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1. A patent database is available at www.patents.ibm.com/ibm.html

Is There an Intrinsic Period of the Circadian Clock?

In their report "Stability, precision, and near-24-hour period of the human circadian pacemaker" (25 June 1999, p. 2177), C. A. Czeisler *et al.* describe that under the experimental conditions of "forced desynchrony," the human endogenous pacemaker exhibits a period averaging 24.18 hours. They further report remarkable precision of the clock and suggest that both are "intrinsic" components of the human circadian pacemaker. Such a characterization may be misleading because it implies (and the authors articulate) that a rhythm measured under any other conditions is merely the expression of an "apparent period" of the biological clock.

It has been recognized since the late 1950s that the free-running circadian periods of laboratory animals depend on the experimental conditions under which they are measured. Indeed, one of the tenets in chronobiology is Aschoff's rule, which defines the differential responses of the circadian pacemakers of nocturnal and diurnal species to changes in light intensity (*1*). Which, then, should we call the intrinsic period of, for example, the finch's clock? That observed under constant lighting with an intensity of 0.4 lux, or the longer period that is observed when the bird is studied under 8 lux? Both are clearly endogenous periods, but it is unlikely that one reflects the essential nature of the pacemaker more so than the other. To the contrary, the essential nature of the pacemaker is reflected in its capacity to adapt to changing conditions.

As Czeisler *et al.* point out, the average free-running period of the human circadian clock (as determined by body core temperature) has been measured in a range from 24.2 to 25.1 hours. What distinguishes these various estimates of period length is the experimental conditions under which they were obtained. For example, we showed that when individuals in an otherwise traditional time-free environment took advantage of instructions to "eat and sleep when so inclined," by averaging at least one nap per subjective day, they exhibited an average period length of 24.22 hours, compared with an average period of 24.73 hours for individuals who seldom or never napped (*2*). One interpretation offered at the time to explain this finding was that some aspect of the traditional paradigm (which prohibits napping) might be responsible for "artificially lengthening the intrinsic free-running period" (*2*, p. 640). Seven years later, it seems clear that neither period estimate reflects the intrinsic period of the clock. Rather, both reflect the clock's intrinsic response to a distinct set of environmental or experimental conditions.

The forced desynchrony protocol used by Czeisler *et al.* presents the circadian system with yet another set of experimental conditions under which it must function. In this paradigm, the clock is forced to free-run against a strictly controlled background of reduced ambient light and altered subjective day lengths. The result is a strictly maintained (that is, "precise") rhythm with a characteristic period. For the authors to conclude that this particular set of conditions in some way evokes a more accurate reflection of the pacemaker's intrinsic period than other paradigms seems to beg the question.

As Aschoff emphasized 40 years ago, "The free-running period we can observe in an organism is, of course, nothing like a physical constant. Organisms as open systems are always correlated to the environment. The actual value of the rhythm, the frequency, is determined by all circumstantial conditions—external as well as internal" (*3*). To avoid any inference as to the intrinsic nature of an observed period, Aschoff suggested that an endogenous rhythm observed under specific experimental conditions may best be referred to as the "spontaneous" frequency of the pacemaker. Such a designation seems to capture more clearly the essential nature of the biological clock.

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Response

Circadian period is a fundamental, genetically inherited property of the circadian pacemaker. We do not agree with Campbell that observed circadian periods in humans are highly dependent on environmental or experimental conditions and that the forced-desynchrony protocol no more accurately reflects the intrinsic period of the human circadian pacemaker than a classical free-running paradigm. We also note that the late Jürgen Aschoff began conducting free-running studies in humans around 1960, shortly after the cited reference (*1*), and with Wever concluded more than two decades later that, "With a sample of 147 subjects, the overall mean of the [free-running circadian] period...was found to be 25.0...± 0.50 hr. The period of a free-running rhythm [in humans] is furthermore quite independent of conditions..." (*2*). In fact, on the basis of those findings, the concept that humans have an internal clock with a 25-hour period is included in numerous biology, physiology, and psychology textbooks.

Yet, in a *Science* review article shortly after the initial human free-running studies, Aschoff allowed that one (of three) possible causes of the considerably longer than 24-hour free-running circadian periods that he and others had observed in their now classical free-running experiments was "feedback between the subject's endogenous activity cycle and the self-selected periodic stimuli—that is, turning the lights on and off" (*3*), as he had seen in birds (*4*). He recognized that more data were required to "allow a final decision" on this matter (*3*).

The goal of our study was to provide those data. Individual neurons composing a central neural pacemaker of the mammalian circadian timing system (located in the suprachiasmatic nucleus of the hypothalamus) each contain a transcriptional/translational feedback oscillator or oscillators displaying a circadian period that is under genetic control; when coupled together, these ~10,000 neurons and their core oscillators form a pacemaker (*5*). We attempted to estimate the intrinsic circadian period of this central circadian pacemaker in humans, as measured immediately upon release from entrainment to the 24-hour day, by using a forced desynchrony protocol and measuring output rhythms directly driven by the pacemaker, such as melatonin. By "intrinsic," we mean the period originating from within (*6*) the circadian pacemaker, as distinct from other observed circadian periods influenced at the time of study by extrinsic resetting stimuli continuing to act on the pacemaker. This pacemaker is a dynamical system that rarely shows its intrinsic properties in humans, because it is nearly always being perturbed by light, changes in the timing of the sleep-wake cycle, transmeridian travel, etc. The pacemaker's responses to these perturbations compose the adaptiveness of the circadian pacemaker to which Campbell refers.

This adaptiveness is directly related to the wide range of observed circadian periods previously reported in humans, because in those experiments, factors that modulate the period of the pacemaker were not adequately controlled. The pacemaker's intrinsic period can only be assessed under conditions in which the main external and internal factors that have been shown to affect the clock (that is, the driving terms of

the dynamical system) are absent or distributed uniformly across the circadian cycle. Thus, for example, the circadian period of the perch-hopping rhythm in the finch observed under 8 lux is further removed from the "intrinsic" circadian period of the finch's circadian pacemaker than the period observed under 0.4 lux, because light imposes a direct drive onto the dynamical system. This is why most genetic studies of circadian period in mammals are carried out in constant darkness.

The efficacy of the forced desynchrony protocol in removing or uniformly distributing these driving factors—as predicted by a mathematical model of this dynamical system (*7*)—is demonstrated by our observation that the observed period of the pacemaker was nearly identical in forced desynchrony protocols with markedly different cycle lengths—for example, 11, 20, 28, or 42.85 hours—and with markedly different levels of physical activity. This is in contrast to the cited "eat and sleep when so inclined" paradigm, in which Campbell *et al.* reported that even though all of the participants were given the same instructions, the circadian period averaged 24.73 hours among those who chose to not nap during the experiment and 24.22 hours among those who did nap (*8*). As Campbell *et al.* noted at the time, their observation thus raised the possibility that intrinsic circadian period differed in nappers compared with non-nappers. In contrast, we consistently observed a near-24-hour intrinsic circadian period (averaging 24.18 ± 0.04 hours), despite the fact that none of the individuals in our experiments was allowed to nap. We thus conclude that the reason Campbell *et al.* observed the near-25-hour period among non-nappers (*8*) was because their sleep episodes and associated light-dark cycles were less evenly distributed across circadian phases, resulting in feedback resetting effects on the circadian pacemaker, rather than representing a systematic difference between those population groups.

Although we claimed to have estimated the intrinsic period of the human circadian pacemaker using this protocol, we do not contend that the period of the human circadian pacemaker is invariant. It has been known for 30 years that prior entrainment influences the intrinsic period of the pacemaker (an aftereffect of entrainment that can last for months) in mammalian species. In fact, as we noted in our report, the slightly longer circadian period observed in blind individuals may in part be a reflection of the absence of such an aftereffect of entrainment to the 24-hour day in some blind people. Such aftereffects do not invalidate the concept of a genetically determined circadian period; natural selection most certainly acted on the parameters of circadian pacemakers in organisms that were entrained to a 24-hour light-dark cycle. We chose to assess the intrinsic period of the human circadian pacemaker immediately on release from entrainment to a 24-hour day because it is this "aftereffected" period that is most relevant for understanding entrainment to the 24-hour day.

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CORRECTIONS AND CLARIFICATIONS

Letters: Response by John Olney under title "Induced damage in the developing brain" (12 May, p. 977). It was an editorial error to list only John Olney as the author of the response to letters by R. W. Montgomery and by A. Sharma and S. Kumar. Olney was the corresponding author for the report under discussion by C. Ikonomidou *et al.*, "Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome" (11 Feb., p. 1056). The author list should have been as follows: C. Ikonomidou, P. Bittigau, M. J. Ishimaru, D. F. Wozniak, C. Koch, K. Genz, M. T. Price, V. Stefovska, F. Hörster, T. Tenkova, K. Dikranian, J. W. Olney. *Science* regrets the error.

News Focus: "Science and policy clash at Yucca Mountain" by Richard A. Kerr (28 Apr., p. 602). John Greeves is with the Nuclear Regulatory Commission (NRC). All appearances of "NRC" referred to the Nuclear Regulatory Comission.

News of the Week: "Pruned sanctions list points to closer ties" by Jeffrey Mervis (14 Apr., p. 244). The director of the Aeronautical Development Establishment, a defense institute that remains on the banned list, was misidentified. His correct name is Krishnapuram Gopalakrishnan Narayanan.