months. The decreased incorporation of glucose was not due to a dilution of the specific activity of the precursor pool [glucose disintegrations per minute (dpm) per nanomole]. The incorporation of [$^2H_4$]choline into acetylcholine also declined with senescence. In the BALB/c strain, the estimated rate of synthesis from [$^2H_4$]choline decreased by 50.4 and 75.9 percent in 10- and 30-month-old mice. Neither the specific activity of the precursor pool nor the uptake of [$^2H_4$]-choline into the brain accounted for the decreased incorporation.

The depressed acetylcholine synthesis in senescent mice was correlated (*15*) with behavioral deficits as measured with a string test (r = .98; Fig. 1), which quantitates the ability of a mouse to traverse an elevated taut string (*16*). Scores for 10- and 30-month-old mice of both strains were 35 to 42 and 77 to 78 percent lower than those of the 3-month-old animals. In thiamine deficiency, a decrease in the string-test score seems attributable to a central cholinergic muscarinic lesion (*15*). Whether this is also a causative factor in aged animals remains to be determined. Decreased acetylcholine synthesis correlates well with a decrement in geriatric memory deficits previously reported (*2*). The latencies in passive avoidance tasks declined 31 to 40 percent in 12-month-old mice (acetylcholine declined 32 percent) and by 58 to 64 percent in 30-month-old mice (acetylcholine declined 59 percent). Other studies in rats by Lippa *et al.* (*4*) suggest that memory impairment may not be detectable until 20 months.

Our studies directly demonstrate reduced acetylcholine synthesis in senescent mice. This decrease is correlated with the development of progressive behavioral deficits and may underlie some of the brain dysfunctions which complicate senescence. The mechanism linking these two findings requires further investigation.

GARY E. GIBSON
CHRISTINE PETERSON
Department of Neurology,
Cornell University Medical College,
Burke Rehabilitation Center,
White Plains, New York 10605
DONALD J. JENDEN
Department of Pharmacology,
UCLA School of Medicine,
Los Angeles, California 90024

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17. The aged animals were from Charles Rivers Breeding Laboratories, which is under contract with the National Institute on Aging. They are kept in our temperature- and humidity-controlled animal facilities for no longer than 3 days. This work was supported in part by NIH grants NS16997, MS15649, and MH17691; the Winifred Masterson Burke Relief Foundation; and the Will Rogers Institute.

25 November 1980; revised 26 March 1981

Short-Term Variations in Diet Composition Change the Pattern of Spontaneous Motor Activity in Rats

Abstract. *The nocturnal activity patterns of rats changed significantly within 3 days after they were given unrestricted access to isocaloric diets in which the ratio of carbohydrate to protein was systematically varied. As the ratio increased, the rats were more continuously active. The subjects showed similar responses to variations in this ratio whether the diet contained 15 or 45 percent fat. No correlation was found between the number of calories an animal ate and its activity pattern.*

Does diet composition affect the behavior of omnivores like rats and humans? Learning, sleep, and spontaneous motor activity can be altered experimentally by starvation (*1*), malnutrition (*2*), or excesses or deficiencies of various dietary components (*3*). Animals or humans may occasionally be exposed to severe and protracted dietary changes; however, they usually are able to search for and choose among a variety of foods (*4*). The behavioral effects of normal short-term variations in diet composition have not, to our knowledge, been studied.

The proportions of protein and carbohydrate in each meal can affect the amounts of tryptophan and tyrosine taken up into the brain (*5*) and, consequently, synthesis of serotonin and the catecholamine neurotransmitters. Similarly, dietary lecithin or choline content can affect neuronal acetylcholine synthesis (*6*). There is evidence that these neurotransmitters participate in brain mechanisms underlying behavior such as spontaneous motor activity (*7*). We now report that short-term changes in diet composition, similar to those that may occur naturally, can modify patterns of spontaneous motor activity in rats.

Male Sprague-Dawley rats (Charles River) were housed singly for several weeks in specially constructed plexiglass cages (14 by 14 by 10 inches) that allowed them unrestricted access to food and water. The cages were kept in an isolated room and could be cleaned without disturbing the animals (*8*). Between 4 a.m. and 4:40 p.m. daily, the cages were lighted by fluorescent bulbs (Vita-Lite, Duro Test Corp.) emitting a spectrum

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close to that of sunlight. A Z80 microcomputer (Zilog Corp.) was programmed to record the number of times an animal interrupted either of two crossed infrared photocell beams generated by infrared phototransistors mounted on the sides of the cage (*9*). The total number of beam interruptions in successive 20-minute periods was printed by teletype (Olivetti TE 318). Animals were given 1 week to adjust to the cages, and, except as noted, had access to Charles River rat/mouse/hamster formula during that time. They were then provided with one of several agar-based synthetic diets (*10*) for 3 days each. One group of 20 rats received food containing 18 percent casein, 57 percent carbohydrate (dextrose, sucrose, and dextrin), and 15 percent vegetable fat (*10*); a second group of 20 rats received no protein, 75 percent carbohydrate, and 15 percent fat. Weight gain and food consumption were recorded daily.

A typical activity record for a rat consuming the 18 percent protein diet (Fig. 1A) shows that activity increased with the onset of darkness and decreased with the onset of light. In rats consuming the 0 percent protein diet (Fig. 1B), a similar preponderance of activity occurred during the dark period. Animals consuming the 18 percent protein diet showed a highly irregular activity pattern during the dark period; periods of intense activity were followed by periods of complete quiescence. In contrast, animals consuming the 0 percent protein diet were almost continuously active during the entire dark period, with few or no periods of quiescence.

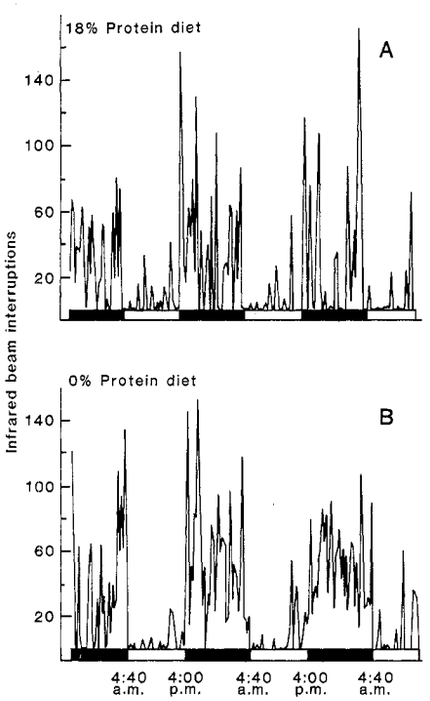

Fig. 1. Effect of diets of different protein content on rat activity patterns. The 18 percent protein diet (A) also contained 57 percent carbohydrate and 15 percent fat; the 0 percent protein diet (B) also contained 75 percent carbohydrate and 15 percent fat. Black bars indicate dark periods. Each data point is the number of infrared beam interruptions occurring in successive 20-minute periods.

Using the raw data, we constructed frequency histograms (*11*), choosing three regions to represent the percentage of 20-minute intervals during which a rat registered a specified level of activity. Activity levels were classified as low (0 to 9 infrared beam interruptions), moderate (10 to 59 interruptions), and intense (90 or more interruptions) (*12*). We evaluated the level of activity for each animal on each diet.

On the average, the 20 animals consuming the 18 percent protein diet registered low activity during 42 percent of the dark period, while rats consuming the 0 percent protein diet registered low activity during no more than 26 percent of the dark period. Animals consuming the 0 percent protein diet shifted instead to 57 percent moderate activity, versus 37 percent moderate activity for rats on the 18 percent protein diet. We compared the two groups by Hotelling's T^2 test (*13*); the result was highly significant ($P << .01$). We compared individual regions using t-tests with a conservative value of 3.01 (*14*); the decrease in low activity levels and the increase in moderate activity levels were both significant at $P < .05$. The percentage of periods during which the animals were intensely active did not increase significantly when they consumed the 18 percent protein diet (Fig. 2). Rats given the 0 percent protein diet ate significantly fewer total grams or calories than those consuming the 18 percent protein diet (33 ± 1.1 g versus 47.5 ± 1.3 g; $P < .01$). Furthermore, animals eating the protein diet gained weight during the 3 days (12 ± 0.4 g), while those on the no-protein diet lost weight (−2 ± 0.5 g) ($P << .01$).

To determine how quickly rats modify their activity patterns in response to dietary changes, the activity of another group of 22 rats was followed for 7 days. On days 1, 2, 6, and 7 the animals consumed the 18 percent protein diet; on days 3 to 5 they ate the 0 percent protein diet. Once animals began to eat the no-protein diet, low activity decreased on successive nights (32 percent the first night, 27 percent the second, and 20 percent the third), while moderate activity increased from 45 percent the first night to 49 and 52 percent on the following nights. The activity patterns during the third proteinless night differed significantly from those recorded on the second night of the protein regimen ($P << .01$). By the seventh night (after animals had again eaten the protein diet for 2 days), the activity pattern did not differ significantly from that observed during the second night. The activity patterns in the daylight period did not change significantly during the week.

To examine dose-response relations between dietary protein content and activity patterns, 27 rats were fed the 18 percent protein diet for 3 days and then given food containing 0, 6, 18, or 30 percent protein (*10*) for 3 days. A one-way multivariate analysis of variance (*15*) indicates that the overall changes in activity which resulted were significant and dose-related ($P < .01$); the percentage of low activity increased and the percentage of moderate activity decreased as dietary protein content increased (Fig. 3A).

We next examined the effect of variations in fat content on nocturnal activity patterns. Nineteen rats were given the 18 percent protein diet (containing 15 percent fat) for 3 days. Then, over consecutive 3-day periods, they were given access to diets containing (i) no protein, 45 percent carbohydrate, and 45 percent fat (*10*); (ii) 18 percent protein; (iii) 22.5 percent protein, 22.5 percent carbohydrate, and 45 percent fat (*10*); (iv) 18 percent protein; and (v) 45 percent protein, no carbohydrate, and 45 percent fat (*10*). The overall changes in activity were significant ($P << .01$; analysis of variance); the percentage of low activity

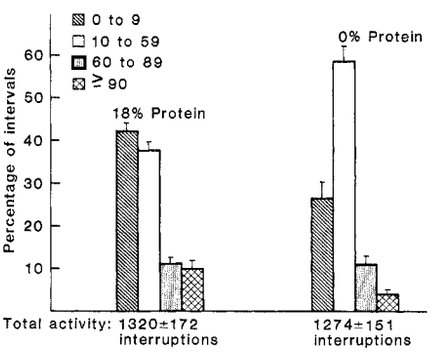

Fig. 2. Histograms showing percentage of 20-minute intervals during which rats on different diets interrupted infrared beams 0 to 9, 10 to 59, 60 to 89, or ≥ 90 times. Twenty rats ate the 18 percent protein diet, which also contained 57 percent carbohydrate and 15 percent fat; and 20 rats ate the 0 percent protein diet, which was 75 percent carbohydrate and 15 percent fat. Error bars indicate standard errors of the mean. Overall differences between the two groups, as determined by Hotelling's T^2 test, were highly significant [$F(3, 36) = 9.1$; $P < .01$].

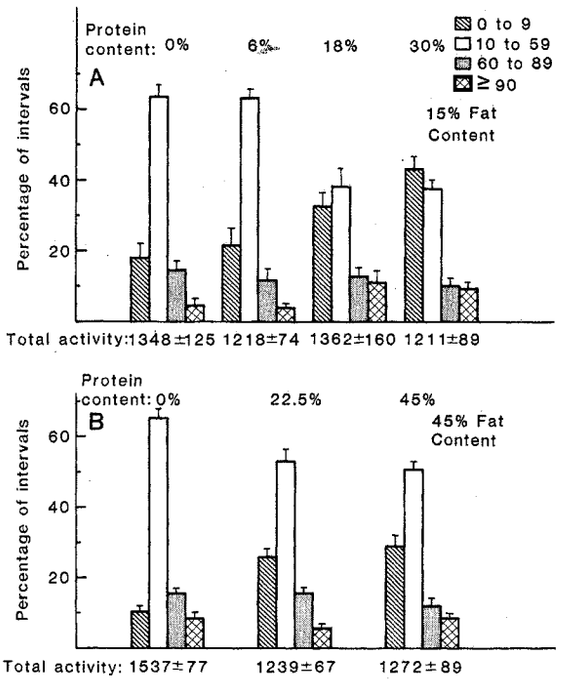

Fig. 3. Interaction of dietary fat and protein content effects on nocturnal activity patterns. Rats consumed 15 percent (A) or 45 percent (B) fat diets containing various proportions of protein (as indicated) and carbohydrate. One-way multivariate analyses of variance indicated that the overall changes in activity patterns were significant for both groups at $P < .01$.

increased and the percentage of moderate activity decreased as protein content increased (Fig. 3B). Hence, variations in dietary protein and carbohydrate content still caused changes in activity patterns when the fat content of the diet was increased.

Our observations did not rule out the possibility that diet composition affects activity pattern by changing the amount of food an animal consumes: protein-poor diets resulted in reduced food intake if the diets also contained 15 percent fat, but not if they contained 45 percent fat. We tested the possibility that the amount of food animals consumed generated the activity changes by doing a pair-feeding study. One group ate 53 ± 1 g of the 18 percent protein diet, while the second group was given only 30 g of the same diet (that is, the amount rats consumed on the 0 percent protein diet). The activity patterns for the two groups did not differ significantly. We also examined the relation between each animal's weight gain and its activity pattern, as indicated by the percentage of time that it displayed low, moderate, or intense activity. Multiple linear regression analyses of data for animals given the 18 percent ($N = 8$) or 0 percent ($N = 7$) protein diets demonstrated poor correlations between activity and weight gain and between activity and total food consumption.

Our data demonstrate that short-term variations in diet composition can affect the pattern of spontaneous motor activity within 3 days. The changes are not caused by variations in the amount of food an animal eats, by changes in its weight, or by the fat content of the diet. Rather, the dietary protein-to-carbohydrate ratio, which controls availability of the neurotransmitter precursors tyrosine and tryptophan to the brain (*5*), may alter the synthesis and release of monoamine neurotransmitters utilized by neurons affecting motor activity.

The pattern of infrared beam interruptions provides no insight into the specific nature of the behavioral changes that occur as diet composition is varied. The total amount of certain behaviors could change (for example, grooming may decrease and rearing increase when animals consume a particular diet); or the sequencing of certain behaviors [for example, the likelihood that rearing will be followed by sniffing instead of grooming might depend on the protein content of the most recently consumed meal (*16*)]. The changes we observed may reflect changes in the behavioral strategies animals use to obtain meals.

HILLEL J. CHIEL*
RICHARD J. WURTMAN
Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

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8. The cage bottoms were wire mesh so droppings could fall onto trays underneath.
9. Two photodiodes (TIL 31, Texas Instruments) mounted on adjoining walls of the cage generated infrared beams, which were sensed by two phototransistors (TIL 81, Texas Instruments) on opposite walls of the cage.
10. All synthetic diets contained 40 g of Rogers-Harper's mineral mix (Teklad Test Diets), 35 g of agar, and 22 g of vitamin mix (Nutritional Biochemicals) per 1000 g (dry weight). One liter of water was used to make up the agar gel for each 1000 g (dry weight) of food. The diets that were 15 percent fat contained 150 g of Crisco per 1000 g (dry weight). Of these diets, the diet that contained no protein had 264 g of dextrose, 225 g of sucrose, and 264 g of dextrin; the diet that contained 6 percent protein had 60 g of casein, 247 g of dextrose, 199 g of sucrose, and 247 g of dextrin; the diet that contained 18 percent protein had 180 g of casein, 204 g of dextrose, 165 g of sucrose, and 204 g of dextrin; and the diet that contained 30 percent protein had 300 g of casein, 161 g of dextrose, 131 g of sucrose, and 161 g of dextrin. The diets that were 45 percent fat contained 450 g of Crisco. Of these diets, the diet that contained no protein had 161 g of dextrose, 131 g of sucrose, and 161 g of dextrin; the diet that contained 22.5 percent protein had 225 g of casein, 81 g of dextrose, 66 g of sucrose, and 81 g of dextrin; and the diet that contained 45 percent protein had 453 g of casein and no carbohydrate.
11. We could not use parametric statistics because the raw data were not normally distributed. We determined that the regions of frequency histograms for several animals were normally distributed and that the variances of each region were of comparable magnitude. The regions were chosen after looking at a small sample of the data. Therefore, we analyzed the regions of the frequency histograms by multivariate statistical techniques.
12. By definition, the area of a frequency histogram must be 100 percent. Trying to analyze a set of numbers that add to a fixed constant introduces a spurious correlation into the data. One can avoid this problem by arbitrarily choosing a smaller number of regions that do not add to 100 percent [R. J. Harris, *A Primer of Multivariate Statistics* (Academic Press, New York, 1975), p. 76].
13. Hotelling's T^2 test is the multivariate analog of the t-test, making it possible to compare the differences between two groups for several dependent variables [R. J. Harris (*12*), chap. 3].
14. Hotelling's T^2 test can be used to determine a value of t for comparisons between individual variables; this guarantees that, no matter how many individual comparisons are made, only 5 percent of those that appear to be significantly different will be due to random fluctuations [R. J. Harris (*12*), p. 104].
15. R. J. Harris (*12*), chap. 4.
16. C. J. Latham and J. E. Blundell [*Life Sci.* **24**, 1971 (1979)] demonstrated that subtle changes in meal pattern can be determined by examining the length, and the time between, "bouts" of eating.
17. We thank F. Romero, R. Spinella, L. Phebus, and B. Sahakian for helping to design and build the cages, W. Rand and H. Chernoff for advice on statistical analysis, and T. Deboissiere for analyzing much of the data. Supported in part by NIH grant AM-14228, NASA grant NGR-22-009-627, and the Center for Brain Sciences and Metabolism Charitable Trust.
* Present address: Division of Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York 10032.

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