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The Significance of Circadian Organization for Longevity in the Golden Hamster

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Abstract While functional roles for biological clocks have been demonstrated in organisms throughout phylogeny, the adaptive advantages of circadian organization per se are largely matters of conjecture. It is generally accepted, though without direct experimental evidence, that organisms derive primary benefits from the temporal organization of their physiology and behavior, as well as from the anticipation of daily changes in their environment and their own fluctuating physiological requirements. However, the consequences of circadian dysfunction that might demonstrate a primary adaptive advantage and explain the natural origins and apparent ubiquity of circadian systems have not been documented. The authors report that longevity in hamsters is decreased with a noninvasive disruption of rhythmicity and is increased in older animals given suprachiasmatic implants that restore higher amplitude rhythms. The results substantiate the importance of the temporal organization of physiology and behavior provided by the circadian clock to the health and longevity of an organism.

Key words biological clock, suprachiasmatic, life span, transplantation, neural graft, locomotor activity

INTRODUCTION

Circadian clocks regulate the temporal organization of daily physiological and behavioral events and are critical for appropriate seasonal responses, reproduction, migration, and orientation. When their apparent ubiquity across phylogeny is considered, it is easy to conclude that clocks endow organisms with major adaptive advantages, the most basic or primitive of which would be realized through the temporal organization of physiology and behavior and the ability to anticipate regular events or changes that are relevant to the organism (Pittendrigh, 1960, 1993; Daan, 1981). However, while arguments based on ecological strategies are compelling, the direct experimental evidence that supports this contention is sparse (Daan, 1981; DeCoursey et al., 1997; DeCoursey and Krulas, 1998). Life expectancy ultimately may be reduced for some organisms if they are raised in light-dark cycles with periods that differ substantially from that of their own circadian rhythm (Went, 1959, 1960; Ketellapper, 1960; Aschoff et al., 1971; Pittendrigh and Minis, 1972) or if photoperiods are substantially disturbed (Halberg and Cadotte, 1975; Perret, 1997). Most of this work has never been replicated. In addition, altered circadian timekeeping accompanies various mental and physical disorders (Kripke et al., 1978; Weitzmann et al., 1981; Ehlers et al., 1988; Lewy et al.,

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1988; Healy and Waterhouse, 1990) and deficits associated with time zone changes (Monk and Aplin, 1980; Minors and Waterhouse, 1987), shift work (Eastman et al., 1995), and aging (Pittendrigh and Daan, 1974; for reviews, see van Gool and Mirmiran, 1986; Brock, 1991; Turek et al., 1995). As yet, research has provided little insight into the issues of causality or the mechanism underlying these associations. Rhythm disorganization may be a reflection of aging or pathology rather than a contributing factor.

To test the hypothesis that circadian organization has a primary influence on health and longevity in mammals, we used hamsters carrying the period mutation, *tau* (Ralph and Menaker, 1988), to examine first whether a specific environmentally induced rhythm disruption in these animals (see Osiel et al., 1998) reduces longevity. Then we tested whether the reverse were also true—that an increase in longevity follows the restoration of more youthful patterns of behavior (Hurd, 1996) and physiology (Cai and Wise, 1996; Cai et al., 1997a, 1997b) produced by grafts of the fetal SCN into aged adult hosts.

METHODS

Locomotor Activity Recording and Analysis

Animals were housed in translucent, polypropylene cages with food and water freely available and with free access to a running wheel mounted in the cage. Activity was monitored continuously as wheel running using a Dataquest III data acquisition system (Minimitter Co., Inc., Sunriver, Oregon). Wheel turns were registered as closures of a microswitch once per wheel revolution. These were accumulated into 6-min bins for analysis using a program written for this purpose (Ezpaste, NSF Center for Biological Timing, University of Virginia).

Animals

Hamsters (*Mesocricetus auratus*; *tau*^{s}) have been maintained in our breeding colony in an LD 14:10 light cycle since 1991. The colony is outbred every second generation with wild-type (+/+) females obtained from Charles River Laboratories, Quebec. Routinely, these females are crossed with *tau*^{s}/*tau*^{s} males to produce *tau*^{s}/+ (F_1), which are intercrossed to produce F_2 experimental animals. Some F_2 *tau*$_s$/*tau*$_s$ males are retained as replacement breeders. Examination of 3 years of breeding records (1991-1994) indicated that *tau*^{s}/+ animals were about 20% shorter lived than either homozygous group.

Hamster Longevity

Three F_2 litters were produced from *tau*^{s}/+ × *tau*^{s}/+ crosses. At weaning, offspring were moved to individual cages. They were maintained in colony conditions without further experimental disturbance until their demise. Technicians who were blind to the experimental conditions provided animal care and were instructed to remove animals that had died or when death was imminent. Postmortem examination indicated that animals died from pervasive organ dysfunction associated with age. There were no indications of pathogenic causes.

Selection of Aged Hosts and Suprachiasmatic Transplantation

Hamsters that were to serve as transplant hosts were raised in the breeding colony (LD 14:10) to 9 months of age before beginning a process of selection for those with age-dependent fragmentation of rhythmic locomotor behavior (cf. Fig. 2a-d, first 2 weeks of each record). Animals whose total activity per circadian cycle (tested in constant dark for > 14 days) had dropped below 10% of the young adult levels were selected as potential transplant recipients. Using 17 cm diameter wheels, hamsters typically will run about 9000 wheel turns per night when first introduced to the wheel. In our hands, this drops to a steady state of 3000 to 5000 wheel turns after about 6 weeks and is maintained for many months thereafter. Between about 9 and 15 months, activity may become fragmented and less predictable. Animals are considered elderly when the amount number of turns has dropped below 10% of the young adult value (i.e., about 500 to 1000 turns/cycle).

Those meeting the established behavioral criteria were assigned at random to 1 of 4 groups: SCN graft, control graft, sham operated, and unoperated. All *tau* genotypes were included in the host pool so that direction of the transplant might be randomized, and all were represented in each experimental group. Tissues for grafting were always of a different genotype from the host (either +/+ or *tau*^{s}/+), so that changes in host-driven periodicity or the expression of a new graft-driven rhythm (if any) could be distinguished. As a routine, this type of experiment is performed

using hosts and tissue donors of different *tau* genotypes. This allows different behavioral effects of the grafts to be distinguished in the subsequent locomotor records. These effects include the following: (1) direct expression of graft-derived rhythmicity, (2) relative coordination between graft- and host-derived rhythms, and (3) modulation of host amplitude (Hurd et al., 1995; Hurd, 1996). Each effect involves potentially a distinct mechanism and potentially 3 different ways in which longevity could be altered following SCN grafting. In particular, the appearance of a graft-driven periodicity has the effect of disrupting the expression of host periodicity. Because we were interested specifically in the reconsolidation of the host rhythm, and because an effect on host rhythm amplitude is difficult to separate from the other overt effects of SCN grafts, only those records with no overt graft-driven periodicity were included in the analysis.

Continuous activity records were maintained for all animals until their demise or until an animal care technician concluded, using independent judgment, that death was imminent. Animal care technicians were blind to the experimental conditions but were asked to remove animals when they appeared sick or dying. In addition, particular attention was paid by the technicians to animals that had become arrhythmic according to activity records. The data presented here show that once animals exhibit the behavioral criteria for selection, their remaining life expectancy is less than 50 days. Post hoc examination of running records for animals that were found dead suggests that arrhythmic behavior was a predictor of the animal's imminent death. A definition of the onset of arrhythmia needs to be established. Viability of a graft was assessed as described previously (Hurd et al., 1995) by detecting the presence of neuropeptides within the graft during subsequent immunocytochemical analysis. Because animals had been selected based on the lack of overt rhythm expression in their posttransplant locomotor records, the behavioral indication of graft viability was missing. In this experiment, the detection of AVP or VIP immunoreactive cells demonstrated only the presence of a live graft and not necessarily the presence of pacemaker cells.

Statistics

For both experiments, differences among group means were tested for significance using ANOVA, followed by Scheffé's post hoc test for differences between individual groups. Changes in activity levels were examined using the Wilcoxon test. Survival times (cumulative survival probabilities) were estimated by the product limit method (Kaplan and Meier, 1958) and tested for significance using the Cox method (SPSS for Windows, SPSS Inc. Chicago, Illinois).

RESULTS

Experiment 1: Environment-Induced Rhythm Disruption and Longevity

In previous studies, we have found that heterozygous (tau^s/+) hamsters are able to synchronize with the 24-h LD cycle but have early onsets of nocturnal behavior and highly fragmented patterns compared with the wild type (Osiel et al., 1998). Examples are shown in Fig. 1a-d. Homozygous tau^s/tau^s mutants (free-running period = ca. 20 h) do not entrain to this light cycle in our hands. Ostensibly this is due to the larger (4-h) difference between the period of the tau^s/tau^s rhythm (20 h) and the entraining cycle (24 h), such that the daily adjustment of the endogenous cycle is difficult to maintain. Under these conditions, the periods of the animals' rhythms may be influenced but not entrained, and the pattern of activity of each circadian cycle remains consolidated and similar to the constant dark condition (Osiel et al., 1998).

In the LD 14:10 light cycle, the life span of tau^s/+ mutants was significantly shortened by almost 7 months ($p < .05$ vs. +/+; $p < .07$ vs. tau^s/tau^s). However, the average life span for tau^s/tau^s animals was not different from +/+ (Fig. 1e). The rate of demise was similar for each of the groups (between 8% and 9% per month, following the first death in each group), indicating that the differences among the means were not produced by the inclusion of 1 or 2 substantially long- or short-lived animals (Fig. 1f). The experimental results reflected our breeding colony records, which had indicated a reduction of > 20% in the life span of tau^s/+ animals in LD 14:10 conditions compared with the other 2 genotypes, regardless of reproductive history or genetic background.

In a related study, animals raised in colony conditions but in constant dim light (LL, 20-40 lux), had shown no differences in longevity across genotypes. A small test colony had been established for the main purpose of testing lighting conditions that would produce the greatest overall fecundity for hamsters where all 3 *tau* genotypes were included as breeders. The average life span for groups maintained in LL from 10

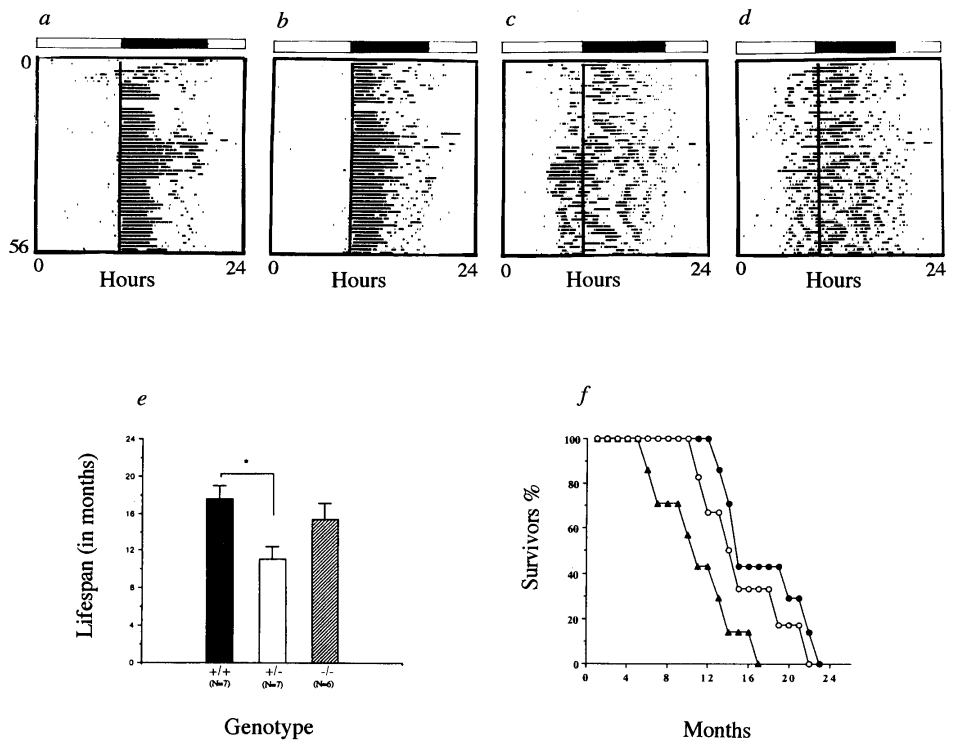

Figure 1. Rhythm disruption and longevity in wild-type and period mutant hamsters. (a-d) Locomotor activity in LD 14:10. Representative actograms from animals of 2 *tau* genotypes: (a, b) wild type, (+/+); (c, d) heterozygous, (*tau*^{s}/+). Light-dark cycle is indicated by the horizontal bar above each record. Vertical lines indicate the beginning of the 10-h dark period each day. (e) Mean life spans for each genotype. * = $p < .05$. Difference between *tau*^{s}/+ and *tau*^{s}/*tau*^{s} was not significant at $p = .08$. (f) Survival plots for each genotype. Mean survival times: +/+ (●)= 17.5 months, *tau*^{s}/+ (○) = 10.9 months, *tau*^{s}/*tau*^{s} (▲) = 15.8 months.

weeks of age were the following: +/+; 17 ± 2 months; (n = 12), *tau*^{s}/+; 18 ± 2 months; (n = 12), *tau*^{s}/*tau*^{s}; 15 ± 4 months; (n = 6). These LL data should be viewed with caution as there was no consideration for relatedness within groups. Five litters (F_2) are represented in the population, but only 3 contributed homozygous mutants.

Experiment 2: Restoration of Rhythm Amplitude and Activity Levels by SCN Grafts

To address the issue of causality, we examined the effects of fetal SCN grafts on the longevity and circadian organization of adult hamsters whose locomotor rhythms had become fragmented naturally during the process of aging. Control animals that were given grafts of cortical tissue or that were sham operated or unoperated continued to perform low levels of activity following the manipulation (Fig. 2a,b,e). In contrast, the presence of an SCN graft was associated with a significant increase in the total amount of locomotor activity (Fig. 2c,d,e). This occurred in the absence of either overt donor-driven periodicity or changes in the host's circadian period. Of the 11 animals with viable SCN grafts according to subsequent histological examination, 4 were wild type (*tau*^{s}/+ grafts), 6 were heterozygous, and 1 was homozygous mutant (+/+ grafts). Of the 11 with viable cortex grafts, the numbers were 4, 5, and 2. The intact control group comprised 4 of each genotype.

Animals with viable SCN grafts survived significantly longer following transplantation than either operated or unoperated controls, with a mean increase in longevity of more than 4 months (Fig. 2f). Furthermore, the rate of demise was reduced to less than 10% per month for the SCN recipient group compared with the rates (20% to 30% per month) for all control groups as well as for SCN recipients for whom viable grafts could not be verified (Fig. 2f). The increased survival time and reduced rate of demise show that, on average, SCN graft recipients lived more than twice as long as predicted from their pretransplant behavioral state. Moreover, in addition to becoming more vigorous

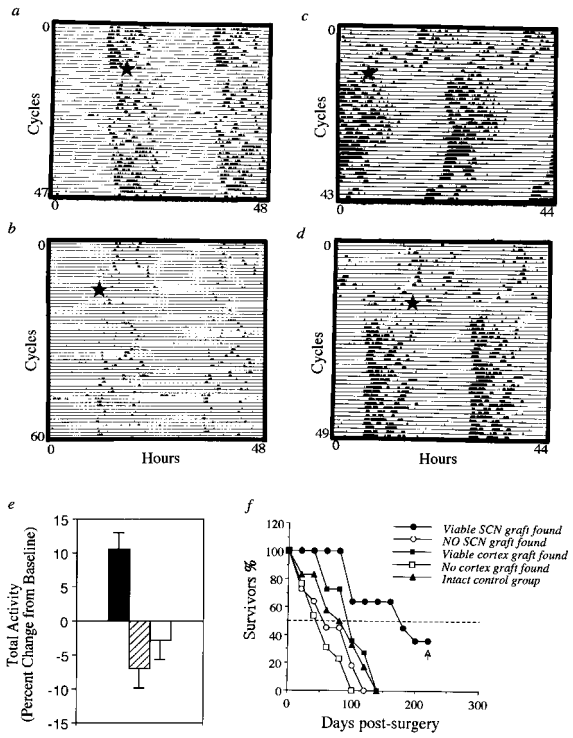

Figure 2. Effects of suprachiasmatic transplantation on activity and longevity. (a-d) Actograms from adult hosts given control implants (a, b) or SCN implants (c, d). Day and time of transplant surgery are indicated by the stars on each record. (e) Percent change in wheel-running activity (wheel turns per circadian cycle) following transplantation into adult hosts. Histograms represent SCN-grafted (solid bar), cortex-grafted (hatched bar), and sham-operated (open bar) groups. Changes in activity following SCN transplantation were significant ($p < .01$; Wilcoxon test). (f) Survival plots for animals given SCN grafts (circles) or cortical grafts (squares). An unperturbed control group selected using the same criteria as operated groups is also included (triangles). Data have been plotted as percent survival at 20-day intervals following surgery; 50% survival probability is indicated by the horizontal line. Mean survival time is significantly increased for SCN-grafted animals only ($p < .01$, ANOVA) over all other groups. The curve for SCN graft recipients differs significantly from the group survival and the intact control curves at $p = .005$ and $p = .0001$, respectively (Cox regression). Kaplan-Meier analysis placed the mean survival time for the SCN graft recipients at 164 days (95% confidence interval [CI] = 125-202 days), cortex graft recipients at 95 days (95% CI = 77-113 days), and the intact controls at 77 days (95% CI = 54-100 days). The experiment was terminated for unrelated reasons at the point indicated by the upward arrow.

behaviorally, animal care observations suggest that the general appearance and food consumption of the animals improved following SCN grafting. While this needs to be verified experimentally, it does suggest that health, as well as longevity, is improved in aged animals with SCN grafts.

DISCUSSION

Taken together, the results of the 2 experiments indicate that circadian rhythm disruption is not merely associated with aging but that rhythm integrity is a major contributing factor in the determination of longevity. *Tau*^{s}/+ mutants, whose behavioral rhythms are fragmented in LD 14:10, relative to +/+ and *tau*^{s}/*tau*^{s}, were found to have reduced life spans compared to both their wild-type and homozygous *tau* mutant siblings; fetal brain grafts containing the SCN both reversed the natural decline of behavioral rhythmicity associated with age and extended the expected longevity of adult hamsters by almost 20%. In our animals, the decline in rhythm amplitude and total amount of activity was rapid, and these occurred together—although only the amount of running was quantified. It is important to recognize that both the rate of demise and the mean life expectancy following this behavioral change were essentially the same for all the control groups—including the unoperated controls. This allows us to be reasonably confident that the activity criterion used here was sufficient to predict remaining life expectancy to within a few weeks. The

effectiveness of the SCN grafting is best illustrated by the fact that all 11 SCN recipients with restored rhythms outlived 50% of all controls, and 4 of these recipients were still alive and healthy 3 months after the last control animal had died.

The mechanisms that are affected by the SCN grafts that could be responsible for the changes in longevity are likely to be numerous. The immediate target is most likely the host SCN since in previous studies, we have found that the period of the host rhythm may be modulated by the graft (Hurd et al., 1995), and in this study, the rhythmic output of the host nucleus is the most obvious observed change. It is still possible that the graft influences the host SCN through a third site, although hypothesizing this level of complexity seems unwarranted at this time. Because the transplants are made into intact animals, the neural connections of the host SCN remain intact. Any change in the amplitude of the host rhythm then would be reflected in a similar change in the signals to target organs and tissues. Neuroendocrine rhythms that are lost during aging may recover following SCN grafting, whereas they are not restored to hosts with SCN lesions.

An attractive but unsupported notion is that the clock speed is directly related somehow to the life span of an organism—that life span and circadian period are mechanistically related in such a way that longevity reflects a set number of ticks of the clock. Hence, by speeding up the clock (e.g., the *tau* mutation), life span would be reduced proportionally. Our data do not support such a notion, as the longevity of intact animals with period mutations appears to depend on the environment in which they are housed.

However, the data do indicate that the organization of physiology and behavior provided by the clock is a significant factor. When interactions between clock and environment produced disrupted rhythms and abnormal entrainment, longevity was reduced. Conversely, when the disorganization of behavior that accompanied aging was ameliorated, longevity was increased. Generalizing from these results, therefore, it may be concluded that any situations that produce rhythm disruption are potentially deleterious to both health and longevity, and improved health and increased longevity may be obtained with manipulations that promote daily or circadian organization.

An immediate question that is raised from this is whether longevity would be affected by complete SCN lesions. This issue is being approached by DeCoursey and Krulas (1998), who have reported a reduction in the long-term survival of SCN-lesioned chipmunks released into a natural environment. Survival in this case was related to differential predation rather than to changes in longevity, although the latter cannot be discounted. Unfortunately, it is often difficult to reach firm conclusions regarding fitness from single experiments such as these. Damage to a system that has co-evolved with others to work optimally as a whole (in this case, the SCN and its effector mechanisms) may produce disruptions that are more profoundly devastating than the lack of the system per se. Likewise, our first experiment is encumbered with the same argument. While the most likely explanation for reduced longevity of *tau* heterozygotes is rhythm disruption, it is still possible that the unnatural phase relationship exhibited by these animals in LD 14:10 compromises the animals' physiology in ways that are unrelated to the clock's adaptive value. Though the lack of a circadian clock might be disadvantageous, it may be worse to have a clock that is wrong.

Our conclusion regarding the importance of rhythm integrity, therefore, is based on the combined results of both experiments, but its strength is derived from the second. Whether the effect of the graft is to restore the integrity of the host clock or to improve responsiveness to its output remains to be seen. In either case, the strength lies in the fact that a rejuvenation of circadian rhythmicity is accompanied by an increase in life expectancy.

Why longevity is influenced by circadian organization is not certain. It is possible that a metabolic cost associated with maintaining circadian organization for the whole organism is increased when entrainment is compromised. More likely, perhaps, is the possibility that health and longevity are compromised by desynchronization of rhythmic physiological and behavioral processes whose temporal relationships have been optimized over evolutionary time. In either case, the potential implications for humans engaging in shift work, or for those who are confined to other situations where rhythms may be disrupted, are readily apparent. Humans, like other organisms, are rhythmic in nature, and the contribution of rhythm maintenance to overall health, longevity, and energy—especially in the elderly—should not be undervalued.

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