the 2DG method picks up attention-related activity and motor activity as well as activity associated with the retrieval of learned information, yet John *et al.* (*1*) treat only retrieval of learned information as a relevant variable.

During the first test, only the green lens hemisphere of the split-brain cat receives information about visual discriminanda. If the cat performs the task at levels approaching criterion, it is clear that the green lens hemisphere is the executive hemisphere, initiating and controlling the motor activity that moves the cat through the correct door. During the second test, we cannot know whether the green lens hemisphere, the red lens hemisphere, or both—stimultaneously or in alternation—control the cat's movements. Unless the green lens hemisphere is also the dominant or sole executive hemisphere during the second ^{18}F test, the comparison of the two experimental conditions is not valid, because a difference in metabolic activity would not merely reflect a difference in information processing, but also a difference in motor-related activity.

Because differences in neural activity between the hemispheres, or between the same hemisphere during the first and second test, may have been determined by a diversity of variables, and not just by the presence or absence of familiar information, it seems virtually impossible to decide which of the activities measured by the 2DG method was, in fact, relevant to the storage or retrieval of specific learned information.

John *et al.* state, "No conceivable neuron or set of neurons, no matter how diffuse its synaptic inputs, can evaluate the enormous amount of neural activity here shown to be involved in retrieval of even a simple form discrimination. Memory and awareness in complex neural systems may depend upon presently unrecognized properties of the system as a whole, and not upon any of the elements that constitute the system." Perhaps. Alternatively, unrecognized properties of their paradigm may have yielded results irrelevant to the hypothesis that John *et al.* thought they were testing.

FERNANDO NOTTEBOHM
HEATHER WILLIAMS
Field Research Center for Ecology and Ethology, Rockefeller University, Millbrook, NY 12545

REFERENCES AND NOTES

1. E. R. John, Y. Tang, A. B. Brill, R. Young, K. Ono, *Science* **233**, 1167 (1986).
2. The manner in which John *et al.* arrive at this figure appears on p. 1173 of their article (*1*). The 15-million estimate corresponds to the number of neurons in pixels, where activity is significantly greater than the control at a level of $P < 0.05$.

17 October 1986; accepted 12 February 1987

E. R. John *et al.* (*1*) propose a method for sequential double-labeling with 2-deoxyglucose (2DG) in which ^{14}C and ^{18}F are used. Sequential double-labeling with 2DG involves injecting a bolus of [^{14}C]2DG, applying stimulus A for 45 minutes, injecting a second bolus of 2DG either labeled with ^{3}H or ^{18}F and applying stimulus B for a second 45-minute period. By taking advantage of the physical properties of the radioisotopes, the relative inability of ^{3}H to expose coated x-ray film in the case of tritium or a short half-life in the case of ^{18}F and with the use of image-processing techniques, John *et al.* ascertains the relative contributions of each form of the labeled 2DG. These experiments offer the potential for determining the effects of two different stimuli in a single animal or for using an animal as its own control. The primary assumption is that the original [^{14}C]2DG does not relocate in response to stimulation during the second labeling period.

Given the importance of this assumption, we were surprised at what little attention John *et al.* give the assumption in their article. Two abstracts are cited in support of the notion that 2DG does not relocate (*2*) both of which reported studies in which 2DG labeled with ^{3}H and ^{14}C were used. The possibility that errors could occur in differentiating the relative contributions from each isotope is not discussed. It seemed that a simpler and cleaner approach would be to inject a single bolus of [^{14}C]-2DG at time 0 and to stimulate animals 45 to 90 minutes after injection. No assumptions or complicated image processing would be required, since we were measuring the contribution from only one radioisotope.

Rats were prepared for self-stimulation (*3*) and given [^{14}C]2DG (80 μCi per rat) in an intraperitoneal injection. In contrast with normal procedures, self-stimulation began 45 minutes after injection and proceeded until 90 minutes after injection. Examination of the autoradiograms indicated responses to stimulation in the ventral limb of the diagonal band of Broca and the right posterior medial forebrain bundle were comparable in these animals to those seen in animals stimulated from 0 to 45 minutes after injection.

In a further assessment of the stability of labeled 2DG during stimulation from 45 to 90 minutes after injection, young male rats were prepared for full quantification procedures (*4*). At time 0, 50 μCi were injected intravenously. From 0 to 45 minutes after injection, the left C3 whisker was stimulated by the tactile whisker method, while from 45 to 90 minutes after injection, the right C3 whisker was stimulated in exactly the same manner (*5*). Blood was collected during the entire 90-minute procedure, and the autoradiograms were analyzed for local cerebral glucose utilization (LCGU) with the DUMAS imaging system (*5*).

Preliminary comparison of the right and left cerebral cortices in two rats indicates that between 49 and 51% of the increase in LCGU over background levels recorded in response to stimulation applied from 0 to 45 minutes after injection occurred in response to stimulation given from 45 to 90 minutes after injection. This confirms the results from the self-stimulation experiments and suggests that the primary assumption in sequential double-labeling with 2DG may be invalid.

DONALD L. MCEACHRON
Image Processing Center, Drexel University, Philadelphia, PA 19104
C. R. GALLISTEL
Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104
PETER J. HAND
Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania Philadelphia, PA 19104

REFERENCES

1. E. R. John, Y. Tang, A. B. Brill, R. Young, K. Ono, *Science* **233**, 1167 (1986).
2. H. R. Friedman, C. J. Bruce, P. S. Goldman-Rakic, *Soc. Neurosci. Abstr.* **10**, 1002 (1984); J. L. Olds, K. A. Frey, R. L. Ehrenkaufer, J. Patoki, B. Agranoff, *ibid.* **11**, 1002 (1985).
3. Gallistel *et al.*, *J. Neurosci.* **5**, 1246 (1985).
4. L. Sokoloff *et al.*, *J. Neurochem.* **28**, 897 (1977).
5. M. Kossut and P. Hand, *Neurosci. Lett.* **46**, 7 (1984).
6. D. L. McEachron, O. J. Tretiak, E. Feingold, *Func. Photog.* **22**, 30 (1986); *ibid.*, p. 26.

12 June 1987; accepted 17 August 1987

Response: We appreciate the thoughtful criticisms of our experiment by Nottebohm and Williams. The major issue raised in their comment indicates that they consider "novelty" and "familiarity" to be properties inherent in the encoding of sensory input by the brain. They imply that stimuli are perceived as "unfamiliar" (triangles) or "familiar" (circle and stars); familiar cues "trigger" a search for associated memories but unfamiliar ones do not. We contend that the novelty or familiarity of a stimulus input can only be established by a memory search, which must be an inherent and continuous part of the perceptual process. Both hemispheres of our split-brain animals must engage in this process equally. The difference