

## Spatial Learning in Mutant Mice

A. J. Silva *et al.* (1, 2) and S. G. N. Grant *et al.* (3) found that two types of mutant mice showed learning impairment, relative to wild-type mice (the control group), on an experimental task. These researchers argue that such mutant mice suffer from a memory deficit that is specific to spatial learning. Silva *et al.* state that their work "demonstrates that a mutation in a known gene is linked to a specific mammalian learning deficit, and indicates that single genetic changes can have a selective but drastic impact on learning and memory" (1, p. 210). Grant *et al.* state that "[m]utations in the *fyn* gene in mice result in an impairment of both LTP [long-term potentiation] and spatial learning" (3, p. 1908). However, these interpretations in terms of a memory deficit are still open to question in view of the data presented in the two articles.

Two versions of the Morris water task were used by both groups of researchers (1–3) to measure behavioral differences between mutant and wild-type (control group) mice. In the "hidden-platform task," a fixed platform was submerged in a round tank of water that was rendered opaque; the task of the mice was to swim to the platform repeatedly and thus learn its location. In the "visible-platform task," the mice swam to a submerged platform, positioned in random locations, marked with a flag above water. Success in learning in both tasks was assessed by measuring the time it took for mice to reach the platform.

In the study by Silva *et al.* (1), mice were first tested on the visible-platform task. Mutant mice (4) "initially took longer than the wild-type mice to reach the platform," but "they were able to overcome this deficit by training" (1, p. 207). Silva *et al.* tentatively attribute this initial deficit to a "jumping response" by the mutant mice that led to fatigue on the first day of training (1, p. 207). On day 2 the mutant mice "did not show the jumping response and hence were not fatigued before the trials." This explanation is contradicted by figure 1B of the research article by Silva *et al.* (1, p. 207) and by their statistical analysis (1, p. 211). The mutant mice had large and significantly higher escape latencies (the time required to reach the platform) even on the first block of the second day, when they no longer showed a jumping response. The acquisition curve shows no sudden shift on day 2. As the poorer performance of the mutant mice occurred before learning had taken place, one can infer that the mutant mice suffered from deficits that were not related to memory. These deficits manifested themselves in the visible-platform task, which is held to be equiv-

alent to the hidden-platform task except that it "does not require a spatial map"; (3, p. 1906). If so, then nonmemory factors could also be responsible for deficits in performance in the hidden-platform task.

In the hidden-platform task in the study by Silva *et al.*, the mutant mice again showed a large deficit in performance at the outset of training that could not be a result of spatial or other memory. This deficit remained nearly constant throughout the experiment as shown by the statistical analysis performed by Silva *et al.* (1). This revealed a main effect, that of genotype, which was highly significant. However, "[t]he interaction between genotype and trial block was not significant" (1, p. 211). This means that no reliable difference in the rate of learning between the mutant and control mice could be detected in the hidden-platform experiment. Only such a difference in rate could provide evidence for a possible difference in memory between the two genotypes in this experiment.

Further experiments by Silva *et al.* showed that the mutant and control mice relied on different strategies to find the hidden platform (1, p. 209)

This may mean that the mutant mice are impaired in learning the spatial relations between distal cues and the escape platform (true impaired spatial learning). However, [maybe] the mice are impaired in another process (or processes), such as the ability to see and attend to distal cues, or to make an association between the distal environment and the escape platform. In order to exclude these latter possibilities, we tested the mice in a water-filled plus (+) maze. . . . The plus maze is a four-armed (+) Plexiglas maze filled with opaque water. An escape platform is placed in one arm of the maze with its top 1 cm below the surface of the water. . . . Because the maze is clear the animal can use prominent distal cues in the room to locate the platform.

Just because the animals can use prominent distal cues, they do not necessarily do so. The introduction of the Plexiglas maze could have provided proximal cues that were inconspicuous to human observers. Silva *et al.* present no control or transfer test data about what cues the mice might actually have used. Thus, the plus maze experiment may be irrelevant.

Silva *et al.* reject the hypothesis that the mutant and control mice differed in their performance in the plus maze on the grounds that it was not statistically significant ( $P = 0.326$ ).

However, the plus maze experiment leaves unresolved the question of whether there is some intermediate impairment in a nonmemory process that is sufficient to produce the differences observed in the plus

maze task. The performance of the mutant mice was 25% worse in this task, which, while not significant, is still the best estimate available. How the scores on the plus maze task translate into choice of the solution strategy used by the mice in the hidden platform task is unknown.

The results from the plus maze task do not exclude the possibility of nonmemory disabilities, nor do they indicate them; the plus maze task is too insensitive an instrument to do either. The scores of the mutant mice would have had to be at least twice as high as those of the control group to reach any level of statistical significance. Also, it is not clear why only five mice per group were used in this task as against 12 in the hidden-platform experiment.

In the visible-platform task, the mutant mice (1, p. 207) showed a clear nonmemory deficit, even though the visual cues toward which they had to learn to navigate were nearer than in the plus maze. Unless they were hyperopic, it would be unlikely that the mutant mice were unimpaired in the plus maze if or when such cues were more distant.

Grant *et al.* published a related study (3). In the main experiment, all the mice were trained for 7 days, with four trials per day (plus four more trials after the first transfer test) during which the animals would unlearn. Wild-type mice showed an eventual reduction in the time taken to find the hidden platform. This reduction did not occur with *fyn* mutant mice (3, p. 1905). To show that this difference was a result of a specific deficit in spatial learning in the mutants, Grant *et al.* performed a visible-platform (control) experiment. The results of this task were similar to those obtained in the same task in the study by Silva *et al.* (1). The wild-type mice at first performed with a latency of escape one-half that of the *fyn* mice; the latter improved so that they performed as well as the wild-type mice by day 6.

Grant *et al.* appear to regard the initial impairment of the mutant mice in this task as genuine and not an artifact of order: "In fact, both the *fyn*<sup>-</sup> and CamKII<sup>-</sup> mice show an initial impairment in the single-cue association task . . ." (3, p. 1908). If so, the control experiment reveals strong nonmemory factors. However, because the same mice were used in the main and control experiments without a counterbalanced design (3), one cannot exclude order effects as a possible cause of the initial difference in latencies in the performances on the visible-platform task or any other difference found in performances between the hidden and visible-platform tasks.

Grant *et al.* say (3, p. 1906) the visible-platform results demonstrate "that *fyn*<sup>-</sup> mice can learn some tasks" presumably in contrast to their inability to learn in the

hidden-platform task, thus showing that their deficit in spatial learning is specific. However, in the hidden-platform task, the control group showed no significant learning for 20 trials and the mutants showed none for 28 trials, at which point the training experiment was terminated. In many tasks, mice learn after a much larger number of trials. The questionable rationale for this early termination at 28 trials appears in note 29 of the research article by Grant *et al.*: "We used a training procedure that avoided overtraining the mice, because, in pilot experiments, overtraining masked the *fyn*<sup>-</sup> learning defect." Thus, it seems that with a larger number of trials, the mutant mice do learn the hidden-platform task, albeit more slowly than the wild-type mice. This resembles the pattern that emerges in the visible-platform task, which was run for 48 trials.

In summary, we find no evidence that the mutant mice in either set of studies (1–3) suffered from a specific impairment in spatial memory. The interpretable evidence shows instead that nonmemory deficits played an important role in the performance of the mutant mice. Nevertheless, these are important experiments. The mutant mice, in spite of gross derangement of long-term potentiation, were clearly capable of learning. These are pioneering studies in disrupting targeted genes in order to elucidate the physiological bases of learning and behavior.

**J. Anthony Deutsch**

Department of Psychology,  
University of California, San Diego,  
La Jolla, CA 92093-0109

## REFERENCES AND NOTES

1. A. J. Silva, R. Paylor, J. M. Wehner, S. Tonegawa, *Science* **257**, 206 (1992).
2. A. J. Silva, C. F. Stevens, S. Tonegawa, Y. Wang, *ibid.*, p. 201.
3. S. G. N. Grant, T. J. O'Dell, K. A. Karl, P. L. Stein, P. Soriano, E. R. Kandel, *ibid.* **258**, 1903 (1992).
4. Silva *et al.* (1) used mutant mice defective in the  $\alpha$  isoform of calcium-calmodulin-dependent kinase II.

27 July 1992 and 19 January 1993; accepted 15 June 1993

**Responses:** The criticisms by Deutsch focus on the evaluation of performance variables that may be important in determining whether mutant mice are impaired (as compared with wild-type litter mates) in spatial learning performance. The criticisms relate to two issues; first, the use of latencies (the time taken to escape to a platform) to evaluate performance, and second, the statistical analysis and interpretation of the plus maze experiments.

The Morris water task is a learning task that is frequently used to assess spatial learn-

ing performance in rodents. However, to interpret performance in this task, several measures must be used. The major thrust of Deutsch's criticism with regard to the use of the Morris water task is based on the presumption that success in learning was evaluated by measuring the time it took for mice to reach the platform. On the contrary, we conducted several tests—which provided multiple measures of spatial learning performance—to compare wild-type and mutant mice. Escape times, or latencies, during acquisition phases of the hidden platform task were not the only measures we relied on because they do not address the issue of spatial selectivity in the task.

Our experience with the hidden platform task (gained during the testing of at least 30 different strains of mice in the last 6 years) has indicated that latencies are not a good measure of the spatial learning strategies of mice in the Morris water task. Similarly, others have shown that latencies decrease as a function of training in rats with hippocampal lesions during acquisition training despite the fact that the rats showed impairment on other, better measures of spatial selectivity that have been derived from probe trial, or transfer tests (1). Stated simply, animals incapable of using a spatial strategy will revert to some other type of strategy to escape to the platform.

Deutsch discusses the latency curves in our report and argues that we cannot conclude that the mutant mice are impaired in spatial learning because no reliable difference was detected in the rate of learning between the mutant and control mice. However, conclusions with regard to spatial learning were not based only on the rate of learning in the hidden platform task, but on the results of probe trials and data acquired by assessing the behavior of mice when the platform was moved to new sites. In the trial the differential latency (the difference between the time taken to reach the original site and a new, randomly located, site) provides important information. First, each animal serves as an internal control for swim speed. Second, the trial measures further the selectivity of the animal's search with procedures identical to those used during task acquisition (except for the location of the platform).

Deutsch states that nonspecific behavioral impairments in  $\alpha$ -CAMKII mutants could have lead to increased latencies on the visible platform version of the task. We attributed these longer latencies, which occurred on the first day of training, to fatigue caused by "jumpiness." The fact that we said that the mutants were better habituated to the task by the second day is not in conflict with the data. It is true that their average latencies on the first block of

trials were also longer on the second day. This might be expected if fatigue interfered with their using the information presented to them on the first day of training. The important point in this aspect of the study is that they caught up with the wild-type mice in their performance by the second block of trials on the second day. Thus, any performance factors that were problematic in the mutants were quickly overcome during the second day of visible platform training. The training in the hidden platform version of the task was accomplished in 3 days, and these same interfering factors should have been diminished by the second day. If such factors were a problem, a precipitous drop in latencies would again be expected in the mutants. This was not the case. In fact, to rule out this possibility, some animals were given an additional 2 days of training. Again, in the total 5 days of training, latencies on the hidden version remained different between the mutants and wild-types, which suggests that there was impaired spatial learning in the former. This impairment was then further verified by probe trial and random platform trial data.

Last, Deutsch suggests that the additional use of the plus maze to evaluate differences in performance between wild-type and mutants may be irrelevant. Deutsch is correct that a transfer test was not performed with the plus maze. However, the position of the maze was rotated between trials, and the start position varied such that it was unlikely that the animals could have used intramaze cues. Our data analysis, with the use of a two-tailed t-test, yielded a *P* value of 0.652, which suggests that a statistically reliable difference between the performances of the two genotypes on the plus maze was not observed. In support of this conclusion, we did a power analysis (2) of our data, based on an 80% chance of detecting a difference in the performance of wild-type and mutant mice in the plus maze. The analysis indicated that more than 170 animals would need to be tested for one to detect a difference. Regardless of how the two genotypes are solving the plus maze task, a heroic effort would be required before these slight differences would register as statistically significant.

In summary, we used the Morris water task with at least the same degree of stringency that has been applied in other studies of the effects of lesions and pharmacological agents on spatial learning performance, and we showed differences in performance on this task between the mutant and wild-type mice on the basis of various measures of spatial selectivity. As indicated in our research article, the mutant mice have other behavioral defects, and we evaluated the impact of such impairments on spatial learning performance. We stand by our

conclusion that the  $\alpha$ -CaMKII mutant mice are impaired in spatial learning.

**Alcino J. Silva**

Center for Learning and Memory,  
Cold Spring Harbor Laboratory,  
Cold Spring Harbor, NY 11724

**Richard Paylor**

**Jeanne M. Wehner**  
Institute for Behavioral Genetics  
University of Colorado at Boulder,  
Boulder, CO 80309-0447

**Susumu Tonegawa**

Howard Hughes Medical Institute  
at Massachusetts Institute of Technology  
Center for Cancer Research  
Cambridge, MA 02139

## REFERENCES

1. R. G. M. Morris, P. Garrud, J. N. P. Rawlins, J. O'Keefe, *Nature* **297**, 681 (1982); R. J. Sutherland, B. Kolb, I. Q. Whishaw, *Neurosci. Lett.* **31**, 271 (1982).
2. J. Cohen, *Statistical Power Analysis for the Behavioral Sciences* (Academic Press, New York, 1969); E. L. Wike, *Numbers: A Primer of Data Analysis* (Merrill, Columbus, OH, 1985).

25 August 1992; accepted 15 June 1993

**Response:** Deutsch generously notes that the studies of mice with targeted disruptions of the  $\alpha$ -CaMKII (1) and *fyn* (2) genes are pioneering in attempting to elucidate the physiological bases of learning and behavior. However, he seems to misinterpret some of the results of these studies and attributes to us conclusions to which we do not subscribe. Specifically, Deutsch addresses three issues in our paper (2) on *fyn* mice. First, he states that "Success in learning in both [hidden- and visible-platform] tasks was assessed by measuring the time it took for mice to reach the platform." This is not completely correct. Escape latencies in the hidden-platform version of the Morris maze (3) are by themselves poor indicators of spatial learning. A better measure comes from the additional use of the transfer test, a variation of the Morris maze specifically designed to measure spatial learning (3-6). We therefore also carried out this test. Here again, we found that wild-type mice had learned: they showed a significant spatial bias toward the quadrant of the pool where the platform was located during training. By contrast, the *fyn* mice showed no such bias, thus indicating impaired spatial learning in this more specific task as well.

Second, Deutsch inaccurately attributes to us the conclusion that "mutant mice suffer from a memory deficit that is specific to spatial learning." Although we found that *fyn* mice had a spatial learning deficit, we did not conclude that this defect is specific to spatial learning. Rather, we point out that *fyn* mice initially showed longer escape latencies than did wild-type

mice in the visible-platform test. This led us to emphasize that other (nonspatial) forms of learning may also have been impaired (2, p. 1908). We wrote

both the *fyn*<sup>-</sup> and CaMKII<sup>-</sup> mice show an initial impairment in the single-cue association task, a task that requires nonhippocampal regions. . . . This finding suggests either that the hippocampus can be involved in simple associative learning or that these kinases may be important for learning processes that require regions other than the hippocampus.

Thus, although *fyn*<sup>-</sup> mice eventually performed as well as wild-type controls in the visible-platform task, we considered this initial difference significant.

We agree with Deutsch that without a counterbalanced experimental design we cannot exclude the possibility that the initially longer escape latencies in *fyn*<sup>-</sup> mice represent an order effect and not a real behavioral phenotype produced by the *fyn* mutation. However, an order effect would not explain the results of the transfer test, which indicated significant differences in performance between mutant and wild-type mice, as the mice were experimentally naive before hidden-platform training. Moreover, Silva *et al.* (1, 2) observed a similar difference between mutant and wild-type mice in the visible-platform task despite their using the reverse order of experiments (visible-platform training first).

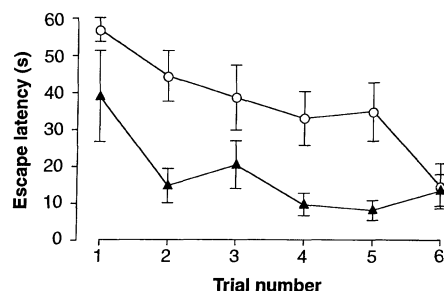
Third, Deutsch argues that, because *fyn*<sup>-</sup> mice learned the visible-platform task more slowly than wild-type mice, there is a defect in visual discrimination or motivation, which casts into doubt whether the results in the hidden-platform task really reflect a memory deficit rather than a non-memory deficit. We disagree. While the *fyn*<sup>-</sup> mice showed a higher initial escape latency at the start of training in the visible-platform task than did wild-type mice, they showed a significant improvement on the very first day of training after as few as three trials ( $P < 0.05$ , Duncan's multiple range test). By the sixth training trial, they performed as well as wild-type mice (Fig. 1). Thus, the same *fyn*<sup>-</sup> mice that made no progress over 7 days of training on the hidden-platform task showed immediate learning within the first few trials when the platform was visible. Because the *fyn*<sup>-</sup> mice learned to perform as well as wild-type mice in the visible-platform task, they did not appear to have gross sensory, motor, or motivational abnormalities that would preclude learning. This suggests that the difference we detected between the wild-type mice in the transfer tests (which followed training in the hidden-platform configuration) was specific to learning, although not necessarily to spatial learning.

Finally, Deutsch objects to the fact that

we did not use the same number of training trials in the visible-platform task (48 trials; 8 days of six consecutive trials each at 30-second intertrial intervals) as in the hidden-platform task (28 trials; 7 days of four trials separated by 60-minute intertrial intervals). But why should one use the same training protocol (number of trials, intertrial interval) for tests that explore distinctly different types of learning? Although the transfer test is a sensitive assay of hippocampal-dependent spatial learning (3, 4), even animals with hippocampal lesions (that normally show large deficits) can compensate and exhibit spatial learning when they are overtrained (4, 5). Overtraining can mask a variety of learning deficits in other forms of learning as well.

To optimize our detecting potential differences, we therefore conducted pilot experiments to determine a training protocol that was most likely to avoid overtraining. This was particularly important in our case, as we had found that LTP was reduced but not completely absent in *fyn*<sup>-</sup> mice, and the degree of reduction was dependent on the stimulation protocol used to induce LTP (3). We were thus concerned that this defect in LTP might lead to a subtle learning deficit that would go undetected if animals were overtrained. Our pilot studies also indicated that the time interval between training trials, rather than number of trials, was an important variable. We found that the performance of *fyn*<sup>-</sup> mice improved when training trials were closely spaced together, perhaps because such spacing is less taxing on long-term memory. To optimize the detection of any learning defect that *fyn*<sup>-</sup> mice might have, we selected longer intertrial intervals and fewer training trials.

Deutsch concludes by saying that in both the *fyn*<sup>-</sup> and  $\alpha$ -CaMKII mutant mice, despite "gross derangement of long-term potentiation, [the mice] were clearly capa-



**Fig. 1.** Training in the visible-platform task on the first day of the 8-day training protocol. Mice were placed into the pool at random locations and escaped by swimming to a flagged platform, also at a random location. Both *fyn*<sup>-</sup> (○) and wild-type (▲) mice showed immediate improvement after the first trial and performed equally by the sixth trial. Error bars show mean and SEM.

# Gravitational Separation in Polar Firn

ble of learning." Our results (2) and those of Silva *et al.* (1) do not suggest, as Deutsch implies, that there is a dissociation between LTP and learning, and that therefore these processes are not linked. The data (1–3) indicate that interference with LTP can impair spatial learning. We believe it is naive to think that deficits in LTP in the CA1 region of the hippocampus will eliminate spatial learning in an all-or-none fashion, because even rodents with hippocampal lesions or with a complete pharmacological blockade of LTP can demonstrate some spatial learning, and in both the *fyn*<sup>−</sup> and  $\alpha$ -CaMKII mutant mice, LTP in the CA1 region is impaired but not completely abolished.

We would simply emphasize that our initial behavioral studies of transgenic mice bearing specifically engineered mutations were not meant to be a definitive description of the behavioral repertoire of these animals. Instead, they represent the first steps toward exploiting the power of target gene disruption in a combined molecular, physiological, and behavioral study of learning and memory. We believe that our studies (2), as well as those of Silva *et al.* (1), demonstrate the usefulness of gene targeting techniques for investigating the molecular components of the signaling pathways responsible for long-term potentiation as well as providing a new approach to the study of behavior.

Seth G. N. Grant  
Thomas J. O'Dell  
Kevin A. Karl

Center For Neurobiology and Behavior,  
Howard Hughes Medical Institute,  
College of Physicians and Surgeons,  
Columbia University,  
New York, NY 10032

Paul L. Stein  
Philippe Soriano

Program in Molecular Medicine  
Fred Hutchinson Cancer Research Center,  
Seattle, WA 98104

Eric R. Kandel  
Center for Neurobiology and Behavior,  
Howard Hughes Medical Institute,  
College of Physicians and Surgeons,  
Columbia University

## REFERENCES

1. A. J. Silva, C. F. Stevens, S. Tonegawa, Y. Wang, *Science* 257, 201 (1992). A. J. Silva, R. Paylor, J. M. Wehner, S. Tonegawa, *ibid.*, p. 206.
2. S. G. N. Grant, T. J. O'Dell, K. A. Karl, P. L. Stein, P. Soriano, E. R. Kandel, *ibid.* 258, 1903 (1992).
3. R. G. M. Morris, E. Anderson, G. S. Lynch, M. Baudry, *Nature* 319, 774 (1986).
4. R. G. M. Morris, *Cold Spring Harbor Symp. Quant. Biol.* 50, 161 (1990).
5. ———, F. Schent, F. Tweedie, L. E. Varrard, *Eur. J. Neurosci.* 2, 1016 (1990).
6. S. Davis, S. P. Butcher, R. G. M. Morris, *J. Neurosci.* 12, 21 (1992).

2 March 1993; accepted 15 June 1993

D. Raynaud *et al.* (1) describe the processes affecting the composition of trapped air in polar ice. They state (1, p. 927)

It was recently shown that the composition of the air column sampled at different depth levels in the open porosity of the firn, before its enclosure as air bubbles in ice, essentially reflects diffusive and gravitational equilibrium with the atmosphere at the surface of the ice sheet. . . .

In figure 2 of their article (1, p. 927), Raynaud *et al.* state that

the air column tends to reach a state of diffusive equilibrium, in which the heaviest components become enriched as a result of gravitation at the bottom of the air column. . . .

Raynaud *et al.* refer to a paper by Schwander *et al.* (2, p. 2836) as the authority for their position. I pointed out in the paper with the original theory and data for the effects of gravitational separation in polar firn (3), that an attribution such as that in (1, 2) of the heavy isotope enrichments in firn columns is premature. From observations, one cannot distinguish enrichments by gravitational settling from the effects of effusion through partially sintered micropores. That is, the enrichment of a heavy species (*i*) relative to a lighter component is proportional to  $(M_i - M)$ , the atomic mass difference between the components, multiplied by  $gZ/RT$ , where  $g$  is the gravitational acceleration,  $Z$  is the depth in a firn layer,  $R$  is the gas constant, and  $T$  is the absolute temperature; while in effusion through porous barriers, the heavy component enrichment is proportional (for small enrichments) to  $[(M_i - M)/2M_i]F$ , where  $F$  is the fraction of gas lost in the actual fractionating process. The observed isotopic enrichments in <sup>15</sup>N and <sup>18</sup>O at the base of a 75-m column of firn are consistent with either gravitational separation or with an effusive loss of approximately 2% of the gas (3). For large mass differences, the expected enrichments diverge for the two processes, but for isotopic species the enrichment factors are indistinguishable for these processes (3).

Raynaud *et al.* (1) and Schwander *et al.* (2) base their attribution of the enrichment process on their analyses of gases within the firn column above the nascent ice (2, p. 2836), in which <sup>15</sup>N/<sup>14</sup>N, <sup>18</sup>O/<sup>16</sup>O, and O<sub>2</sub>/N<sub>2</sub> enrichments were measured in two sets of samples from five depths, and the measured ratio enrichments were compared with those predicted by gravitational settling. However, good agreement was found with only one set of N isotope results. The second set of N data, and both sets of O isotope data, were systematically more enriched at all depths than the predicted

values: O<sub>2</sub>/N<sub>2</sub> ratios (predicted to be enriched by 0 to 1.4 per mil from the surface to 75 m) actually clustered at "−6 per mil" in one set and ranged from 0 to 4 per mil, below 60 m, in the second set. If one assumes that the fractional loss of gas by effusion varies from 0 to 2% down the depth of the firn (a reasonable postulate given that air is actually removed from the firn), then gravitational or effusional fractionation equally predict the isotopic N and O enrichments.

Raynaud *et al.* (1) follow Schwander *et al.* (2, p. 2832), who write the equation for their model of the flux of air out of the firn column to the atmosphere as the sum of chemical and gravitational free energy contributions (after J. Willard Gibbs); however, there is an advective loss of air upward through the firn (3) as a result of its compaction with depth and its decreasing porosity. Thus it is a priori possible that effusion through the compacting firn is the process that is responsible for the isotopic enrichments observed (1, 2, 3). The negative O<sub>2</sub>/N<sub>2</sub> ratio enrichments (2) are another matter: they cannot be explained by either process because both gravitational and effusional enrichments favor the heavier species. It has been shown (3) that these negative enrichments are a result of differential capillary flow during gas loss from firn either in situ or from stored samples, a process that is dependent on molecular volumes for fractionation of chemical species, but not isotopes. Indeed, the relative losses of O<sub>2</sub>, Ar, and N<sub>2</sub> based on experimental calibration, are observed (3, 4) to be in the expected order (5) and are almost exactly the negative values measured in (3). Therefore the O<sub>2</sub>/N<sub>2</sub> ratios used by Raynaud *et al.* cannot be used to support the gravitational theory. As noted above, the isotopic data are not sufficient to distinguish two physically plausible (though perhaps not equally aesthetic) physical processes.

The only discriminant for these two processes is the use of two species of the quasi-isotopic inert gases with a large mass difference. This has now been done by measurement of <sup>84</sup>Kr/<sup>36</sup>Ar ratios, species that differ in atomic weight by fully 48 atomic mass units. For this large mass difference, the predicted enrichments in Greenland ice are 12 per mil for gravity, but only 6 per mil for effusion. The first data (6) for ice at 70 to 150 m have a mean value of 13.4 per mil, with a standard deviation of 3 per mil ( $n = 5$ ). This is the only set of measurements that support the postulate that gravity is responsible for the isotopic enrichments observed in recent ice cores (3).