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.

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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 $P < 0.05$.

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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.

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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

between the two hemispheres is that the search for circle-star memories will activate a representational system established by the earlier discrimination learning experience, which cannot be activated by the search for triangle memories for which no discriminative representational system had been built. Thus, we do not consider it reasonable to argue that the increase in neural activity in the hemisphere receiving input about learned cues is due to "general processes of memory retrieval." Nottebohm and Williams suggest further that there exists informational asymmetry in the second test period; the triangles are then familiar for the red lens because they were encountered in the first test period, but remain novel for the green lens hemisphere. This ignores that (i) triangles, whether novel or familiar, have no discriminative cue value, and (ii) the confidence limits for the difference image in the green lens hemisphere (discriminated circle-star versus novel triangles) were defined by the statistics of the difference image in the red lens hemisphere (novel versus familiar triangles). The effect they are suggesting should increase the red lens hemisphere differences and thus bias against significant findings in the green lens hemispheres. The goal of our study was to map the memory of a discriminative response, and the design compensated for effects such as possible differences between novel and not-so-novel noncontingent stimuli by the Z-transformation of difference images.

Nottebohm and Williams argue that the motor activity which moves the cat through the correct door must be controlled by a dominant "executive" hemisphere, which must be the green lens hemisphere in the first period and is unknowable but may be either hemisphere in the second period. The consequent increase in the red lens hemisphere difference image would again bias against significant findings in the green lens hemisphere. In fact, the smooth locomotion displayed by these split-brain cats leads us to believe that both hemispheres participate in moving the four limbs and that the "executive control" Nottebohm and Williams attribute to one or the other hemisphere is more probably exercised by some integrative system in the diencephalon, mesencephalon, or cerebellum, where striking asymmetries in metabolism were observed.

It took several weeks for each cat to reach criterion. Because cats tend to nibble rather than gulp their food, free access to food for some time after each session is good practice; it keeps body weight up and anxiety down. Cats trained in this manner, if placed on the laboratory floor will sometimes jump into the apparatus and wait in the starting box; if satiated in the home cage before the session, they will often work but not ingest the reward. Response latencies were not measured, but were usually about 5 seconds, since the cats performed the response, ate the food, and walked back to the starting box in less than the 1 minute between trials. Incorrect responses consisted of choosing the wrong door. Failure to choose either door was rare after the initial days of training and, if repeated, caused the session to end. In the actual 2DG uptake periods, no response failures occurred.

Nottebohm and Williams raise questions about the performance during the two sessions. As stated in our article, each cat performed exactly the same number of trials in both sessions, ran the same distance in the same time, and received (ate) the same amount of food. Possible differences in motivation, attentiveness, response latency, and motor activity between the two uptake periods about which they speculate are nonspecific influences on both hemispheres, exactly the sources of variance estimated by the difference image of the red lens hemisphere.

Nottebohm and Williams conclude that, because of the ostensible shortcomings in design pinpointed by their critique, it seems virtually impossible to decide what part of the observed differences in uptake by the two hemispheres during the two uptake periods are due to nonspecific influences that our paradigm failed to consider and what part reflects the activation of a specific memory about the learned discrimination. Most of the factors they discuss can be expected to contribute equally to the difference image obtained from each hemisphere, while the remainder would increase the red lens hemisphere differences and thereby bias against positive findings in the other hemisphere. We believe we were correct to use split-brain cats and the dual tracer strategy, and we point out that increases in red lens hemisphere variance must decrease the pixel Z-scores of the other hemisphere. Our paradigm supports exactly the conclusions we drew.

The degree of late redistribution of 2DG has been and continues to be a matter of serious concern, and despite much attention to the problem, the issue remains unsettled. The data provided by McEachron *et al.* speak to this point, and the authors suggest a methodological approach that takes advantage of such redistribution as occurs. Their data are taken as indicating that as much as 50% of the amount of tracer accumulated in regions activated during the first 45 minutes after injection can be accumulated, presumably largely by redistribution, during the period from 45 to 90 minutes in response to a second stimulus. It will be important to determine the locations from which the redistribution occurs. If it is largely from extra neuronal tissue, where most of the 2DG accumulates, the two-phase stimulus model he proposes could be of great benefit for brain research protocols. The use of the strategy they suggest, if valid, could simplify the analytic problem in autoradiographic studies where each animal is its own control, a methodologically elegant approach. The computation of LCGU, however, requires the measurement of the arterial blood clearance curve, as well as tracer content in the region of interest. In experiments such as we conducted, in which mobile animals walked through a maze-like appparatus, such blood sampling could not be accomplished readily without interfering with the study itself.

If one assumes that the observation of McEachron *et al.* is generally valid, it is incumbent upon us to assess the effect it would have on the interpretations we made in the mapping study of cat brain memory we reported. In our study, the first experimental period was the test period in which the stimulus was presented and imprinted with [^{14}C]2DG. In the second period, the short-lived tracer [^{18}F]2DG was administered along with background stimulation. Thus, redistribution of activity from phase 1 during phase 2 would have diminished the strength of the differences we observed by taking activity away from imprinted areas and would not have been expected to create artifactual increases. Had the order of the study been reversed, significant redistribution would have raised serious questions concerning the validity of the results we reported. Since this was not the case, such redistribution as occurred would not invalidate the conclusions we reached in our studies, which we believe remain intact.

The observations made by McEachron *et al.* are important and need to be extended. In particular, it will be important to know the source and extent of redistribution and the magnitude of redistribution from other brain regions. The methodological approach they suggest is interesting and could be a useful additional strategy for autoradiographic studies.

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