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REVIEW ARTICLE

The Ticking Clock of Cayo Santiago Macaques and Its Implications for Understanding Human Circadian Rhythm Disorders

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The circadian clock disorders in humans remain poorly understood. However, their impact on the development and progression of major human conditions, from cancer to insomnia, metabolic or mental illness becomes increasingly apparent. Addressing human circadian disorders in animal models is, in part, complicated by inverse temporal relationship between the core clock and specific physiological or behavioral processes in diurnal and nocturnal animals. Major advantages of a macaque model for translational circadian research, as a diurnal vertebrate phylogenetically close to humans, are further emphasized by the discovery of the first familial circadian disorder in non-human primates among the rhesus monkeys originating from Cayo Santiago. The remarkable similarity of their pathological phenotypes to human Delayed Sleep Phase Disorder (DSPD), high penetrance of the disorder within one branch of the colony and the large number of animals available provide outstanding opportunities for studying the mechanisms of circadian disorders, their impact on other pathological conditions, and for the development of novel and effective treatment strategies. Am. J. Primatol. 78:117–126, 2016.

Key words: DSPD; desynchrony; rhesus monkey; genetic; primate; night eating

INTRODUCTION

Circadian Rhythms and Their Disorders

Sunlight is arguably one of the most important factors defining our existence, with photosynthesis responsible for virtually all the organic matter on the planet, as well as our oxygen rich atmosphere. The periodicity of the Earth's day-night cycle, dictated by the planet's rotation, necessarily influences essentially all life on Earth. Over hundreds of millions of years of evolution, different organisms have adapted in different ways to Earth's environment, including a diversity of adaptations for coping with the daily cycle of sunlight. Among animals, diurnal species are tuned to concentrate their activity during daytime, while nocturnal species are specialized for nighttime activity. Other species sidestep this dichotomy and become crepuscular, limiting their activity to twilight, at both dusk and dawn. However, no matter the daily timetable of activity, effective adaptation of relatively slow biological processes to diurnal environmental cycles (often including temperature and other variables that are correlated with sunlight), requires accurate prediction of when each critical phase change will occur, so that individual animals may anticipate and prepare. This adaptive value of successfully predicting the day-night cycle has necessitated the evolution of an intrinsic biological clock with a period close to 24 hr in length.

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Indeed, almost every living cell, whether existing as a single cell or part of a complex multicellular organism, has an autonomous molecular 24-hour clock. This is called circadian, coming from the Latin *circa* meaning "approximately" and *diem* meaning "day", because its intrinsic period typically deviates somewhat from an exact 24 hr. In mammals, coordination of these cell-autonomous oscillators is achieved through response to signals coming from a "master clock", driven by the paired suprachiasmatic nuclei (SCN) of the hypothalamus in the brain [Moore & Eichler, 1972; Stephan & Zucker, 1972]. Unlike other central or peripheral structures, the SCN can effectively couple oscillations of its individual cells, synchronizing them [Aton et al., 2005; Honma et al., 2000; Mohawk &Takahashi, 2011; Ono et al., 2013; Welsh et al., 2010; Yamaguchi et al., 2003]. The resulting rhythmic signal is then conveyed by the SCN to other cells of the body through neuronal and endocrine pathways, entraining numerous cellular oscillators to one stable intrinsic period (tau or τ) [Dibner et al., 2010]. In humans, τ can range roughly between 23 and 25 hr [Duffy et al., 2011]. In the absence of environmental time cues (mainly light), the SCN and the clock-controlled physiological and behavioral systems "free-run" following an individual's τ. To adjust to the environmental rhythm of sunlight, the SCN processes photic information reaching it via a monosynaptic glutamatergic pathway of the retino-hypothalamic tract, with the melanopsin-containing axons, and entrains its own rhythm and those that depend on the SCN to the natural 24-hour period. Remarkably, even under entrained conditions, intrinsic rhythms can still powerfully affect our behavior and physiology. Individuals with short τ (less than 24 hr) tend to wake up and fall asleep earlier than those who have longer τ, and are sometimes described as "larks" versus "owls," i.e., those with morningness and eveningness chronotypes, respectively [Duffy et al., 2001].

In humans, various elements can go wrong within the circadian clock system. Its molecular mechanisms, involving a network of multiple clock and clock-controlled genes, can suffer mutation and thus disruption of normal functionality. The coupling of oscillators within the master clock, or the ability of the master clock to accurately perceive the environmental light-dark cycle, can be compromised. Processes downstream of the master clock, those that normally carry its signal to other cells of the body, can be altered by an array of pathological processes. Any of these changes can result in some form of a circadian clock disorder, associated with dampened or absent circadian rhythms, shorter or longer τ, altered entrainment to light and/or phase shift between several body rhythms.

The most prevalent of the known human circadian disorders is the Delayed Sleep Phase Disorder (DSPD), which affects people of different ages, but especially adolescents [Ancoli-Israel et al., 2001; Gradisar & Crowley, 2013; Kripke et al., 2008; Weitzman et al., 1981]. Phase delay is also documented as a symptom of several mental disorders [Glozier et al., 2014; Karatsoreos, 2014; Li et al., 2013]. Moreover, recently, investigators have begun to appreciate that circadian alterations are contributing to a wide range of other major human ailments, from insomnias to cancer, metabolic syndrome, neurodegenerative, immune, and cardiovascular disorders [Gery & Koeffler, 2010; Kissling et al., 2008; Lewy, 2009; Maury et al., 2014; Rosenwasser, 2010; Xu et al., 2010].

The development of new treatments or preventive therapies for these circadian conditions requires a deep understanding of the mechanisms of the underlying circadian pathology [Sack et al., 2007]. However, studying circadian disorders in humans is challenging for a number of reasons [Duffy & Wright, 2005]. Compared to the majority of periodic biological processes, the circadian oscillations are very slow, e.g., 86,000 times slower than the heart rate. Their modification by photic and non-photic environmental time cues or the masking of natural rhythms by the acute effects of light, darkness, posture, locomotor activity, or scheduled food intake, means that characterizing human rhythms requires many days of observation under strictly controlled conditions. This favors the use of whole animal models in circadian research and, indeed, studies in Drosophila, mice, rats, and hamsters have produced the principal breakthroughs in our understanding of the clock mechanisms.

However, the dichotomy between nocturnal and diurnal species must be considered as researchers delve more deeply into the molecular mechanisms of circadian function and dysfunction. We suggest that in order to understand the nature of some human circadian disorders, and to investigate their impact on other pathological processes or to search for effective treatment strategies, the translational value of an animal model depends to a significant degree on whether that species is diurnal or nocturnal. In nocturnal species, the activity, sleep, heart rate, blood pressure, hormonal secretion, or metabolic processes are on the inverse schedule relative to diurnal species. The SCN neurons of nocturnal animals are, like those of diurnals, more active during the day and the principal circadian hormone, melatonin, is produced only at night [Challet, 2007; Smale et al., 2003]. However, it remains largely unknown how this similarity in central signal is then translated into the opposite physiological and behavioral outputs in diurnal versus nocturnal species [Sack et al., 2007].

Given that humans are diurnal, our understanding of the role of the circadian system in human health and disease will greatly benefit from, and

must be validated through, a detailed examination of circadian physiology in diurnal mammals [Lowrey & Takahashi, 2004]. Model species that are phylogenetically close to humans provide systems that are critical for determining whether the basic knowledge gained through studies of nocturnal models (e.g., mice and rats) or phylogenetically more distant diurnal vertebrates (e.g., zebrafish) [Zhdanova, 2011] can be effectively translated to understanding complex circadian regulation of body functions in humans, and role of the clock in human disorders. Genetics and physiology count here, and controlling for variation in these systems as much as possible, but employing phylogenetically close species is strongly justified.

The rhesus macaque (*Macaca mulatta*) is arguably one of the best available diurnal laboratory animals for translational research into the organization of the human clock and consequences of its pathological conditions on human health [Haley et al., 2009; Labyak et al., 1997; Lambert & Weaver, 2006; Lemos et al., 2009; Masuda & Zhdanova, 2010; Reppert et al., 1984; Schwartz et al., 2011; Urbanski, 2011; Zhdanova et al., 2002,2011, 2012]. In addition to the fundamental similarity of rhesus macaques to humans in neurobiology, endocrinology and physiology, the genes and overall genomic organization of macaques is much more similar to humans than that of rodents or non-mammals [Phillips et al., 2014; Rogers & Gibbs, 2014].

Discovery of the First Familial Circadian Disorder in Non-Human Primates

Through our studies of the circadian rhythms of sleep, activity and food intake in diurnal primates, and the modifications that occur in those processes with aging, we (I.Z.) built our first Primate Circadian Research Laboratory (PCRL-BU) at Boston University School of Medicine. We developed specialized circadian chambers to prevent masking or entraining effects of environmental time cues, and employed a number of behavioral techniques and custom software programs to study animals obtained from several National Primate Research Centers [Masuda & Zhdanova, 2010; Zhdanova et al., 2011]. For months at a time, we have evaluated the rhythms of sleep and activity of individual macaques using image analysis and polysomnographic techniques under entrained and constant conditions (i.e., in light-dark cycle or constant dim light, correspondingly).

By documenting the timing of each gram of food that an animal acquired through operating a touch-screen connected to an automatic feeder, we also characterized the circadian patterns of food intake and cognitive performance in young and aged monkeys, ranging from 5 to 28 years of age. This allowed us to reveal substantial inter-individual and age-dependent variability in the intrinsic rhythms of rhesus monkeys and the ways those entrain to light or melatonin. However, the diurnal life style, morningness and eveningness chronotypes, the length of the intrinsic period (tau) or circadian phase angle between specific body rhythms in these rhesus monkeys proved to be remarkably similar to normal rhythms documented in humans [Masuda & Zhdanova, 2010, 2011].

This was the state of knowledge until 2007 when we began studies of four young male rhesus monkeys that came to our PCRL-BU from the Caribbean Primate Research Center (CPRC). To our surprise, three of these animals demonstrated major circadian alterations [Zhdanova et al., 2012]. Detailed characterization of their entrained and intrinsic circadian patterns showed that one of the animals lacked circadian rhythms (Fig. 1D), while two had a pronounced Delayed Sleep Phase Disorder (Fig. 1B, C). Moreover, a delay in waking up in the morning and falling asleep in the evening in these animals was associated with nighttime eating, reminiscent of the Night-Eating Syndrome (NES) in humans. This analogy to NES was especially clear in one of the monkeys that displayed dissociation between the onset of activity and substantially later onset of food intake in the morning.

Studying food intake patterns in rhesus monkeys automatically involves evaluating their cognitive performance around the clock. Each circadian chamber is equipped with a touch-screen monitor, connected to an automatic feeder. By touching a moving on-screen target, a monkey can self-administer regular chow in 1 g portions. This food access paradigm was termed “free-food”, documenting the time each food pellet is dispensed. However, whenever a monkey would not touch its monitor for over 30 min, a new icon would appear on the screen announcing that to get back to the “free-food” a monkey would need to complete a cognitive test. The choice routinely involves guessing between the two pictures, one of which has been shown some seconds earlier, serving as an example of what has to be touched when two pictures are presented, the so called Delayed Match to Sample Test (DMST, 20 sec fixed delay). Once a defined number of correct choices is reached, each of which is rewarded with the same 1 g food pellet, a program switches back to the “free food” paradigm. Not every monkey, however, wants to complete the test every time it initiates it. This led to our second rule. If an animal interrupts the DMST for over 5 min, it looses all the “points” acquired and the test is reinitiated. This allows us to test cognitive performance around the clock, reflecting the success rate and reaction times, but also providing a measure of motivation for acquiring food and measure of attention, through monitoring test interruptions.

Our monkeys with the circadian disorder exhibited not only delays in motivation to acquire food

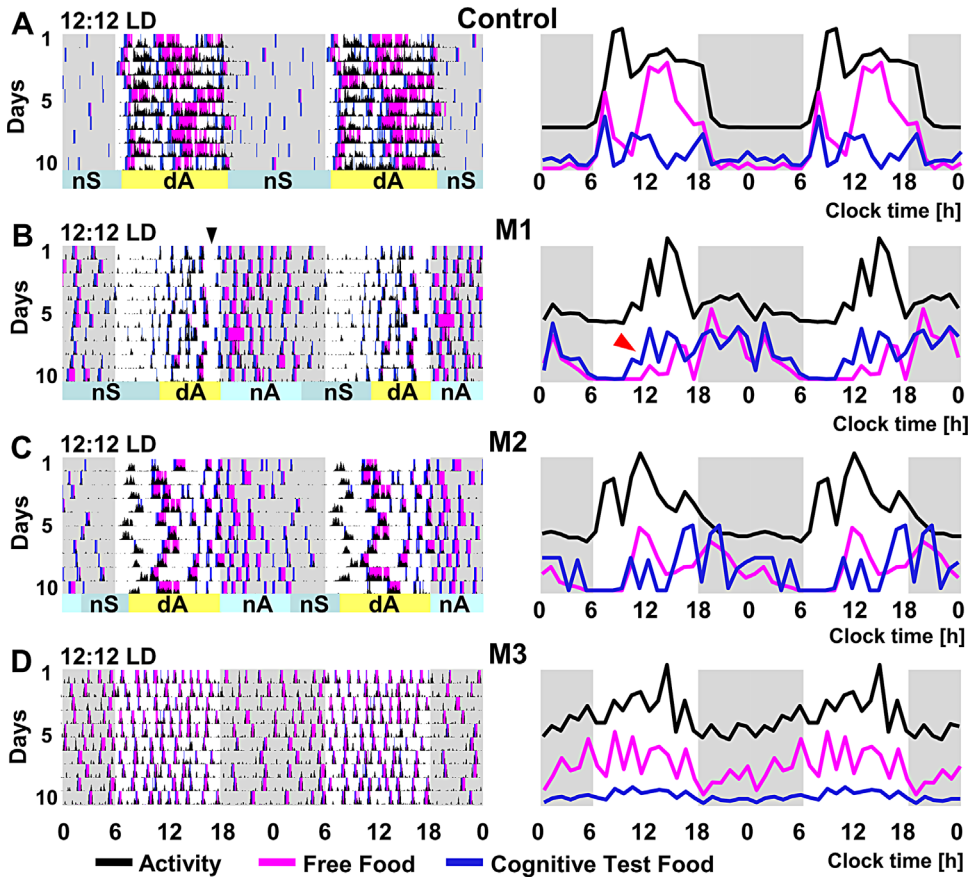

Fig. 1. Delayed sleep phase, altered daytime cognitive performance and increased nighttime eating in group-M in Light-Dark cycle. A–D: Double plots of activity and food intake in 12:12LD, in (A) representative control (animal C1, t = 25.1 in CDL) and (B–D) in group-M monkeys, M1, M2, and M3. Left panels: 10-day activity (black), free-food intake (magenta) and food through cognitive test (blue) recordings. Horizontal bars: sleep (blue) and active (yellow) periods. dA, daytime activity; nA, nighttime activity; nS, nighttime sleep. Black arrow head at the top of B-left panel points out a regular end-of-a-day nap (dS); Right panels: corresponding daily profiles, with Y-axis representing change in mean value per hour for each measure shown. Background: white, light period; gray, dark period: Red arrow (B, right panel), earlier onset of "food through cognitive test" intake, relative to "free-food intake", reflecting high incidence of incomplete cognitive tests during the day. (modified from Zhdanova et al., 2012).

in the morning but displayed lower success rate and lower percentage of completion of initiated tests during the day, when compared to night. This is an unusual pattern for rhesus monkeys. At night, however, they demonstrated quite normal cognitive performance, proving that it is the timing function rather than cognitive abilities that are deficient in these animals.

Shortly thereafter it was discovered that all three affected animals came from the same large group (Group M, n ~ 300) of Cayo Santiago-derived rhesus monkeys housed at the CPRC's Sabana Seca Field Station (SSFS). The ancestors of this group were among the 409 founders of the Cayo Santiago (CS) colony brought from India to the shores of Puerto Rico in 1938 by Clarence R. Carpenter [Rawlins & Kessler, 1986]. As the population grew in size and fissioned, Group F was formed. By 1972, it contained over 120 monkeys and gradually fissioned between Groups F and M by 1973 [Chepko-Sade & Sade, 1979].

The core of the new group was formed by the lowest-ranking female of group-F, an aged female called O22, and all her progeny (11 females and 4 males). Several animals of relatively low rank but belonging to two other Group-F matrilines joined them as well (7 females and 11 males). Interestingly, not one animal of the three highest ranking families, out of total of six, became a member of Group-M. Thus overall, the animals of Group-F that split and formed Group-M were low ranking males and females, typical of fissures that occur in rhesus monkey groups [Chepko-Sade & Sade, 1979]. Over the next decade, Group-M continued its independent identity as a well-defined social group within the Cayo Santiago population.

In January 1984, Group-M (n ~ 145) was relocated from CS to the SSFS west of San Juan [Bercovitch & Lebron, 1991; Kapsalis, 1985; Kessler et al., 1984] to help reduce population size on CS and to provide an intact social group of CS macaques in which studies that were not feasible on CS could be

conducted [Kessler pers. comm.]. Since then, Group M has continued to produce offspring, and each new infant can be assigned to one of four matrilines originating from Cayo Santiago. The group was placed in a large outdoor corral at SSFS, and here it has remained as an isolated group, with many group members removed for studies conducted at other institutions or transferred to the specific-pathogen-free (SPF) colony at SSFS. Over the past 30 years, Group-M animals that remained at SSFS were part of several important studies concerning social factors that mediate the interactions between hormones and behavior, with special attention to how these processes affect male reproductive maturation [Bercovitch,1989, 1997]. These monkeys were also subjects in studies of macular degeneration [Hope et al., 1992], bone and joint diseases [Pritzker & Kessler, 2012], periodontal disease [Ebersole et al., 2008, 2014; Gonzalez et al., 2011, 2013, 2014], and serologic response to gut pathogens [Kienesberger et al., 2012]. Finally, 34 years after the spontaneous formation of this group on Cayo Santiago, we discovered that some of its members present a phenotype remarkably similar to a known human circadian disorder.

The first three animals with the documented circadian disorder [Zhdanova et al., 2012] belonged to the same matriline; that is, all of them were the progeny of that particular female (O22) that originally led the split of Group-M from Group-F [Chepko-Sade & Sade, 1979]. Two animals, representing different phenotypes, with loss of rhythm and DSPD, were closely related, having a common maternal great-grandmother and a common maternal grandfather. The third animal, also with DSPD, was a distant relative of the other two. That said, although the paternity in Group-M has been recently characterized through genotyping, not all the sires have been identified and thus the degree of relatedness between some animals might be underappreciated.

From an Ammunition Bunker to the State-of-the-Art Primate Circadian Research Laboratory

The decision to initiate in-depth studies of the inheritance pattern, penetrance and underlying mechanisms of the discovered circadian disorder in Group-M was based on two principal factors. First, the translational value of the model is high, considering the close similarity between the DSPD phenotype in the Group-M animals and the increasing prevalence of human DSPD. Second, because Group-M and the entire CPCR colony serves as a major resource of experimental animals to NIH-funded and industry research, we (IZ, JGM) believed that it would be important to determine which lineages within this colony carry the circadian disorder and which have normal rhythms.

To achieve both goals, and with the support of NIH, we constructed a high throughput PCRL close to the Group-M corral at SSFS, so that animals could be moved from the main colony, phenotyped and then placed in small group corrals for planned breeding and further studies on the impact of circadian abnormalities on individual and social behaviors, reproduction and early development.

The history of the SSFS site provided yet another twist to this story. The site of a former naval base, SSFS has several ammunition bunkers, dating to World War II. We decided to renovate one of those, since it provided an ideal environment for a laboratory devoted to circadian research, providing massive soundproof walls that ensure constant conditions of sound and light attenuation, absolutely necessary for effective circadian studies. A fitting historical parallelism would not escape circadian biologists who know that, in the 1960s, Jürgen Aschoff, a principal co-founder of the entire field of chronobiology, conducted his pioneering studies on human circadian rhythms in a World War II bunker, at the Max Planck Institute for Behavioral Physiology.

In our case, a dilapidated bunker (Fig. 2A) was converted into the second Primate Circadian Research Laboratory (PCRL-SSFS), a state-of-the-art high throughput facility, with 24 individual circadian chambers, each equipped with multiple video cameras, image analysis systems for monitoring activity and sleep, personal "monkey" computers, touch screens allowing them to play video games or watch video clips, and to operate automatic feeders or conduct cognitive tests (Fig. 2B,C). In this unique facility, a large cohort of Cayo monkeys started day-by-day revealing to us the mysteries of their internal circadian rhythms. At this point, we have completed evaluation of circadian rhythms in a total of 25 animals of Group-M. While a separate research article will detail the overall findings, which exceeded our expectations, we found that 20 out of 25 subjects are affected by the disorder, and it is present in all four matrilines comprising Group-M. This suggests a pattern of genetic inheritance with potentially high penetrance.

Genetic Hypotheses

More data and analysis are required before we can definitively establish the nature of the risk for DSPD or any other form of circadian rhythm disorder among Cayo Santiago rhesus macaques and the impact of specific genetic differences. However, the available evidence strongly suggests a genetic basis for individual risk among CS animals in general and Group M animals in particular. Among the 25 Group M animals that have thus far been intensively studied for circadian rhythm patterns, including the first 3 animals evaluated at PCRL-BU using

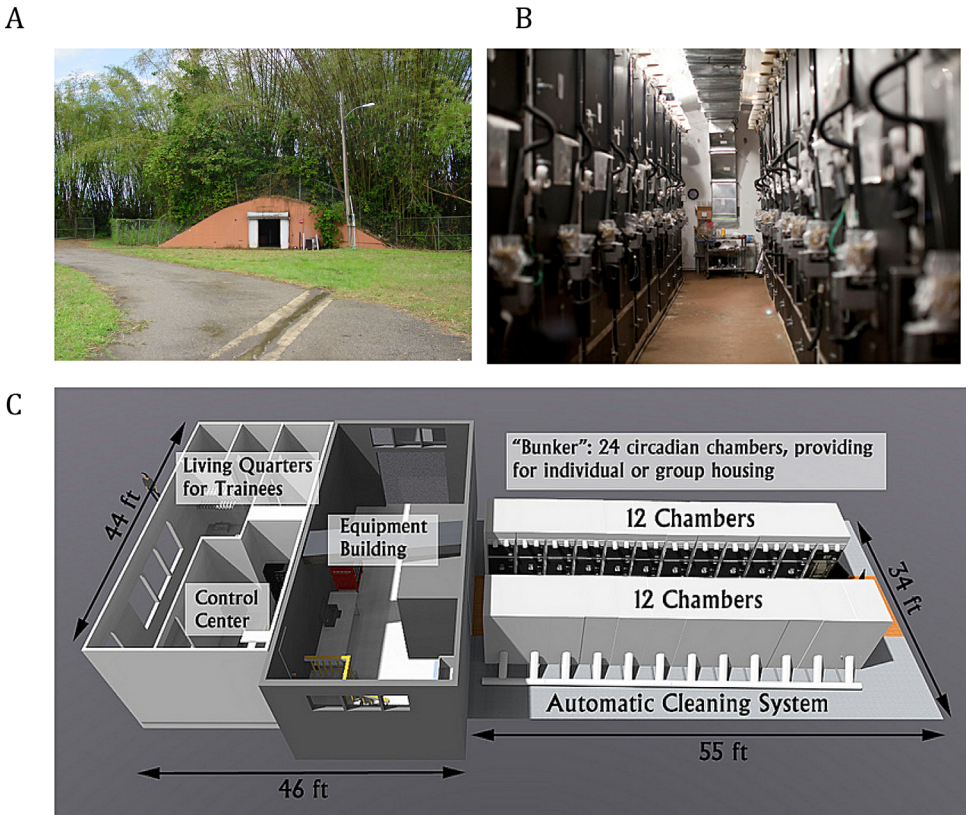

Fig. 2. From a World War II era bunker to a state-of-the-art high throughput Primate Circadian Research Laboratory (PCRL) at the Sabana Seca Field Station, Puerto Rico. A: The bunker before the PCRL construction started. B: The PCRL bunker: 24 specialized chambers for studying circadian rhythms in non-human primates. C: Schematic of the PCRL at Sabana Seca, including the 24 circadian chambers within a bunker area, Control Center, Training Center and living quarters for BU and UPR students, and Equipment building.

same methodological approaches, 13 have been found to exhibit DSPD, in addition to 7 that either had a loss of rhythmicity or dissociation between activity and feeding rhythms, the latter similar to NES in humans. Within that DSPD group of 13, there is one adult male that has produced a large number of progeny in the group. Among his offspring, we have already identified 7 sons/daughters affected by DSPD, and one grand-offspring similarly affected. All the affected offspring are half-sibs. This concentration of affected individuals (half-sibs and grand-offspring) in one lineage provides strong justification for further genetic analysis designed to conclusively demonstrate a genetic basis for DSPD in this species, and then to pursue molecular studies designed to identify the gene or genes involved.

Why the CS Population is a Valuable and Beneficial Context for Studying Circadian Disorders

In view of our unique finding of a high penetrance circadian disorder in rhesus monkeys originating from the Cayo Santiago colony, further in-depth investigation of the CPRC colony and its subgroups would be of major benefit to translational circadian research. The sheer number of animals in Group-M, with already confirmed high prevalence of the circadian disorder, and their relatives that remained on CS is one important consideration. In principle, any of these group mates could also carry this or other circadian abnormalities. In addition, the specialized high throughput laboratory provides an outstanding resource for conducting studies of the intrinsic circadian rhythms in these primates that are phylogenetically so close to humans.

In contrast, high throughput phenotyping of intrinsic circadian rhythms is impractical to perform in humans. Those are exceptionally long-term studies to be conducted under special highly controlled environmental conditions, including constant dim light, lack of environmental time cues or regular social interactions [Duffy & Wright, 2005]. Even under such circumstances, due to decades of entrainment, habitual meal schedules or dietary variables can interfere with the sleep-activity patterns controlled by the SCN, due to the existence of a mechanism independent of the SCN and involving a thus far enigmatic food-entrainable circadian oscillator [Mistlberger, 2011]. As an illustration of the complexity of such

investigations, the discovery of the first familial circadian disorder in humans, an Advanced Sleep Phase Disorder (ASPD) in an isolated family in the US led to an intrinsic rhythm to be evaluated in only one family member [Jones et al., 1999].

Thus, our validated methodological approaches and a high-throughput PCRL can provide effective methods for in-depth investigation of not only the genetic basis of the human-like circadian disorders per se but of the molecular modulators and modifiers that contribute to differences between normal circadian phenotypes and to variations in pathological circadian patterns.

Moreover, a combination of our circadian evaluations and the wide spectrum of behavioral and morphological studies conducted on Cayo Santiago or at SSFS by investigators from other academic institutions would be beneficial in several ways. Collaborations among behaviorists, morphologists and circadian researchers could provide insight into the potential role of circadian abnormalities in the development of specific pathological conditions or, alternatively, a contribution of other disorders to altered circadian rhythms. For example, there is a remarkably high incidence of retinal drusen, a sign of age-related macular degeneration (AMD), in CS and CS-derived populations [Anderson et al., 2006; Dawson et al., 1989, 2008]. Dawson et al. [1989] sampled individuals from eight social Cayo Santiago groups and found that animals from Group-M were more likely to have retinal drusen (70%) than others. Hope et al. [1992] then documented that the CPRC rhesus colony was, in general, uniquely predisposed to developing drusen and reported that the prevalence of monkeys with drusen in CPRC colony was up to nine times that of monkeys from US mainland facilities (57.7%, vs. 6.1%). Considering the major role of the circadian clock in eye physiology, known to affect light adaptation, disk shedding and other functions [McMahon et al., 2014], intrinsic clock abnormality may contribute to increased frequency of retinal pathology. Conversely, in view of major role of the retina in the entrainment of the SCN to the environmental light-dark cycle, pathological changes in the retina may also contribute to an overall circadian pathology or sub-phenotypes we observed in CS-derived animals of Group-M. In fact, recent studies in humans suggest that specific to circadian signal transmission melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) suffer in AMD patients [Feigl & Zele, 2014].

Another major advantage of large hierarchically structured social groups of rhesus monkeys, such as CS or corral-based colonies at SSFS, is a potential for studying the role of intrinsic and entrained circadian rhythms in social interactions. Social cohesion depends to a great degree on temporal cohesion, so that similar activities are conducted by different animals at the same time, thus providing conditions for social bonding and alliances. The extent to which the intrinsic clock affects these interactions, through similar preference of certain activities to be conducted at certain hours, remains unknown for humans or other species. Common sense, however, suggests that a person suffering from morning anorexia associated with a night-eating syndrome would not be at the table at the same time with one who enjoys his morning breakfast. Moreover, it is likely that the ongoing studies on social behaviors and modeling symptoms associated with autism-spectrum disorders in CS colony [Brent et al., 2013] could benefit from the knowledge of which animals have similar, different or pathological circadian rhythms, or lack those entirely, as some of the Group-M animals we studied.

Because the circadian clock plays a significant role in the regulation of almost every physiological and behavioral function, further studies could address the impact of the circadian disorder on immune, cardiovascular or cognitive functions, maternal behavior or infant development. In all these studies, knowledge of the circadian phenotype would contribute to, and may be necessary for thorough understanding of the genetic basis of those phenotypes.

Two important issues addressed in human studies are the role of the environment in the development of circadian disorders, and interaction between environmental variables and genetic predisposition to circadian abnormalities. While studies in diverse populations of non-human primates could provide major contributions to answering these questions, CS monkeys have already demonstrated that major circadian disorders could be of outstanding value in this respect.

Virtually any experimental model has some limitations or caveats. Although the CS population is living under semi-natural conditions, the density of animals on the island is higher than in the wild. Therefore, any feature of the animals that is affected by population density per square kilometer (e.g., rates of aggressive behavior or infection by natural pathogens) should be investigated and correlated with circadian phenotype very cautiously. Similarly, given the demographic history of the island, the amount of genetic variation present in the current population may not reflect natural (normal) levels of variation for this species. However, the level of variation in any other captive breeding colony will also be affected by the demographic history and breeding structure of that colony, so CS is not unique in this feature. Widdig and colleagues present a review, in this volume, which summarizes more than 40 years of genetic research carried out on CS, from early blood group typing and the genetic characterization of skeletal material to population-wide paternity testing using DNA fingerprints and short-tandem repeats (STRs) to the genotyping of the

highly polymorphic *DQB1* locus within the major histocompatibility complex (MHC).

In summary, our discovery that the circadian clock is not ticking normally in some members of CS-derived groups of rhesus monkeys provides an outstanding opportunity to explore an impact of pathological circadian rhythms on multiple aspects of physiology and behavior in diurnally-active primates, with sleep and circadian rhythm patterns remarkably similar to those in humans. This unparalleled rhesus monkey colony exhibiting a clear circadian disorder has the potential to help understand the ways in which circadian oscillators, both central and peripheral, interact, entrain and define the daily propensity for homeostatic processes in diurnal primates, including humans.

The Cayo Santiago macaques have been used for longitudinal studies of behavior, genetics, and population dynamics. The demographic and genetic databases allow for the examination of genetic consequences of individual behavior and population dynamics. Cayo Santiago is also a living laboratory to search for biological markers that model and predict human disease. One benefit of genetic analysis of CS animals is that they can engage in natural social interactions and natural development of infants. This influences many traits of biomedical importance (immune function, behavioral, and neurobiological development, feeding behavior) and therefore, this population provides a valuable addition to the research resources for nonhuman primate research.

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