mice and controls, respectively). Unbiased stereologic analysis (3) and our own previous studies show that this region contains 6000 to 8000 cholinergic neurons in adult rats. The septal/diagonal band complex of mice is smaller than that in rats, and it is clear from brain sections that mice have about half the cholinergic neurons per section of equal thickness as do rats. Our numbers and those reported by others (4) for mice (3000 to 4000 total) are consistent with this observation.

The greater volume and total cholinergic neuron number in the Peterson *et al.* study may be indicative of some systematic error in their calculations. One factor that could have contributed to the discrepancy would be the possibility that Peterson *et al.* used tissue sections that were not representative of the medial septum/diagonal band or used a sampling grid that did not give neurons an equal likelihood of being counted. However, it is unclear from their comment how many sections were analyzed, what internal reference point was used to ensure that similar sections were sampled between animal groups, from which rostro-caudal level the sections were taken, and how the sampling grid was applied (interpoint distance and so forth). Cholinergic neurons are not distributed in a homogeneous fashion, and small differences in the sampling area in any of the three dimensions would result in substantial numerical differences. If these sampling errors exist, they could be exaggerated if a brain region that includes large areas that are not occupied by cholinergic neurons is used as the reference volume. In addition, some mouse strains are known to lack (to various degrees between animals) fusion of the corpus callosum, which can prevent accurate determination of the genu of the corpus callosum (one of the boundaries used by Peterson *et al.*), which would make accurate volume determination difficult.

In conclusion, we have confirmed our previous published results by using the optical disector method for counting neurons in the entire coronal plane of the medial septum in every third section through the septal nucleus (which represents the entire 3-dimensional extent of the nucleus). Therefore, the apparent discrepancy between our results and those of Peterson *et al.* is most likely not due to differences in quantification methods, but may reflect a genetic difference between the two mouse colonies that is not related to p75. Our results (1), that p75-deficient mice have more basal forebrain cholinergic neurons than control mice and that they do not show the apoptosis observed in control mice, probably more closely reflect the (death-mediating) role of p75. This was further strengthened by our finding (1) that a p75-interfering peptide mimicked the phenotype of the p75-deficient mice, that is, prevented the postnatal death of the cholinergic neurons in control mice. Our results also are consistent with the observations by others that p75 can mediate cell death (5). To resolve the issue of which results (which mice) most closely represent the role of p75 in the basal forebrain, it will be of great interest to analyze the recently created p75-deficient mice, which are on a different genetic background (C57BL/6J; Jackson Laboratories).

Note added in proof: A study (6) from the laboratory of Frank Longo (University of California, San Francisco) replicates our results that adult p75-deficient mice have 50% more cholinergic neurons in the basal forebrain. They obtained p75-deficient mice from Jackson Laboratories and, through backcrossing, produced deficient and wild-type control littermates. With the use of highly controlled stereological methods, the numbers of neurons were blindly assessed by people from two laboratories (F. M. Longo and W. C. Mobley, also at University of California, San Francisco).

Theo Hagg
Catharina E.E.M. Van der Zee
Department of Anatomy & Neurobiology, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada
Gregory M. Ross
Richard J. Riopelle
Department of Medicine (Neurology), Queen's University, Kingston, Ontario K7L 2V7, Canada

REFERENCES

1. C. E. E. M. Van der Zee, G. M. Ross, R. J. Riopelle, T. Hagg, *Science* **274**, 1729 (1996).
2. M. Abercrombie, *Anat. Rec.* **94**, 239 (1946); N = n x T/(T+D), where N = total number, n = counted profiles, T = section thickness, and D = longest cell diameter.
3. G. Leanza, O. G. Nilsson, R. G. Wiley, A. Bjorklund, *Eur. J. Neurosci.* **7**, 329 (1995).
4. J. C. Hornberger *et al.*, *Neurobiol. Aging* **6**, 269 (1985).
5. S. Rabizadeh *et al.*, *Science* **261**, 345 (1993); G. L. Barrett, P. F. Bartlett, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6501 (1994); C. S. von-Bartheld *et al.*, *Neuron* **12**, 639 (1994); G. L. Barrett and A. Georgiou, *J. Neurosci. Res.* **45**, 117 (1996); P. Casaccia-Bonnefil, B. D. Carter, R. T. Dobrowsky, M. V. Chao, *Nature* **383**, 716 (1996); J. M. Frade, A. Rodríguez-Tébar, Y. A. Barde, *ibid.*, p. 166.
6. T. T. Yeo *et al.*, *J. Neurosci.*, in press.

18 February 1997; accepted 5 June 1997

Measuring Memory in a Mouse Model of Alzheimer's Disease

K. Hsiao *et al.* (1) describe evidence of a memory deficit that is correlated with an elevation in amyloid-containing plaques in a transgenic mouse that overexpresses the so-called Swedish mutation of amyloid precursor protein. This comment questions whether the evidence actually shows such a memory deficit.

First, although the performance of the transgenic mice was impaired, they did show signs of learning. Figure 2E in the report demonstrates that the 9- to 10-month-old transgenic mice performed more poorly than did wild-type mice in the visible platform subtest of the Morris water maze, a result which is usually taken to indicate a sensory-motor impairment, not a memory deficit. On two of the four individual days of the subtest, such differences were statistically significant; but it is not shown whether an overall analysis would also produce a significant result. In the absence of basic measures of locomotor activity, vegetative functions, or sensory capacity, perhaps these 9- to 10-month-old animals are sluggish. By the fourth block of trials, the transgenic mice improved, although they remained significantly slower than controls. Thus, the transgenic animals were learning something. This conclusion is confirmed in figure 2A in the report, in which improvement in latency for learning the task of finding the hidden platform is similar on a percentage basis for old transgenic mice (40 to 20 s) and young nontransgenic mice (20 to 10 s). Hsiao *et al.* emphasize the absolute latency difference, suggesting that this represents a memory deficit. But the "AD mice" actually improve more than nontransgenics. From the visible and hidden platform test data, one can conclude that the transgenic animals required more time to perform, yet learned over days (decreasing their mean latency) at a rate similar to that of controls.

Second, there appears to be no relation between the appearance of plaques in aging and the decline in performance. In figure 2B in the report, which shows time in each quadrant with the platform missing, there is essentially no change shown with aging in the target quadrant for the transgenic animals (39% at 2 months, 35% at 6 months, and 34% at 10 months), although table 1 in the report indicates a significant increase in the number of plaques with aging.

Third, apparently no neurofibrillary tangles were seen; thus, this transgenic mouse has serious limitations as a model of AD.

Fourth, there are inconsistencies in the presented results that are possibly related to the small sample size. For example, there is an age-related improvement in the performance of control mice, as shown in figure 2B in the report (35%, 33%, and then 45% improvement at 9 months), which may have skewed the analysis of this particular data set. Figure 2C, showing targeted platform crossings on probe trials, should roughly correlate with the results in figure 2B, which shows percentage of time spent in each platform quadrant. This correlation holds in five of six cases, but not for the old transgenic mice. This disparity is puzzling. Figure 2D on the right casts the same data set as in figure 2C, but are the result of a different analysis.

Fifth, because spontaneous alternation varied little from chance performance, little is added by this analysis. Figure 2F shows percentage alternation, a hippocampal-sensitive task. Neither control nor transgenic mice showed significant alternation to start with, which is consistent with a problem recently raised concerning mouse spatial ability (*2*). Both 3- and 10-month-old control mice demonstrated a 58% alternation (chance is at 50%), while 3-month-old transgenic mice had a 51% and a 47% alternation. The lack of a developmental aging effect in these transgenic mice raises questions about the utility of the task and the effects observed.

I would disagree with Hsiao *et al.* that they have created "transgenic mice with robust behavioral . . . features resembling those found in AD" (*1*, p. 102). Their data show, in mice that harbor amyloid-containing plaques, improvement in performance that is characteristic of learning. Plaque presence, then, is no harbinger of cognitive deficit. Perhaps when a tangle-bearing and plaque-forming mouse is created, convincing memory deficits will be evident.

Aryeh Routtenberg
Northwestern University, Evanston, IL 60208, USA

REFERENCES AND NOTES

1. K. Hsiao *et al.*, *Science* **274**, 99 (1996).
2. R. K. McNamara, U. Namgung, A. Routtenberg, *Mol. Brain Res.* **40**, 177 (1996).
3. I thank several colleagues who provided valuable input.

26 December 1996; accepted 28 May 1997.

Response: My colleagues and I appreciate the time and effort taken by Routtenberg in analyzing the data in our report (*1*). He questions the nature of the behavioral deficit in the TgHuAPP.SWE mice by addressing measures taken of individual behavior, but does not take into account the overall effect. These mice, after reaching an appropriate age, do not perform as well on learning and memory tasks as do control mice. Moreover, several pieces of evidence argue that their sensorimotor function is not grossly impaired.

The first concern is with performance on the visible platform version of the water maze. As we point out in the report (*1*, p. 100), there was no significant difference between the transgenic and control mice on the first day of visible platform training, and the latencies were quite short (<20 s). By the fourth day of training, old transgenic mice were finding the platform in an average of 10 s, a latency value that compares favorably with those observed for control mice in numerous other studies (*2*, *3*). These mice, however, took significantly longer to find the platform on day four, which suggests the possibility of a genuine learning impairment as detected by the visible platform task. This raises an important issue: should we expect to see the exact same pattern of deficits in a mouse that models Alzheimer's disease as we expect to see following selective lesions of the mouse hippocampus? The pathology found both in human Alzheimer's disease and in the TgHuAPP.SWE mice extends well beyond the hippocampus; so, too, should the behavioral deficits.

Routtenberg's second point concerns the various measures of performance on the hidden platform version of the water maze. We made several sets of measurements (escape latency, percentage of time in quadrant on probe trial, and platform crossings on probe trials) because no single measure presents a reliable picture of performance. Thus, Routtenberg's observation that escape latencies improve over time is certainly accurate, but there is more than one way to improve escape latencies (for example, overcoming the tendency to swim near the walls) without specific knowledge of the platform location. We did not claim that the transgenic mice learn nothing; we merely sought to understand what their performance deficits were with the use of standard behavioral tasks. In a similar way, the percentage of time spent in the training quadrant during a probe trial is a useful measure of the animals' knowledge of platform location, but it is not always well suited to the study of mice, which tend to dart around the pool rather than searching methodically as rats do. Nonetheless, the appropriate comparison (that between 9- to 10-month-old transgenic mice and their nontransgenic littermates) reveals a statistically significant difference that was also found with a second group of 12- to 15-month-old transgenic mice from the N_2 generation, and is an indication of the robustness of the phenomenon.

There are several "inconsistencies" that Routtenberg attributes to small sample size. But the average number of animals per group (11, range 9 to 14) in our study (*1*) is consistent with the majority of behavioral studies that use either rat or mouse in the water maze (*2*–*4*). The inconsistencies mostly arise from the fact that different groups of control mice performed differently, a result that is not unexpected. This is why we compared each age group of transgenic animals with their age-matched non-transgenic littermates, not with other animals tested at a different age from a different litter. Routtenberg then expresses puzzlement that the number of platform crossings exaggerate the trend seen in the percentage of time in the training quadrant. But the former test is known to be a more consistent measure of behavioral impairment in mice; had the platform crossings told the same story as the percentage of time in quadrant, these data would indeed have been wholly redundant.

In dismissing the data displayed in figure 2D in our report as "cast[ing] the same data set . . . [in] a different analysis," Routtenberg underestimates the importance of these data. We shared his concern that the deficits seen in the hidden platform could be a result of slow swimming, wall-hugging, or other behaviors that would obscure learning and memory deficits. But the data displayed in this figure indicate that the average number of times the transgenic mice crossed the center of a quadrant (the point where platforms would be located) did not differ from that of the littermate controls. If the mice swam sluggishly, for instance, they would cross very few quadrant centers in 60 s. It is only the percentage of total platform crossings over the target platform location that differed between old transgenic mice and their littermate controls.

There is certainly considerable work remaining in our efforts to characterise the behavioral deficits produced by the HuAPP.SWE transgene in mice. It is most appropriate at this point, however, to look at the overall pattern of results in all the behavioral tests applied to these animals (*5*). That pattern suggests that, in these mice, learning deficits appear at approximately the same age as does Alzheimer's-like brain pathology. For this reason, the TgHuAPP.SWE transgenic mouse will continue to be a useful rodent in which to study Alzheimer's disease.

Karen Hsiao
Department of Neurology, University of Minnesota, Minneapolis, MN 55455, USA
Paul Chapman
Physiology Unit, University of Wales, Cardiff CF1 3US, United Kingdom
Steven Nilsen
Department of Neurology, University of Minnesota
Chris Eckman
Yasuo Harigaya
Steven Younkin
Mayo Clinic, Jacksonville, FL 32224, USA
Fusheng Yang
Greg Cole
Veterans Administration Medical Center, Sepulveda, CA 91343, USA and University of California, Los Angeles, CA 91343, USA

REFERENCES AND NOTES

1. K. Hsiao *et al.*, *Science* **274**, 99 (1996).
2. S. G. N. Grant *et al.*, *ibid.* **258**, 1903 (1992).
3. A. J. Silva, R. Paylor, J. M. Wehner, S. Tonegawa, *ibid.* **257**, 206 (1992).
4. R. G. M. Morris, P. Garrud, J. N. P. Rawlins, J. O'Keefe, *Nature* **297**, 681 (1982).
5. A. J. Silva and P. F. Chapman, *Semin. Neurosci.* **6**, 53 (1994).
6. Our transgenic mouse is available to other researchers, with no scientific restrictions.

14 February 1997; accepted 28 May 1997