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Activity Measures in Rhesus Monkeys on Long-Term Calorie Restriction

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WEED, J. L., M. A. LANE, G. S. ROTH, D. SPEER AND D. K. INGRAM. *Activity measures in rhesus monkeys on long-term calorie restriction.* PHYSIOL BEHAV **62**(1) 97–103, 1997—Calorie restriction (CR), undernutrition without malnutrition, extends the mean and maximal lifespan of several ecologically diverse species. Rodents on CR demonstrate increased activity measured as spontaneous locomotion, wheel running, open field behavior or movement. Activity measures were recorded from 19 male rhesus monkeys (*Macaca mulatta*) as either controls (C) which were fed a nutritious diet to approximate ad libitum levels, or as experimentals (E) which were fed 30% less than age- and weight-matched controls. Within each diet group, some monkeys (n = 10) began CR at 2.3 years of age (range 2.2–2.4 yrs, J Group) while another group (n = 9) began CR at approximately 4.6 years of age (range 4–5.25, A group). Beginning about 6 years after initiation of the study, behavioral activity was measured via ultrasonic motion detectors and recorded on videotape. Diurnal and circadian activity was clearly discernible. Peaks in activity were associated with mealtime and colony husbandry. Compared to Group A, Group J monkeys exhibited higher overall activity as measured by sensors, and also significantly more circling. Compared to AC monkeys, group AE monkeys demonstrated higher rates of gross motor behavior, pacing, stereotypies and grooming. The increases in motor activity observed in one group of monkeys were consistent with results obtained from rodent studies of CR and aging. CR did not significantly inhibit or negatively influence the display of behavior of rhesus monkeys in the laboratory environment. We report here, for the first time, increases in activity due to CR in a model other than the rodent. © 1997 Elsevier Science Inc.

Aging Behavior Diet Nutrition

CALORIE restriction (CR), undernutrition without malnutrition, extends the mean and maximal lifespan of several ecologically divergent genera such as *Daphnia, Lebistes, Mus* and *Rattus* (34,36,38). In addition, in laboratory rodents, CR can reduce the incidence and onset of several age-related diseases and retard age-related changes in numerous parameters, observed at molecular, cellular, and physiological levels (34,36,38). The terms caloric restriction, dietary restriction and food restriction are used somewhat interchangeably and imply operational distinctions. However, each paradigm consists of a reduction in food intake such that calorie consumption is reduced in experimental animals compared to ad libitum fed controls (19).

Because CR studies have been conducted almost exclusively among species with short lifespans, (rat maximal lifespan about 3 years in captivity), effects of this nutritional intervention on aging processes in higher organisms had not been addressed. To address this paucity of information, the National Institute on Aging in 1987 initiated studies in rhesus monkeys (*Macaca mulatta*) implementing a 30% reduction in caloric intake relative to control levels (14). Macaques have an estimated maximal lifespan of 40 years in captivity (16) and their physiological response in many parameters more closely approximates that of humans. Research at the National Institute on Aging, as well as in other laboratories, (2,18) has documented significant CR-induced changes in physiological indices in rhesus macaques. These observed changes are potentially related to the mechanisms by which aging rate is altered by this manipulation. For example, CR has reduced body temperature (20), and lowered plasma glucose and insulin of monkeys on the restricted diet (2,17,22).

As one of three independent studies directly examining the effects of CR on aging in rhesus monkeys, the others at Wisconsin (18), and the University of Maryland (2), the focus of the present series of experiments has been on assessment of physiological correlates of aging. Less attention has been given to potential behavioral differences, between monkeys subjected to CR vs ad libitum feeding. One systematic way to assess behav-

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ioral differences is to measure activity and behavior of monkeys resident within a home cage. Activity has been measured typically via ultrasonic detectors, biotelemetry, recorded on videotape or by direct observation (18,25,32,37). In the Wisconsin study (18) which used ultrasonic movement detectors, it was reported that adult rhesus monkeys (mean age 9.3 yrs) on CR, but not controls, evidenced a small, but significant decrease in activity following implementation of the restriction regime. However, initial differences in activity are no longer significant following continued assessments of these monkeys over a period of 66 months (30).

Virtually all other behavioral research involving CR has been conducted with mice and rats (33). As in the primate studies, activity is just one of many variables measured. Activity has been measured via wheel running, changes in locomotion, spontaneous activity, open field behavior, or load applied to a pressure transducer (6,19,13,15,39).

It has been reported that motor activity was higher in both male and female Fischer 344 rats tested under similar CR conditions (6,7). Average daily activity was higher for old male and female mice on CR when compared to controls (4,5). Rats as well as mice on CR and given access to a running wheel demonstrated higher levels of activity than controls (9,10,15). Every-other-day feeding differentially affected wheel running activity across the life span for male Wistar rats (9). Early in life, wheel activity was actually lower in restricted groups. However, these same restricted groups showed higher levels of wheel running later in life, suggesting that CR's effects on wheel running activity may change over time. Progressive decreases in activity with age, measured as spontaneous movement, were reported for ad libitum fed, but not restricted male Fischer 344 rats (39). Regardless of how CR potentially affects behavior when measured as changes in activity, however defined, the most consistent finding in rodents is that activity increases under CR treatment.

Inasmuch as CR has been suggested as one possible intervention providing beneficial physiological protection against the effects of aging in rodents, (34,38) and that changes in activity are evident following induction of CR in rodents, (6,9,13,15,39) the question is whether long-term CR might influence overall activity and behavior in monkeys. To address this issue, the present analysis examined activity patterns in rhesus monkeys to characterize potential behavioral influences of long-term CR.

METHODS

Subjects

Subjects were 19 male rhesus macaques (*Macaca mulatta*). Group ages and experimental design are provided in Table 1. The J group monkeys were obtained from the NIH primate facility at Perrine, Florida with a mean age of 2 yrs (1.9–2.1 yrs) upon arrival at the Poolesville NIH primate facility. The A group monkeys were obtained from a research colony in the People's Republic of China via the Texas Primate Center (Hazelton Research Primates, Alice, Texas) with a mean age of 4.4 yrs (3.7–5 yrs) at arrival. A more detailed description of the housing and husbandry of the colony can be found elsewhere (14).

Subjects were originally pair-housed. Individuals were matched by experimental group and approximate weight. Although pair-housed, animals were separated for individual feedings. Due to increased fighting and experimental protocol, all subjects were subsequently separated and single housed after approximately 3 years on study.

Diet and Formulation

At the beginning of the study, the monkeys were divided into control (C) and experimental (E) groups. Food allotments for C groups were based on age and body weight in accordance with National Research Council (NRC) recommendations for nonhuman primates (28). Regular measurements of consumption documented actual food intake. Examination of food consumption data over the course of the ongoing study has indicated that C monkeys were eating at approximately ad libitum levels. All animals received food at approximately ad libitum levels for one month prior to starting on restriction. The E groups received 30% less ration than age and body weight matched C groups. The target restriction level of 30% was achieved by a gradual reduction (10% per month) of food intake over 3 months. As control animals grew, intake was adjusted based on NRC requirements for monkeys of a given age and weight. Allotments for E monkeys were adjusted to maintain a 30% restriction.

TABLE 1

EXPERIMENTAL DESIGN AND AGE AT TESTING

Group	Diet	*N*	Age at Activity Testing	Age at Video Testing
J	C	4	8.3 (8.2–8.4)	10.3 (10.2–10.4)
J	E	6		
A	C	5	10.6 (10.0–11.3)	11.6 (11.0–12.3)
A	E	4		

C = Control; E = Experimental. Ages are expressed in years (mean and range).

All monkeys were fed individually, twice per day, at approximately 0700 and 1400. A stainless steel screen was located below each individual cage to catch dropped biscuits. Monkeys could retrieve these biscuits throughout the day. All uneaten food was removed following the afternoon feeding. Each animal received a fruit treat as a supplement once per week.

The diet was formulated at NIH as a modification of their high-fiber diet routinely fed to monkeys. Nutrient content of the diet was based on published estimates of requirements for nonhuman primates (28). All monkeys ate the same diet which was identical and supplemented with additional vitamins, minerals and trace elements to prevent nutritional deficiency (14). Animals experiencing the CR regime demonstrated body weight increases and maintained growth, albeit, more slowly, since the inception of this study (23,35). Further detailed descriptions of the diet and composition are available in previously published reports (14,24).

Vivarium and Apparatus

The monkeys were housed in stainless steel primate cages measuring 88.9 × 61.0 × 68.5 cm. All monkeys were housed in a light and temperature controlled vivarium measuring 2.9 × 8.2 m. The vivarium had only artificial lighting, maintained on a 12–12 LD cycle with lights on at 0600. Room temperature (22–28°C) and humidity (50–60%) were under automatic control. Water was provided ad libitum via automatic filtered watering systems. All testing was conducted within the vivarium. Monkeys had visual, auditory and olfactory, but not tactile interactions.

Activity data were collected using both quantitative and qualitative methods. For quantitative analysis, activity sensors (infrared and microwave motion detectors, C & K systems intrusion detection units, model DT450, Folsom, California) were attached to the front of each testing cage which were identical to the monkeys' home cage. These sensors transferred digitized signals to an IBM XT computer which then recorded and tabulated, via a custom designed program, the detection of whole body move-

TABLE 2
ETHOGRAM

Ambulate	3 or more continuous steps in one direction
Bite cage	Mouthing cage parts
Bite self	Any biting of body
Bounce	Jumping up and down in place
Clasp self	Use of hands or feet to hold onto a part of the body
Climbing	Vertical movement on the cage
Circle	Animal turns a complete circle within cage
Drink water	Common usage
Drink urine	Animal drinks own urine
Eat food	Animal picks up food and places in mouth
Eat feces	Animal picks up feces and places in mouth
Float limb	Limb moves upward slowly without animal apparently aware of movement
Groom	Animal pushes hair aside with hand and fingers, separating hairs
Head tossing	Animal throws head back in a repetitive manner
Licking or sucking	Animal places tongue across body parts or places digits or hair into mouth
Manipulate cage	Tactile exploration of cage
Manipulate toy	Tactile or oral exploration of toy
Masturbate	Common usage
Autoerotic	Animal places penis in mouth
Pace	Repetitive back and forth movement within the cage
Passive	Animal sitting quietly, not engaged in other behavior Eyes open or closed
Pluck	Pulling hair out
Rock	Repetitive back and forth motion while seated
Rump display	Animal's rump directed outward from cage at another individual
Salute	Animal brings arm up to face on ipsilateral side
Shake cage	Animal grabs cage and vigorously moves it back and forth
Sleep	Common usage
Somersault	Animal turns vertically around in place
Spin	Animal turns around horizontally in place
Swing	Animal hangs from cage top and repetitively moves back and forth
Other	Any behavior not formally described previously

ments. These summary counts were then accumulated across 24 h for analysis of circadian patterns. Before each session, the sensors were calibrated to record only gross motor movements of the center of gravity. Calibration was accomplished by adjusting the sensitivity of the sensor to detect a 20–30 cm deflection of the experimenter's arm moved in the center of the cage. Calibration was checked by observing a monkey in the test cage and verifying the activity score obtained visually against the sensor recording. Movements such as scratching and grooming were not detected.

For qualitative analysis, a Canon infrared videocamera (model CI-20R), was attached to the wall opposite the test monkeys. The infrared capability allowed recording of behavior throughout the night when vivaria lights were normally off. A cable connected the camera to a videocassette recorder located in an adjacent room. The videocassette recorder was activated and recorded all behavior during the first 15 min of each h over a 24-h period.

Procedure

At least one week prior to actual testing, a pair of monkeys, one control (C) and one experimental (E), were moved into adjacent test cages outfitted with sham activity units to allow for adaptation to monitors and cameras. Following this period, activity monitors were activated. Testing cages were separated by a solid stainless sheet. The camera was mounted on the wall directly across from the test cages. No monkeys were housed directly in front of these cages. Thus, adaptation and testing conditions were the same for all monkeys. Behavior and activity were recorded for 7 days for each animal. During 1993 and 1994, monkeys in the A groups were videotaped and activity monitors used when the animals were approximately 10–12 (range 10–12.2) years of age. J group activity data were collected during 1993 when these animals were approximately 8.2 years old (range 8.2–8.4). These same monkeys were videotaped during 1995 when they were about 10.2 (range 10.2–10.4) years of age. At the time of the first behavioral testing in 1993, both E groups had experienced the restricted feeding regime for at least 6 years.

Data Analysis

The videotaped activity data was scored by three trained observers at a later time. Definitions of scored behavior are listed in Table 2.

Behavior was scored using the focal animal technique (1). Absolute frequencies of all behavior were recorded. Weekly averages were calculated as well as percent of total activity time spent engaged in each behavior. Such an analysis, representing relative measures of activity, permitted a reasonable comparison of groups even though the data had been collected at different

times. Several categories were combined to simplify the analysis or to merge categories for which low frequencies occurred. For example, behaviors considered to be stereotypical (e.g., clasping self, rocking, saluting, etc.) were combined due to the overall low frequency of occurrence. Climbing and ambulating were combined into one category of gross movement. Behaviors occurring less that 2 percent of the total observation time were not included in the overall analysis. Data were subjected to a 2 (Diet) $\times$ 2 (Group) analysis of variance (ANOVA) for individual behaviors. Statistical significance was set to $p < 0.05$.

The activity data collected from the sensor system were subjected to a 2 (Group) by 21 (h) or 2 (Diet) by 21 (h) repeated measures analysis of variance, with hours as the repeated measure. Data of 8, 9 and 1000 h were excluded from the analysis because colony husbandry occurred at this time. Daily human activities have been shown to inflate activity scores artificially (25). Activity monitors were reset at this same time.

RESULTS

Sensor System

Activity data obtained from the sensors are presented in Figures 1 and 2 and show several distinct patterns. The circadian rhythm of activity is shown clearly with low levels obtained during nocturnal hours and high levels during diurnal hours. Activity onset occurred just prior to lights on at 0600 and peaked twice during the day around meal times (0700 and 1400). All ANOVAs yielded significant effects for time, $p < 0.0001$.

Figure 1A reveals a clear group difference with the J group showing higher levels of activity throughout the 24 h period compared to the A group $F(1,20) = 5.05$, $p < 0.04$, with no significant group by time interaction, $p > 0.05$. Figure 1B also shows that monkeys in the E group demonstrated a higher level of activity compared to controls, which occurred primarily during diurnal hours and most prominently during the first meal period. The main effect of diet was not significant $F(1,17) < 1.0$; however, there was a significant diet by time interaction, $F(1,20) = 2.54$, $p < 0.0003$.

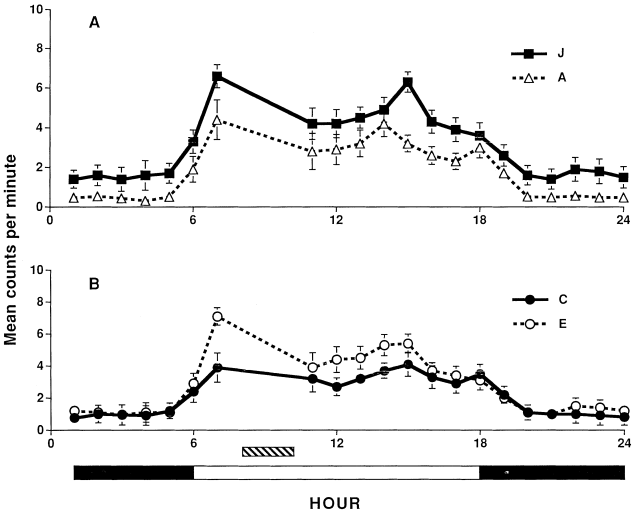

FIG. 1. Circadian patterns of locomotor activity (mean ± SEM), in (A) J vs. A group; (B) Control (C) vs. Experimental (E) groups. Filled bars = lights off. Open bar = lights on. Striped bar = no data collected. A score of zero on the ordinate = no movement, a score of twelve = almost constant movement. Error bars for certain data points are not seen with the scale used.

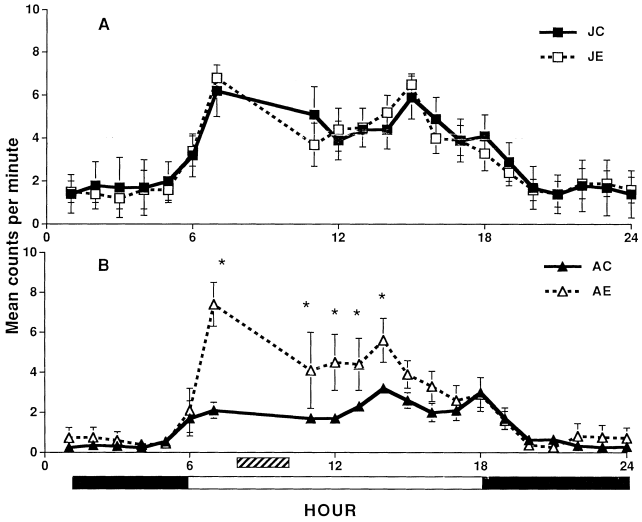

FIG. 2. Circadian patterns of locomotor activity (mean ± SEM) in (A) JC vs. JE group; (B) AC vs. AE group. Filled bars = lights off. Open bars = lights on. Striped bar = no data collected. C = control; E = experimental. A score of zero on the ordinate = no movement, a score of twelve = almost constant movement. Error bars for certain data points are not seen with the scale used. Significant differences in activity are indicated by asterisks.

Although the differences in activity between diet groups appear quite marked, the interpretation of this is complicated by the fact that sampling of the J and A groups occurred at different times. Nonetheless, the general quantitative profile appears comparable.

The cleaner comparisons of activity can be made between diet groups within each age group because the samples were collected at the same time. As seen in Figure 2, these diet group comparisons reveal that the CR regime increased activity only in the AE group of monkeys. In the AE group, results of ANOVA revealed a significant main effect of diet, $F(1,7) = 6.6$, $p < 0.04$, as well as a significant diet by time interaction $F(1,20) = 2.8$, $p < 0.0002$. Post-hoc *t*-tests determined that there were significant differences in activity, with AE monkeys showing significantly higher levels of activity at 7, 11, 12, 13, and 1400 h. E monkeys showed distinctly higher activity during diurnal periods, especially during mealtime. The lack of a significant diet effect in the J monkeys was attributable to the generally higher lever of activity among controls in this group compared to those in the AC group.

Videotaped Activity

Consistent with activity changes seen in rodents subjected to calorie restriction, monkeys in the AE group demonstrated a higher percentage of time exhibiting gross motor patterns (climbing and ambulating combined) and pacing. These data are presented in Figure 3. There was a significant effect of group for gross motor movement $F(1,15) = 10.6$, $p < 0.006$. Neither the diet nor the group by diet interaction were significant. When the effect was computed for each age group separately, no significant diet differences were found. A significant group by diet interaction did emerge for pacing $F(1,15) = 4.6$, $p < 0.05$. No pacing was observed in the JE group.

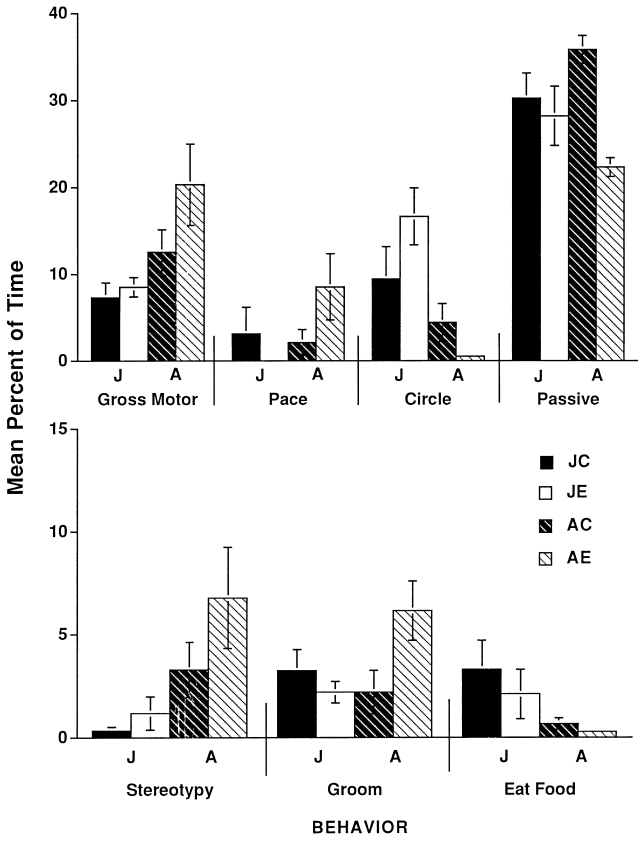

FIG. 3. Relative time distribution of behavior (mean ± SEM) in J's and A's. C = control; E = experimental. No pacing was observed in the JE group. Error bars for certain data points are not seen with the scale used.

Examination of the other behavior patterns associated with movement, or lack thereof, revealed a significant group effect for circling, $F(1,15) = 13.5$, $p < 0.005$. The J group engaged in significantly more circling. Neither the diet nor group by diet interactions were significant. The analysis of passive behavior, scored here as the converse of movement, revealed a significant diet $F(1,15) = 8.1$, $p < 0.02$, and group by diet interaction $F(1,15) = 4.6$, $p < 0.05$. There were no significant differences observed in the J Group. Further analyses indicated that the AC group was significantly more passive than AE's, $F(1,7) = 43.0$, $p < 0.0004$.

Significant differences were also found in other behaviors, not associated with gross movement or activity. Significantly more stereotypies were observed in the Group A monkeys compared to the Group J, $F(1,15) = 10.1$, $p < 0.007$. The diet and group by diet interactions failed to reach significance. The stereotypy score consisted of: bites self, clasp self, urine drinking, head tossing, licking or sucking self, autoerotic behavior, plucking, rocking, saluting and swinging. These scores were combined into one score due to the extreme low frequencies seen in behavior within each category. Examination of the mean scores for each category revealed that the A group exhibited more bouts of rocking and licking. These two behaviors combined were responsible for the significant difference seen in the display of stereotypies.

A significant group by diet interaction was revealed for grooming $F(1,15) = 6.3$, $p < 0.03$. Further analyses indicated a marginal effect of diet on grooming in the AE group, $F(1,7) = 5.14$, $p = 0.057$. There were no differences between J groups in the amount of time spent grooming. Examination of the eating data revealed a significant group effect $F(1,15) = 4.9$, $p < 0.04$. J Group was observed eating significantly more often than the A group. No significant differences were found for any group in amounts of time spent sleeping, bouncing or engaged in aggressive displays (data not shown).

DISCUSSION

Our results demonstrate that behavior in captive housed rhesus monkeys was not adversely affected by long-term calorie restriction. Moreover, behavioral profiles indicate that daily activity was typical for captive housed primates as measured over 24 h (25,37). Peaks in activity were observed near the onset of light and husbandry, i.e., feeding and cleaning. This was true for both experimental, receiving the 30% less food allotment, as well as control monkeys (fed approximately ad libitum). Examining the effects of adult-onset CR in rhesus monkeys, investigators at Wisconsin have also reported daily fluctuations in movement over the 24-h period. These diurnal patterns are typical of rhesus monkeys housed under laboratory conditions as well as in natural environments (18). These same peaks in activity, associated with normal colony procedures, are seen at other primate facilities (25). In the present study, the analysis of both videotaped and sensor-derived activity data documented these same cyclical patterns. The data replicate earlier findings from this laboratory which revealed distinct peak activity associated with mealtime as well as clear evidence of circadian patterns (20).

The long-term objective of our CR study is to assess whether this nutritional regimen can retard specific parameters of aging in a long-lived species. The results indicate that CR had a significant influence on behavior and activity patterns but almost exclusively in the A group. The only behavior that was significantly elevated in the J group compared to the A group was circling. Increased activity was observed among the experimental animals in the A group. Compared to control cohorts, monkeys in the AE group exhibited more pacing, gross movement, stereotypies and were significantly less passive. The differences in behavior associated with movement in the present study are consistent with and in a similar direction as reported changes in activity of rodents on CR. Significantly more grooming was observed in the AE group. The relative influence of CR on grooming remains elusive.

The extent of influence of CR on certain behavioral differences remains unclear. There is little reason to predict that monkeys subjected to CR should exhibit more stereotypies than controls; however, we did observe that CR resulted in increased stereotypies in the AE group when compared to AC monkeys. Examination of the data revealed that this effect was due largely to increased levels of licking or sucking coupled with a higher incidence of rocking among these AE individuals. Rocking may contribute to vestibular self-stimulation (8) and may be another way to demonstrate movement. Licking or sucking furnishes gustatory stimuli. Increased licking or sucking provides non-nutritive oral stimulation, and may be contributory towards satiety. It is reasonable to assume that animals on restriction may be hungrier than ad lib fed controls, and thus engage in more food oriented behavior. No systematic research has been conducted to confirm this assumption. This issue of possible motivational differences can also affect analysis of calorie restriction effects in other behavioral tasks such as learning, memory or psychomotor performance in rodents (12). Thus, the control of such variables will have to be considered for future behavioral analysis in our primate study.

In rodents, long-term CR has not been shown to adversely affect behavioral tasks involving psychomotor performance. Rather, performance in some behavioral assessments was improved for both mice and rats (12). Examination of measures, including more complex behavior, obtained from monkeys on CR, will be required to elucidate the effects of an altered dietary regime on these behavioral propensities.

Because some of our monkeys were obtained from a Chinese source (the A group) and imported into this country when they were at least 4 years old, it is possible that some of the behavioral differences observed between the J and A groups were due to genetic differences (3) as well as differential rearing histories rather than to age differences per se. These potential differences could have also interacted with the effects of calorie restriction. No behavioral data were available on these A group monkeys until they were incorporated into the NIA study. While birth dates are known for these animals, no data are available on the type of housing or structure of social systems, if any, these monkeys experienced from birth through their fourth year, a time coinciding to marked developmental changes measured socially and physically. Thus, the stereotypies observed in these A group monkeys were possibly well developed in individuals prior to shipment to the United States (29). Alternatively, changes in housing may have contributed to the display of stereotypies for individually housed monkeys. All J group monkeys were born at the NIH Perrine facility, and were separated from dams early on. Following separation, monkeys were pair housed with similar aged peers. While the J monkeys experienced differential rearing histories and housing, behavioral adaptations or adjustments to early experience remain unknown. Regarding the general frequency of stereotypies which included a broad range of behaviors, the overall incidence occupied less than 12% of time when considering all monkeys studied.

Our results differ from those reported by the Wisconsin CR study in several respects. Activity levels in the CR monkeys in the Wisconsin study initially declined from baseline values, whereas they increased in the controls. However, the most recent report from this study indicates no significant differences between control and experimental groups in activity levels measured by sensors (30). It is unclear why the activity patterns apparently changed in this study, however, possible explanations include adjustments to the CR regime and dietary composition (17,18). Some CR monkeys in the present study showed increased activity, specifically those with a gross motor component involved, i.e., ambulating, climbing, and pacing. Our results also document that monkeys exhibited food-related increases in activity, but that levels are higher for the J and AE group monkeys. Animals in the present study have been on CR, approximately 9 years, since inception of the project. CR began for the Wisconsin animals when they were adults; whereas, monkeys in the current study were much younger when the study was begun. Examination of the videotaped behavior, coupled with the analysis of sensor data in the present study revealed significant behavioral differences in the monkeys due to CR.

Age at onset, as well as duration of CR, may influence the expression of behavioral differences in rhesus monkeys. Clearly, early onset of CR in rodents, i.e., immediately following weaning, as well as implementation in early adulthood, results in extension of the mean and maximal lifespan, as well as affecting behavior (12,36). Behavioral results obtained from the present series of experiments are preliminary and await further verification, validation and clarification regarding the potential influence that early onset CR has on the expression of behavior of rhesus monkeys in the laboratory.

Regarding possible causal mechanisms of increased activity among CR monkeys, it is important to consider energy metabolism, which has been suggested as one potential mechanism that might affect maximal lifespan. It has been proposed (11,31) that CR affects longevity by lowering metabolic rate. McCarter and colleagues (26,27) tested the energy metabolism hypothesis; caloric restriction results in decreased energy utilization. They found that while short-term CR may decrease metabolic rate, the effect was transient and concluded that reduced metabolic rate per unit of metabolic mass was not required for lifespan extension in CR rodents. Metabolic rate studies in CR monkeys generally agree with these findings (20,21); the reduction in metabolic rate, i.e., energy expenditure, is transient. For example, we have shown (20), that during short-term CR, before lean mass is lost, energy expenditure per lean body mass is reduced. During long-term CR energy expenditure was not different between groups (21). Similar results have been reported in the Wisconsin study as energy expenditure was reduced at 24, but not at 42 or 66 months following CR (30). Thus, the observed changes in activity were not detectably related to alterations in energy expenditure or 24-h energy balance. Although total daily energy expenditure was not significantly altered by long-term CR, it remains possible that certain components of the daily energy budget, such as resting energy expenditure, the thermic effect of food, or energy expended only for physical activity, are affected by CR. Studies to examine these parameters are underway in our laboratory.

As one of three studies of the effects of CR on primates, it is important to document the physiological as well as behavioral changes associated with implementation of a restricted diet paradigm. The majority of research in this area has used the rodent as the primary model of choice. Length of life span and the ability to conduct longitudinal as well as cross-sectional assessments make the rodent model an obvious choice (33). However, the goal of studying the effects of CR on longevity is not to prolong the lives of laboratory rodents per se, but rather to assess empirical manipulations of CR and suggest potential mechanisms responsible for these observed increases in mean and maximal life span (36). Moreover, the choice of nonhuman primates permits investigation of CR in a longer lived species whose physiological makeup more closely approximately that of humans.

Results obtained from the present study are important for several reasons. We report significant changes in behavior following CR in a taxon other than Rodentia. Quantitative and qualitative assessments provided clues to the nature of behavioral changes observed over a 24-h period. Our results are in agreement with previous studies in rodents on CR, which found increased activity in animals receiving a calorically restricted diet. More importantly, there was no evidence of reduced activity due to decreased caloric intake. CR did not significantly inhibit or negatively influence the display of behavior of rhesus macaques in the laboratory environment. Our continued efforts to examine age-related changes in behavioral and physiological correlates of aging in nonhuman primates should provide valuable information on the potential mechanisms of CR and how it influences the aging process.

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