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Enhanced Longevity in *Tau* Mutant Syrian Hamsters, *Mesocricetus auratus*

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Abstract The single-gene mutation *tau* in the Syrian hamster shortens the circadian period by about 20% in the homozygous mutant and simultaneously increases the mass-specific metabolic rate by about 20%. Both effects might be expected to lead to a change in longevity. To test such expectations, the life span of male and female hamsters from three genotypes (wild-type, heterozygous, and homozygous *tau* mutants, all derived from heterozygote crosses to randomize the genetic background) was recorded in constant darkness. Male hamsters lived significantly longer than females: the overall average life span was 96.9 weeks (SE = 2.5, $n = 118$) for males and 82.0 weeks (SE = 2.1, $n = 99$) for females. To our surprise, male and female homozygous mutant hamsters lived significantly longer rather than shorter compared to wild-types. For males, the difference between the two genotypes was on average 14%; for females, the difference was 16%. The mortality rate of wild-type males was significantly different from that of homozygous *tau* males but not different from that of heterozygotes. Overall, survival of wild-type females was statistically distinguishable from both heterozygous and homozygous mutant females. Male and female wild-type hamsters were heavier than homozygote mutants throughout the entire life span, and heterozygous mutants had intermediate weights. There was no correlation between body mass and life span, and the causes of the extended life span in *tau* mutant hamsters remain unresolved.

Key words circadian rhythms, longevity, Syrian hamster, *tau* mutation

The circadian system is involved in the temporal organization of many physiological and behavioral processes. During senescence, eventually leading to death, circadian rhythms of most physiological parameters (e.g., sleep, body temperature, and heart rate) are disrupted. Age-related changes influence the amplitude of rhythms, the ability to entrain to a 24 h light-dark (LD) cycle, or to synchronize different rhythms. Conversely, some studies have suggested that the manipulation of circadian rhythms might affect the life span. Repeated phase shifts of an LD cycle or imposing LD cycles different from endogenous rhythms of the organisms have been shown to affect longevity adversely in plants (Ketellapper, 1960), flies (Aschoff et al., 1971; Pittendrigh and Minis, 1972), and mice (Halberg and Cadotte, 1975; Halberg et al., 1977; but see Nelson and Halberg, 1986). It is not known whether the cycle length of circadian rhythms is in any way involved in determining life span. Interestingly, the acceleration of seasonal rhythms affected longevity in the lesser mouse lemur (*Microcebus murinus*), where life span depended on the number of seasonal cycles rather than on a fixed biological age (Perret, 1997).

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The availability of genetic mutations affecting the period of circadian rhythms provides a tool for further insight in the relationship of the circadian system and longevity. In the Syrian hamster, the single autosomal *tau* mutation induces a 20% decrease in the period of a free-running circadian activity rhythm in homozygote mutants (Ralph and Menaker, 1988). The *tau* mutation also affects body mass and mass-specific metabolic rate, of which the latter changes proportionally to the circadian frequency (Oklejewicz et al., 1997; Oklejewicz et al., 2000). It is known that the metabolic rate, corrected for body weight, scales inversely proportionally with life span when compared between mammalian species (e.g., Calder, 1985). Indeed, the "rate of living theory" (Pearl, 1928) has postulated that energy expenditure plays a direct role in regulating the rate of aging. Several lines of evidence have yielded some support also for a negative effect of the energy demand placed on the individual and its survival. Studies on food restriction have shown that a decrease in energy intake in insects, fish, rats, and mice increased maximum life span (Sohal and Weindruch, 1996; Weindruch and Walford, 1988). Turkish hamsters lived considerably longer when allowed to suppress overall metabolism in hibernation (Lyman et al., 1981). Poikilotherms such as *Drosophila* have decreased life spans parallel with increased metabolic rates under high ambient temperature regimes (Miquel et al., 1976).

Thus, from two different lines of reasoning, one might expect that homozygote *tau* hamsters would live shorter than wild types. A previous study on longevity in *tau* mutant genotypes (Hurd and Ralph, 1998) indeed revealed a shorter life span of heterozygote *tau* hamsters. This might, however, be attributed to the 24 h LD cycle animals were exposed to, which required large daily phase shifts to maintain entrainment. To avoid this problem, we examined life spans of the three genotypes and of both sexes of hamsters exposed to constant darkness and temperature conditions throughout their lives.

MATERIALS AND METHODS

Male and female Syrian hamsters (*Mesocricetus auratus*) were derived from a breeding colony established at the Zoological Laboratory, Haren (Oklejewicz et al., 1997). Wild-type (*tau* +/+), heterozygote *tau* mutant (*tau* +/–), and homozygote *tau* mutant (*tau* –/–) hamsters (F_2 generation) were all obtained from distinct crossings between 80 heterozygous parents (40 males, 40 females; F_1 generation). After weaning (at about 30 days of age), all hamsters were injected with identification transponders (ID100, Trovan, Eid Aaltenbu, Aalten, The Netherlands; dimensions: 2.1 × 11.5 mm). The animals were then transferred to wheel running recording cages in a constantly dimly illuminated room (red light <0.5 lux). Here, the phenotype of each individual was determined by monitoring locomotor activity for at least 1 week. The circadian period was determined by chi-square periodogram analysis of the activity record (Sokolove and Bushel, 1978).

Altogether, 40 litters of the F_2 generation were screened, which yielded 94 *tau* +/+ hamsters, 186 *tau* +/– hamsters, and 67 *tau* –/– hamsters of both sexes. This frequency distribution of the three genotypes significantly differed from the expected 1:2:1 ratio (χ^2 = 6.3, p = 0.04). This is caused by a slightly smaller number of *tau* –/– hamsters than expected. Sixty homozygote mutants, 80 randomly selected heterozygote mutants, and 77 wild types were used in the study. The average circadian period for the *tau* +/+ hamsters used in the experiment was 23.9 ± 0.3 h, 22.1 ± 0.3 h for *tau* +/– hamsters, and 20.2 ± 0.4 h for *tau* –/– hamsters.

After phenotype screening, the experimental animals were transferred to permanent living cages (l × w × h = 45 × 25 × 14 cm) in a temperature-controlled experimental room (25 × 0.5 °C). The room was continuously illuminated by dim red light with intensities varying from 0.1 to 0.6 lux throughout the entire duration of the experiment. The warm ambient temperature was chosen to prevent the occurrence of seasonal hypothermia (see Oklejewicz, Daan, et al., 2001). Hamsters were group housed, with 4 individuals of the same sex and genotype per cage. Food and water were available ad libitum, replenished at weekly intervals. All cages were inspected three times a week for the occurrence of spontaneous deaths. There was no indication of pathogenic causes of dead animals and no indication of fighting wounds due to group housing. All animals were weighed once per week until the age of 20 weeks, then every 1 or 2 months for the rest of their lives.

All data are presented as mean ± SE unless mentioned otherwise. Differences in survival between groups were tested using the Cox regression method with Wald statistic (W). The overall and pairwise comparison in mortality between genotypes was analyzed by the Wilcoxon-Gehan test (SPSS for Windows, Life

Table 1. Characteristics of life span (in weeks) in male and female hamsters.

Sex	*Genotype*	n	*Mean Life Span*	*Age at 50% Survival*	*Maximum Life Span*
Male	*tau* +/+	38	93.5 (4.7)	99	123.4 (2.0)
	tau +/–	40	91.0 (4.2)	99	119.9 (2.9)
	tau –/–	40	106.1 (3.7)	107	133.0 (1.9)
Female	*tau* +/+	39	75.5 (3.6)	79	102.0 (3.9)
	tau +/–	40	85.4 (3.1)	89	106.1 (1.8)
	tau –/–	20	87.8 (4.1)	88	110.2 (6.7)

NOTE: Standard errors are in parentheses. *tau* +/+ = wild-type, *tau* +/– = heterozygous mutant, *tau* –/– = homozygous mutant. Maximum life span = mean age of 20% longest survived hamsters.

Tables). The Pearson product-moment correlation coefficient was computed to test the linear association between body weight and life span. All tests were two-tailed, with significance accepted at $p < 0.05$.

RESULTS

The age-specific survival curves for male and female hamsters of the three genotypes starting at weaning are presented in Figure 1. Female hamsters of all genotypes lived on average shorter life spans than males. The mean life span pooled over genotypes ranged from 28.9 to 129.9 weeks (mean = 82.0 ± 2.1; n = 99) for females and from 23.0 to 144.6 weeks (mean = 96.9 ± 2.5 weeks; n = 118) for males. We tested the effects of sex, genotype, and the interaction between genotype and sex on the rate of survival in a combined model using the Cox regression method. The sex difference, with male hamsters outliving females, was highly significant (W = 37.5, df = 1, $p < 0.0001$). Genotype contributed significantly to the explained variance in life span (W = 8.2, df = 2, p = 0.02) (Table 1). There was no significant interaction between sex and genotype (W = 3.2, df = 2, p = 0.2).

We further tested whether the difference in life span between genotypes was restricted to early or late mortality. Therefore, the data were divided into two groups to establish survival during and after the first year (Table 2). This is an arbitrary partitioning of the data. The data do not allow partitioning in more than two time windows. The partition is chosen to clearly precede the senescent decline in survival (Fig. 1) while still allowing as much time as possible to render it statistically possible to discern differences in presenescent mortality rates. For males, significant genotypic variation in survival rate was restricted to adult (>1 year) age. Overall, *tau* –/— hamsters had

Table 2. Statistical analysis of survival rates in male and female hamsters of the three genotypes divided into three groups: the overall surviving time, before the first year and after the first year.

	Overall		*Before 1 Year*		*After 1 Year*	
	U	p	U	p	U	p
All males	8.46	0.01*	1.88	0.39	7.21	0.03*
Males pairwise						
tau +/+ vs. *tau* +/–	0.43	0.51	0.18	0.67	0.99	0.32
tau +/+ vs. *tau* –/–	4.06	0.04*	1.93	0.16	2.49	0.11
tau +/– vs. *tau* –/–	8.06	0.004*	0.99	0.32	7.31	0.01*
All females	7.04	0.03*	4.28	0.12	3.52	0.17
Females pairwise						
tau +/+ vs. *tau* +/–	5.59	0.02*	3.10	0.08	2.81	0.09
tau +/+ vs. *tau* –/–	4.27	0.04*	1.96	0.16	2.25	0.13
tau +/– vs. *tau* –/–	0.01	0.91	0.01	0.97	0.01	0.91

NOTE: Wilcoxon-Gehan test: U = test statistic, p = chance probability of larger U. *tau* +/+ = wild-type, *tau* +/– = heterozygous mutant, *tau* –/– = homozygous mutant.

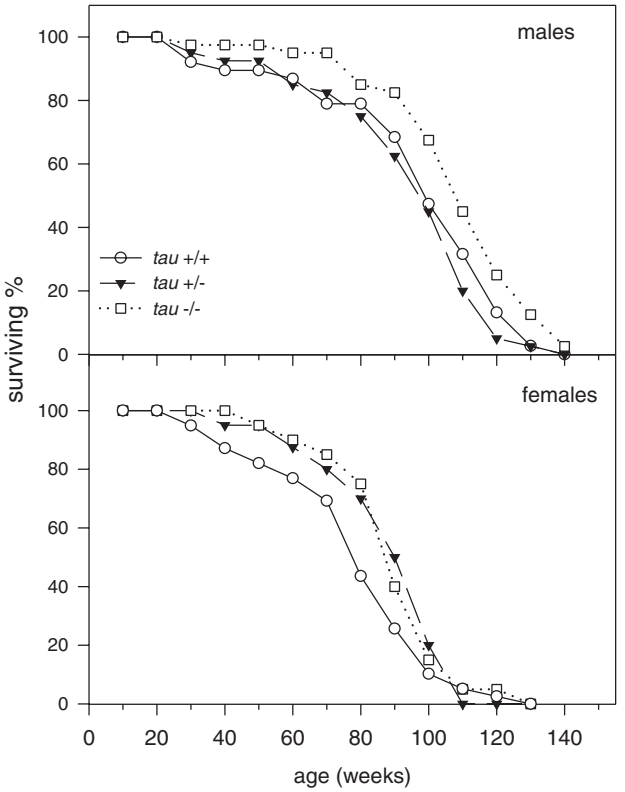

Figure 1. Age-specific survival curves for male and female Syrian hamsters of the three genotypes: *tau* +/+ (wild-type), *tau* +/– (heterozygous mutant), and *tau* –/– (homozygous mutant).

significantly lower mortality than both *tau* +/+ and *tau* +/– hamsters (Table 2). Within females, there was no significant genotypic variation in mortality for the two groups separately. Overall, *tau* +/+ females had

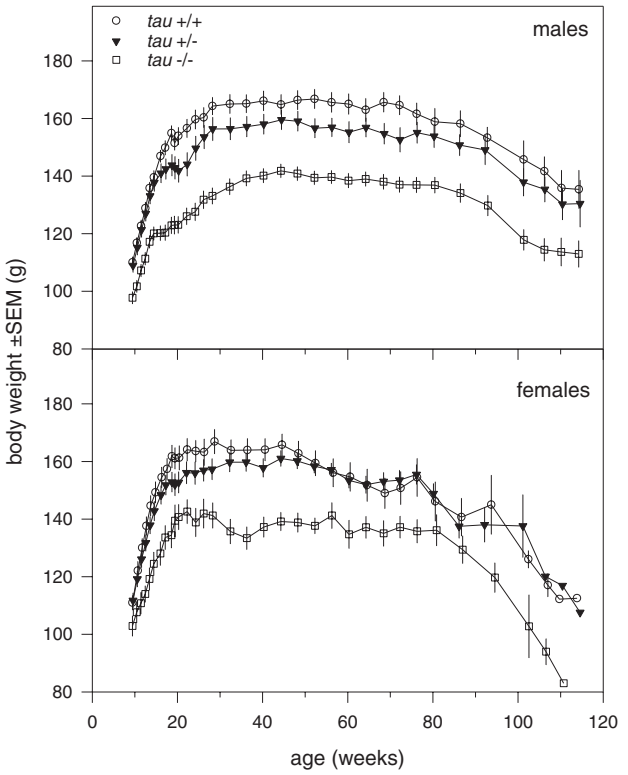

Figure 2. Body weight as a function of age for male and female Syrian hamsters: *tau* +/+ (wild-type), *tau* +/– (heterozygous mutant), and *tau* –/– (homozygous mutant).

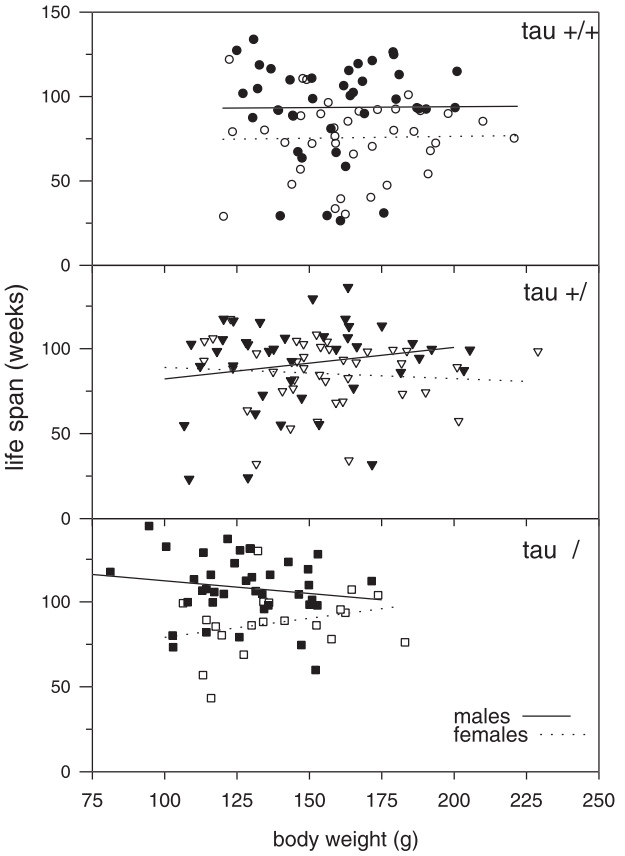

Figure 3. Relationship of individual adult body weight (at 25 weeks) and life span in male (closed symbols) and female (open symbols) Syrian hamsters.

significantly higher mortality than both heterozygote and homozygote mutants (Table 2).

Mean body weight throughout the life span for male and female hamsters is presented in Figure 2. Both male and female hamsters from the three genotypes showed pronounced differences in body weight, confirming previous reports for males only (Oklejewicz et al., 1997; Lucas et al., 2000). Homozygote *tau* mutants developed a lower adult body weight compared to wild-type hamsters. Heterozygote *tau* mutants were intermediate. Among the three genotype males, these variations in adult body weight (taken at about 25 weeks) were significantly different from random. Among females, *tau* +/– hamsters were not significantly different from the *tau* +/+ genotype but homozygotes weighed significantly less. The decline in body weight at the end of life occurred at an earlier age in females compared to males (Fig. 2). This reflects difference in the life span between the sexes.

Because homozygote *tau* mutant hamsters of both sexes weighed consistently less and lived consistently longer, we also analyzed whether there is an interindividual association between these variables within each genotype. The relationship between adult body weight and life span is shown for each sex and genotype in Figure 3. There was no significant correlation in any these relationships ($p > 0.1$). For tau +/– females and tau –/– males, a slight nonsignificant negative correlation between body weight at 25 weeks and life span was observed (Fig. 3).

DISCUSSION

This study confirmed the sex difference in survival rate in the Syrian hamster previously reported by Kirkman and Yau (1972) and Stoll et al. (1997), as well as genotypic differences in body weight (Lucas et al., 2000; Oklejewicz et al., 1997) that persisted throughout their lives. Male and female homozygote *tau* mutant hamsters—whose circadian period is reduced

by 17%—lived on average 15% longer than wild-types. Thus, the hypothesis that life span is proportional to the circadian cycle length is not supported by the results.

The experimental population contained a smaller number of homozygous mutant hamsters than expected from the mendelian ratio of 1:4. It is possible that the weakest homozygous *tau* individuals were eliminated during the preweaning period and that longevity of more viable animals was recorded in the homozygotes. This might be a consequence of selective embryonal mortality or afterbirth infanticide, a common behavior in the Syrian hamster (Day and Galef, 1977; Schneider and Wade, 1991). These alternatives could not be tested in the present study. However, the overall proportion of homozygous *tau* mutants bred from heterozygote crossings in our breeding population showed no significant difference from the expected ratio of 1:4. This suggests a lack of preweaning selection against weaker individuals. Thus, there is a possibility that in our experimental population, the number of homozygous mutant hamsters was underrepresented by chance.

Besides its primary effect on the circadian period, the *tau* mutation influences the timing of other biological processes. In contrast to the circadian effect, the *tau* mutation appears to increase the cycle length of ultradian LH release (time scale of approximately 30 min) (Loudon et al., 1994) in a proportion similar to the increase in life span observed here. In the ultradian food intake cycle, a decrease has been found that is similar to the decrease in the daily activity cycle (Oklejewicz, Overkamp, et al., 2001). Effects of *tau* were found neither in the infradian estrous frequency (Refinetti and Menaker, 1992) nor in the timing of torpor-arousal cycles (Oklejewicz, Daan, et al., 2001). Clearly, the impact of the *tau* mutation on the timing of other processes is not uniform. For some processes, the effect is negative, for some, the effect is positive, and for others, there is no effect. Especially rhythms in the infradian time scale seem not to be affected by shortening of the daily cycle, or inversely, as in the case of life span.

In a previous study on the longevity of the three genotypes (Hurd and Ralph, 1998), heterozygotes died sooner than the other two genotypes. This is clearly opposite to our results. Our study was performed in constant dim light conditions, whereas Hurd and Ralph kept their hamsters in LD 14:10. One might tentatively attribute the differences between the studies to potential negative effects of repeated phase shifts on life span, as reported for flies by Aschoff et al. (1971). Heterozygote *tau* hamsters undergo large phase shifts of the circadian system day after day (Ralph and Menaker, 1988). Although these shifts recur on a daily basis and not once per week as in Aschoff's study, these repeated phase shifts might have been instrumental in causing the decrease in life span observed in the heterozygotes. The circadian system of wild-type hamsters requires much smaller daily phase shifts, whereas homozygote mutants do not entrain to a 24 h LD cycle (Ralph and Menaker, 1988). Thus, the difference in the survival between the three genotypes in the Hurd and Ralph study may reflect the environmental conditions on top of a *tau* mutation effect. The average life span differed substantially between the two studies. In our study in constant darkness, the mean survival of wild-type hamsters was circa 17 weeks longer than in the Hurd and Ralph study in LD. For heterozygote and homozygote *tau* mutants, this difference was about 38 weeks. Although this might suggest higher survival in constant darkness compared to LD for the three genotypes, other differences between the studies may have also contributed. Another difference between the studies is that we kept the hamsters with 4 per cage rather than 1. This may of course have led inadvertently to behavioral antagonistic interactions eventually precipitating death. Although such effects cannot be excluded, the fact that the hamsters lived longer rather than shorter in our study than in that of Hurd and Ralph and that no fighting wounds were ever observed argue against such reasoning.

Among species of eutherian mammals, longevity increases with size with the same slope (exponent about 0.3) as the decline in mass-specific metabolic rate (Daan and Aschoff, 1982; Stahl, 1962). Although there is experimental evidence for negative effects of energy turnover on life span, little is known about the spontaneous intraspecific association between these variables. In a recent study on house mice, Speakman et al. (2001) observed a positive association between individual daily energy expenditure and natural survival. We have previously reported that the mass-specific metabolic rate in homozygote *tau* mutant hamsters is higher than in wild types and intermediate in heterozygotes (Oklejewicz et al., 1997; Oklejewicz et al., 2000). Thus, the intraspecific relationship between energy expenditure and longevity in hamsters is similar to that observed in mice (Speakman et al., 2001). Oxidative stress has been postulated as the major causal factor of senescence limit-

ing life span (e.g., Sohal and Weindruch, 1996). Higher mass-specific metabolic rates clearly would elevate oxidative stress. However, animals with increased energy turnover may also have more energy to invest in antioxidant defenses, so that the outcome in interindividual comparison may deviate from expectations built on experimental data.

The *tau* mutation was the first clock mutation discovered in mammals and so far remains the most extensively studied with respect to timing of behavioral and physiological processes. As usual in the case of single gene mutation with pleiotropic effects, it is difficult to rule out the possibility of genetic linkage with other processes. By consistently deriving our animals from crosses between heterozygous parents, and thus comparing wild-type and heterozygote and homozygote siblings, we maximized homogeneity of the genetic background. Even this strategy does not fully rule out systematic differences between them in genes other than *tau*. With this caveat, it appears that the properties of casein kinase I epsilon (Lowrey et al., 2000), the product of the *tau* gene, unexpectedly show a positive effect on life span in animals with both alleles mutated.

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