Entrainment of the Human Circadian Clock

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Humans are an excellent model system for studying entrainment of the circadian clock in the real world. Unlike the situation in laboratory experiments, entrainment under natural conditions is achieved by different external signals as well as by internal signals generated by multiple feedbacks within the system (e.g., behavior-dependent light and temperature changes, melatonin levels, or regular nutrient intake). Signals that by themselves would not be sufficient zeitgebers may contribute to entrainment in conjunction with other self-sufficient zeitgeber signals (e.g., light). The investigation of these complex zeitgeber interactions seems to be problematic in most model systems and strengthens the human system for circadian research.

Here, we review our endeavors measuring human entrainment in real life, predominantly with the help of the Munich ChronoType Questionnaire (MCTQ). The large number of participants in our current MCTQ database allows accurate quantification of the human phase of entrainment (chronotype) and how it depends on age or sex. We also present new data showing how chronotype depends on natural light exposure. The results indicate the importance of zeitgeber strength on human entrainment and help in understanding the differences in chronotype, e.g., between urban and rural regions.

INTRODUCTION

Entrainment is the most common and most important state for circadian systems. Although clock research has acquired a host of fundamental knowledge about free-running rhythms as well as their entrainment under relatively artificial laboratory conditions, we know fairly little about how clocks are synchronized to their cyclic environment in the real world. Unlike in most laboratory experiments, the onset and offset of light are gradual and are not constant in intensity or in spectral composition throughout the natural day. In addition, alternations of day and night are always accompanied by temperature changes (with a certain lag), whereas light entrainment is recorded in constant temperatures in the laboratory. Natural entrainment is probably a complex interaction of multiple zeitgebers, among them light, temperature, and nutrition.

There is no doubt that light is the most potent zeitgeber for the biological clock, but other environmental signals also entrain, especially in poikilotherms. It has even been hypothesized that temperature is a stronger zeitgeber than light for the *Neurospora* clock (Liu et al. 1998; see also Doyle and Menaker, this volume), but the relative power of these two signals very much depends on their respective strength (Roenneberg and Merrow 2001). It is noteworthy, however, that temperature cycles entrain the circadian rhythm in this fungus even in constant light, which normally renders the *Neurospora* clock arrhythmic (Roenneberg and Merrow 2001). Nutrients can also entrain some circadian systems; nitrate, for example, resets the circadian clock in the unicellular alga *Gonyaulax polyedra* (Roenneberg and Rehman 1996).

The ecology of *Gonyaulax* gives an indication of how multiple zeitgebers may interact in the real world. Light, temperature, and nutrients have all been shown to be zeitgebers for the *Gonyaulax* clock in laboratory experiments. Both quantity and quality of light can act independently as zeitgebers in this alga because as in many plants (Roenneberg and Merrow 2000) and animals (see Doyle et al. 2006; Doyle and Menaker, this volume), light reaches the *Gonyaulax* clock via more than one light receptor (Roenneberg and Hastings 1988; Deng and Roenneberg 1997). In the ocean, *Gonyaulax* migrates daily over large distances from nutrient-poor surface waters to nutrient-rich depths (Roenneberg et al. 1989). Over the course of their natural day and during their daily migration, the algae encounter significant changes in all of the three zeitgebers which potentially all contribute to entraining its circadian system. Due to the lack of experimental possibilities, however, it is unlikely that we will ever be able to understand how these multiple inputs act as a composite zeitgeber signal for *Gonyaulax* in the real world. Similar limitations are true for practically all circadian model systems, except perhaps for one—the human circadian clock.

Circadian research has predominantly investigated self-sufficient zeitgebers, i.e., those that each and by themselves can entrain the clock under laboratory conditions. However, this might not be enough for understanding entrainment in the real world. It is "standard operating procedure" to carefully separate the acute (masking) responses elicited by the organism's environment as well as by its own behavior from the endogenous changes controlled by the circadian system. Yet, masking might be part of entrainment in the real world (Mrosovsky 1999). Although both temperature cycles and periodic feeding can coordinate daily behavior in mammals, this synchronization has often been interpreted as masking, at least for the activity rhythm driven by the SCN (suprachiasmatic nucleus) (Honma et al. 1983). On the other hand, both temperature (Brown et al. 2002) and nutrient (Stokkan et al. 2001; Schibler et al. 2003) cycles can entrain the molecular rhythms in mammalian cells and tissues. Thus,

both endogenous temperature changes and oscillating nutrient levels in the blood may actually be part of the entrainment process.

Nonphotic zeitgeber effects in humans are often studied in blind people. There are several types of blindness: (1) lack of visual perception, (2) lack of residual light perception (conscious or unconscious), and (3) lack of physiological light responses (such as suppression of melatonin). Activity-rest rhythms can still be entrained in the first two types, whereas individuals tend to run free in the last group despite being submitted to strong social time cues. This suggests that the efficacy of nonphotic zeitgebers in humans depends on functional light perception possibly by creating light/dark cycles via behavior (Czeisler et al. 1986; Honma et al. 2003). However, some individuals in the third group still entrain to the 24-hour day (Sack et al. 1992; Czeisler et al. 1995; Lockley et al. 1997). Their free-running periods might be very close to 24 hours, thereby allowing adjustments by nonphotic time cues that would be too weak to entrain longer or shorter rhythms (Klerman et al. 1998; Mistlberger and Skene 2005). The closer a free-running period is to 24 hours, the more readily weak periodic signals, such as activity-dependent temperature changes and/or scheduled food intake, might contribute to entrainment. It follows that "entrained" blind people within the third group would start to run free if they were exposed to schedules longer or shorter than 24 hours and, conversely, that free-running blind people would start to entrain if exposed to schedules closer to their free-running period.

In any case, examples of blind individuals indicate that entrainment will surely involve multiple zeitgebers, some of which may not be self-sufficient and thus, their contribution to entrainment will depend on the presence of other zeitgebers. In view of the many open questions concerning the details of entrainment in the real world, it seems that—if the circadian clock was compared to a car—we certainly know a lot about the engine but little about the car and how it drives it on real roads, in real traffic.

HUMAN PHASE OF ENTRAINMENT (CHRONOTYPE)

The formalisms behind entrainment are surprisingly simple. The circadian system in all its complexity appears to behave very much like a simple mechanical oscillator. Depending on its phase, the circadian clock responds differently to a zeitgeber stimulus (Roenneberg et al. 2003a, 2005a), either by advancing, delaying, or not responding at all.

As with other genetic traits, circadian properties depend on specific genotypes. Different variants of "clock" genes (Young and Kay 2001; Roenneberg and Merrow 2003) are associated, for example, with the period length of the circadian rhythm under constant conditions. In a given population, free-running periods are distributed around a species-specific mean that has been shown in rodents (Pittendrigh and Daan 1976) and humans (e.g., see Wever 1979; Klerman 2001; Dijk and Lockley 2002). Genetic variation also contributes to the interindividual differences of the circadian clock under entrained conditions (Katzenberg et al. 1998; Ebisawa et al. 2001; Toh et al. 2001; Carpen et al. 2006; Hamet and Tremblay 2006; Viola et al. 2007). Individuals adopt a specific temporal relationship to the zeitgeber (e.g., the time difference between dawn and wake-up, core body temperature minimum, or melatonin onset). This relationship between external and internal time is called *phase of entrainment*, and when individuals differ in this trait, they are referred to as different chronotypes (Roenneberg et al. 2003b).

We have developed an instrument, the Munich ChronoType Questionnaire (MCTQ) to assess individual phase of entrainment with simple questions (Roenneberg et al. 2003b, 2004, 2005b, 2007a). The MCTQ asks about sleep and activity times, such as when do you go to bed, how long do you need to fall asleep, when do you wake up. The same set of questions is asked separately for work and for free days. The MCTQ has been validated with highly significant correlations by more than 700 sleep logs, by actimetry, and by correlations to biochemical rhythms such as melatonin and cortisol (T. Roenneberg et al., in prep.). In addition, the phase of entrainment determined by the MCTQ correlates well (Zavada et al. 2005) with the score produced by the Morningness-Eveningness Questionnaire (MEQ) (Horne and Östberg 1976).

To define chronotype quantitatively, a single phase marker has to be extracted from the different times queried in the MCTQ. We initially used mid-sleep on free days (MSF, the half-way point between sleep onset and sleep end) as a definition of chronotype (Roenneberg et al. 2003b). The distribution of MSF within our database (currently more than 60,000 individuals, mainly central Europeans) is almost normal with a slight overrepresentation of later chronotypes (Fig. 1). Although human chronotypes cluster around a mean phase of entrainment, the differences between extreme types span over half of the day.

Average sleep timing and sleep duration are essentially independent traits, i.e., the distribution of sleep duration is similar for early types and late types or vice versa the distribution of chronotypes is similar for short and for long sleepers (Roenneberg et al. 2007b). However, when sleep duration is analyzed separately for work and for free days, striking differences become apparent (Fig. 2). This result suggests that both duration and timing of sleep on free

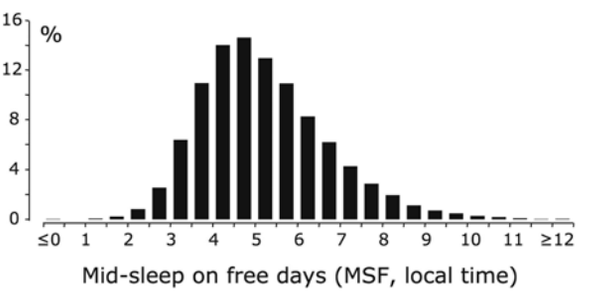

Figure 1. Distributions of chronotypes judged from mid-sleep on free days (MSF). The MCTQ database currently comprises more than 60,000 individuals, mainly from Germany, Switzerland, Austria, and The Netherlands.

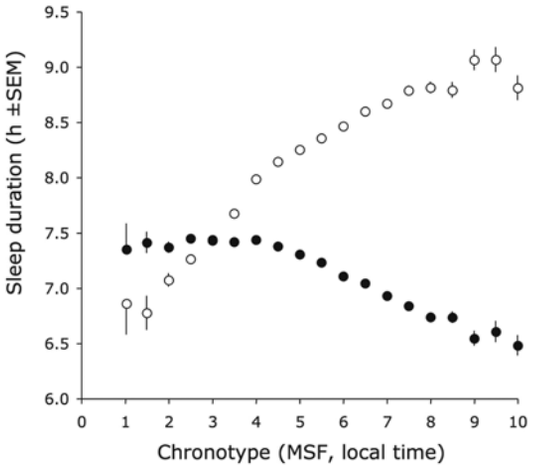

Figure 2. Relationship between chronotype (mid-sleep on free days, MSF) and sleep duration analyzed separately for work and free days (*closed circles* and *open circles*, respectively). Early chronotypes are sleep-deprived on free days, whereas late chronotypes sleep less than their weekly average on workdays. People with an MSF of 3 a.m. (e.g., those who sleep from 11 p.m. to 7 a.m., or midnight to 6 a.m.) are the only chronotypes who show no difference in sleep duration between work and free days. Vertical bars represent the standard error of the mean (S.E.M.) in each category (to avoid overlap, they are in some cases only drawn in one direction); most errors are smaller than the data points.

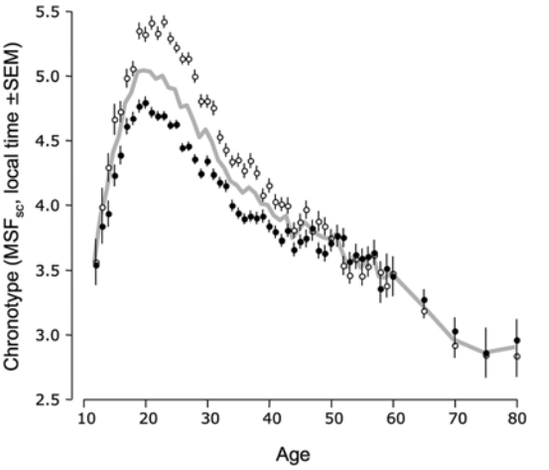

Figure 3. Chronotype (MSF, corrected for sleep debt accumulated over the workweek, MSF_{sc}; see text) depends on age. These changes are highly systematic and are different for males and females: (*closed circles*) females; (*open circles*) males; (*gray line*) averages for the entire population. The first data points represent the averages for subjects aged 12 or younger. Between ages 12 and 60, data were averaged for each year of age, whereas those showing the mean chronotype for subjects above 60 years of age are averaged over groups of 5 years. Vertical lines represent standard error of the mean (S.E.M.).

days are influenced by the sleep-debt accumulated during the work week. Chronotype should therefore be corrected for the confounding influence of sleep debt. Under the assumption that sleep recovery on free days confounds chronotype in a linear fashion, we have adjusted the MSF for sleep debt, thereby creating a theoretical chronotype (MSF_{sc}) estimating the timing of sleep as if subjects did not suffer from lack of sleep on workdays (see supplemental material in Roenneberg et al. 2004). Because the majority of the population is sleep-deprived on workdays, MSF_{sc} lies, in most cases, slightly earlier than MSF.

CHRONOTYPE, SEX, AND AGE

Chronotype depends not only on genetic (Toh et al. 2001; Vink et al. 2001; Archer et al. 2003) and environmental factors (Roenneberg et al. 2003b), but also on age (Carskadon et al. 1999; Dijk et al. 2000; Duffy and Czeisler 2002; Park et al. 2002; Roenneberg et al. 2003). The large MCTQ database accumulated with our survey allows examination of this age dependency as an epidemiological phenomenon (Roenneberg et al. 2004). Within each age group, the shape and width of the chronotype distribution (MSF_{sc}) are similar to that of the general population. Across different age groups, however, their respective means vary systematically (Fig. 3). Children are generally earlier chronotypes, progressively delaying during development, reaching a maximum in "lateness" at about the age of 20, and then becoming earlier again with increasing age.

The general phenomenon that females tend to mature earlier than males in many developmental parameters is also apparent for the ontogeny of chronotype (Fig. 3). Women reach their maximum in lateness at about 19.5 years of age, whereas men continue to delay their sleep until at about the age of 21 (Roenneberg et al. 2004). As a consequence, men are, on average, later chronotypes than women for most of adulthood (see also, Adan and Natale 2002). This sex difference disappears at approximately age 50, which coincides with the average age of concluded menopause (Hollander et al. 2001; Greer et al. 2003). People over 60 years of age are on average even earlier chronotypes than children (are today).

NATURAL DAYLIGHT IS THE PREDOMINANT ZEITGEBER FOR THE HUMAN CLOCK

In our MCTQ survey, addresses and postal codes are provided by the large majority of participants. With the help of this information, we are able to address the question: What zeitgebers entrain the human clock in real life?

Within a given time zone, people live according to a common social time, whereas dawn and dusk progress continuously from east to west. This creates discrepancies between, for example, the time the sun reaches the zenith and noon (by social time). Because we predominantly arrange our lives according to the social clock, we are largely unaware of these discrepancies—but is the human circadian clock similarly oblivious of sun time? If humans were entrained by social time, average sleep-wake behavior should be the same across a time zone from its eastern to its western border. If it was, however, entrained by sun time, a gradual change should be detectable or at least some systematic deviation from the time zone constancy.

A total of 21,600 responses were selected from the

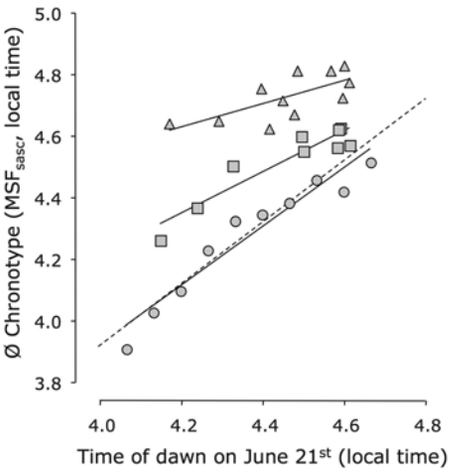

Figure 4. Chronotype, averaged for each of the 11 longitudes from western to eastern Germany (*circles*), highly correlates with the respective time of dawn; for reference, dawn times on the longest day were chosen; the *dashed line* represents the 1:1 relationship. Chronotype is given as the MSF corrected for sleep debt, age, and sex (MSF_{sasc}; see Roenneberg et al. 2007a). The data (*circles*) represent people living in areas with no more than 300,000 inhabitants. Although the average chronotype in larger cities—(*squares*) up to 500,000; (*triangles*) above 500,000—is later and less tightly coupled to sun time, the correlations are still significant.

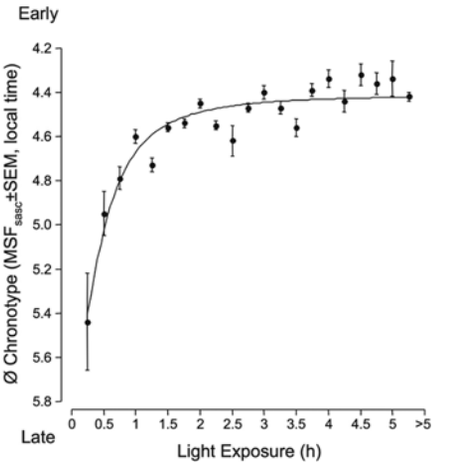

Figure 5. Average chronotype (MSF_{sasc}) depends on the daily light exposure, i.e., the average amount of time subjects spend outside without a roof above their head ($r = 0.96$; $p << 0.0001$; total $N = 41{,}232$). Error bars indicate standard error of the mean (S.E.M.). Note that unlike Figs. 3 and 4, the ordinate showing chronotype is plotted in reverse order from early at the top to late at the bottom.

MCTQ database (at that time comprising slightly more than 40,000 entries) which all contained a German postal code *and* the correct name of the corresponding location to unambiguously allow geographical mapping (Roenneberg et al. 2007a). The comparison between the chronotype distributions for each longitude clearly showed that the human circadian clock is predominantly entrained by sun time rather than by social time. In villages and towns with no more than 300,000 inhabitants (82% of the German population), the average chronotype moves proportionally with the progression of dawn (Fig. 4).

In the 20 larger cities of Germany, the correlation with sun time is weaker (although still significant) and, on average, chronotype is later. This observation could be explained if inhabitants of large cities were exposed to a weaker zeitgeber (less natural light during the day and more artificial light during the night). Weaker zeitgebers predictably lead to a later chronotype (Roenneberg et al. 2003a, 2004) in all individuals whose period—under free-running conditions—is longer than 24 hours (which is the case in the majority of humans).

To obtain some estimate of the zeitgeber strength to which an individual is exposed, the MCTQ also contains a question about how much time participants spend outside without a roof above their head. Although this question can only be answered as a coarse estimate, one can presume that it correlates with reality, especially when based on the high numbers present in our database. Figure 5 shows that chronotype correlates with the amount of time an individual spends outdoors. Chronotype progressively advances by more than an hour when people spend up to 2 hours outside per day; 42% of the population in our database fall into this category, stressing the fact that industrialization means living inside. Beyond 2 hours of natural light exposure, chronotype changes very little. This is important because the sleep debt accumulated by late types over the workweek (see Fig. 2) would be greatly reduced if those individuals could fall asleep an hour earlier by spending more time outside.

Figures 4 and 5 show that sun time is the dominant zeitgeber for entraining the human clock. Sunlight exerts its effects through timing (i.e., creates a longitudinal gradient) and also via zeitgeber strength: People living in larger towns depend less on daylight timing and are, on average,

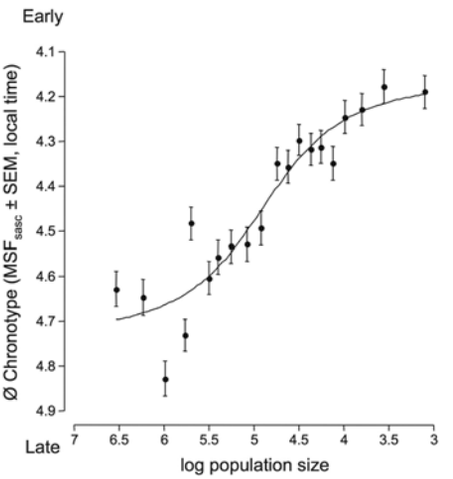

Figure 6. Chronotype depends on population size. The database, sorted by population size, was divided into equal bins of 1080 individuals each, for which average population size and average chronotype (MSF_{sasc}) were calculated. The abscissa is plotted on a logarithmic scale and in reverse order with large cities on the left and villages on the right, assuming that this represents the amount of light inhabitants are exposed to ($r = 0.94$; $p << 0.0001$; total $N = 21{,}600$). Vertical bars represent standard error of the mean (S.E.M.).

later chronotypes. Figure 4 separated the German population into three categories according to the population of their place of residence. A more continuous dependency of chronotype on population size is shown in Figure 6. According to these results, the chronotype of city dwellers is, on average, more than half an hour later than that of people living in the countryside.

THE CIRCADIAN CLOCK: A HYPOTHESIS ABOUT TEMPORAL ENVIRONMENT

Our findings emphasize that individual circadian time rather than social external time should be considered in scientific studies, in school and work schedules, or in medical considerations. Individual time reflects, in turn, the strong dependence of the circadian clock on light, reminding us that this biological system concerns perception in a classical sense. One could argue that its ability to continue without external input (free-run) in addition to its power to anticipate recurring events within the external daily structure are features of an active rather than a perceptive machinery. However, perception is rarely just a passive response; our visual perception of the world, for example, is predominantly based on updating endogenous hypotheses. A good example of hypothesis-driven perception is disorientation upon waking in an unfamiliar setting (e.g., a hotel room). We are disoriented despite the fact that we very well know the room in which we went to bed as well as the room that our brain obviously has provided as the hypothesis to be updated (Roenneberg 1997). After what seems to be a long time, the perceptual deadlock is resolved when the brain provides a new hypothesis leading to an instantaneous recognition of where we are.

This example shows how the brain provides an intrinsic hypothesis about the environment's spatial structures, and the circadian system provides an intrinsic hypothesis of a temporal structure of our environment—the "day." In both cases, hypotheses are continually updated by external and internal information. Not only do these complex interactions result in what we call entrainment, but they can also produce after-effects. Recent, regular time structures of the environment are incorporated into the current temporal hypothesis and thereby shape the responses to new temporal information. The long time it takes to alter an existing hypothesis indicates the robustness of the intrinsic components contributing to perception; this holds for the perception of both spatial and temporal structures. Many factors are integrated to form the "circadian hypothesis," which is then continually updated by the current environmental information.

In view of the complexity and the adaptiveness of such a system, the concept of a stable intrinsic period (Czeisler et al. 1999; Wyatt et al. 1999) seems to be an oxymoron. That we can measure a self-sustained rhythm in temporal isolation may be remarkable to us, but free-running rhythms merely reflect how clocks evolved to optimally fulfill their function as a temporal perception apparatus under natural, entrained conditions. Although free-running period and phase of entrainment are often strongly correlated, natural selection could only act upon the latter.

Most of us consider the relationship between free-running period and entrained phase as causal: Entrained phase *depends* on free-running period. This statement is simply wrong. Phase is merely associated with but does not depend on period! The way individuals behave in everyday life depends on their personalities (this is the analogy to the natural, entrained state). The way individuals behave under the influence of alcohol also depends on their personalities (this is the analogy to the unnatural state of free-running rhythms). But the way individuals behave in everyday life does not depend on the way they behave under the influence of alcohol.

Beyond the erroneous conclusion about a causal period-phase dependency, their strong correlation is a one-way logic that only holds under special conditions: Only if this relationship is investigated in a homogeneous genetic background, a manipulation of components contributing to the trait "free-running period" will also penetrate in the trait "phase of entrainment" (chronotype; see also Merrow and Roenneberg, this volume). Yet, many other external and internal factors, besides free-running period, contribute to chronotype: sensitivity to the zeitgeber stimulus, properties of the transduction pathway, coupling between oscillators and to outputs within the system, and many more. Thus, a generalization of this correlation is built on soft grounds; in genetically heterogeneous populations or even in experiments investigating quantitative trait loci (QTL), one would not necessarily expect this correlation to hold (Michael et al. 2003).

If there was an "intrinsic period," one might also presume an "intrinsic phase." We have shown here the adaptive qualities of human chronotype (for similar results in a simple fungus, see Merrow and Roenneberg, this volume). In humans, phase depends at least on age, sex, and light environment. The latter is influenced by many different factors, such as longitude, latitude, time of year, place of residence, and even profession (office vs. outdoors). To investigate the genetic basis of the human circadian clock, chronotype must be corrected for all of these factors because they all do not work at the genetic level. Because the free-running period reflects (not *is*) part of the mechanism that determines (and adaptively changes) chronotype, its values should be confined to a well-defined and relatively narrow range. However, the term "intrinsic period" only makes sense if it is associated with a distinct value. What are the conditions that produce this value? Forced desynchrony has been used to determine an individual's value of "intrinsic period" (Czeisler et al. 1999). As argued in the Introduction, multiple external factors contribute to entrainment even then if they cannot act as self-sufficient zeitgebers. In addition, entrainment depends on internal factors, both concerning prior history (which influence the current hypothesis) and current internal states. In a forced desynchrony protocol, many of these change and thereby influence the length of the period that has broken away from the imposed schedule. Behavior-dependent light exposure (even if the light changes are too weak to entrain by themselves), activity-dependent temperature changes and regular food intake—to name only few—all influence what is interpreted as "intrinsic period."

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