

Owls and larks in mice

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Humans come in different chronotypes and, particularly, the late chronotype (the so-called owl) has been shown to be associated with several health risks. A number of studies show that laboratory mice also display various chronotypes. In mice as well as in humans, the chronotype shows correlations with the period length and rhythm stability. In addition, some mouse models for human diseases show alterations in their chronotypic behavior, which are comparable to those humans. Thus, analysis of the behavior of mice is a powerful tool to unravel the molecular and genetic background of the chronotype and the prevalence of risks and diseases that are associated with it. In this review, we summarize the correlation of chronotype with free-running period length and rhythm stability in inbred mouse strains, in mice with a compromised molecular clockwork, and in a mouse model for neurodegeneration.

Keywords: circadian rhythms, chronotype, chronobiology, neurodegeneration, stability, activity rhythms

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For all organisms, the ability to respond and adapt to changes in the environment is important to ensure survival (1, 2). Circadian clocks have evolved to fine tune biological functions to specific times within the day or night (3, 4). A disruption of the time keeping system can lead to physiological and psychological problems that can be experienced during transmeridian air travel (jet lag) and include fatigue, often also gastrointestinal and metabolic problems. The psychological symptoms are poor concentration, decreased attention, and problems in memory formations (5). Also, shift work has severe effects on the human time keeping system, and is associated with reduced cognitive performance and an increased risk of cardiovascular diseases, cancer, and sleep disorders (5–8). In mice, chronic jet lag increases mortality levels and accelerates tumor progression (9, 10).

Under natural conditions, phase and period length of the molecular clockwork are entrained to the environmental day/night cycle; but within a given species, there is a substantial variability in the temporal alignment. In order to describe this phenomenon, the term “chronotype” was coined by Ehret (11), and he provided a very succinct definition of the term: “the temporal phenotype of an organism.” Notably, this definition refers to *overt* characters and not to a propensity or inclination. It is somewhat ambiguous with respect to the word “temporal,” as it does not become clear whether it refers to internal (endogenous/circadian, CT) or external (sidereal/*zeitgeber*-, ZT) time. But, Ehret (11) then clarifies this ambiguity by visualizing the “chronotype of the rat.” He plots the acrophases of various behavioral, metabolic, and endocrine activities over diurnal (*zeitgeber*-) time. He implicitly supplies an operational definition of how the chronotype may be measured – by comparing the timing of these acrophases among each other and in relation to the entraining *zeitgebers*, thus permitting to distinguish “early” and “late” chronotypes.

Chronotypes in Humans

The Horne–Östberg questionnaire (12) chronotypes humans based on a scoring system (“morningness–eveningness”). It *does* take into account (psychic) factors, propensities, and inclinations that

may *not* necessarily manifest themselves in the daily behavior. Roenneberg and colleagues (13, 14), on the other hand, relied on an overt character (the “mid-sleep on free days”) to chronotype large populations of humans.

In humans, the chronotypes differ considerably. If the “mid-sleep on free days” is used to quantify the chronotype (13, 14), extreme “larks” and “owls” differ by more than 8 h. Beyond sleep/wake timing, different chronotypes show distinct and different temporal patterns in cognitive performance, gene expression, and endocrine functions (15–17).

The late chronotypes, in particular, are subject to repeated temporal shifts, due to the fact that they are forced into the behavioral patterns of a lark-society (15, 18). The term “social jetlag” has been introduced to denote this shifting entrainment that often results in chronic sleep loss (19, 20). Furthermore, a late chronotype is associated with a multitude of health risks, such as metabolic dysfunctions, obesity, depression, sleep disturbances, and nicotine abuse (15, 16, 21). Social jetlag is also associated with endocrine and cardiovascular risks (22), and negatively correlates with academic performance in undergraduates (23).

Mice might be useful to elucidate the genetic, cellular, and molecular mechanisms that underlie the association between chronotype, time-shifts, health, and disease – if they could be chronotyped. In fact, they can.

Chronotypes in Mice

Most mouse strains used in laboratory research are nocturnal. Under entrained conditions, i.e., under a 12 h light/12 h dark (LD) regime, and food and water *ad libitum*, “lights off” marks the onset of the main activity period (24–28). However, there are also mice that become active already in the light phase, several hours before “lights off” (29).

These results suggest that different mouse strains have different chronotypes. The “mid sleep on free days,” used to chronotype humans, cannot be determined in mice, since their sleep-wake cycles differ radically from those of humans. Instead of having a single continuous sleep phase in a 24 h-interval, mice have numerous short periods of sleep throughout the 24 h cycle (30–32). Furthermore, the activity profiles of mice are not smooth, but show numerous “spikes” and “depressions”; thus, attempts to chronotype mice by calculating the acrophases of their locomotor activity failed to distinguish between the different strains.

Alternatively, chronotypes in mice may be quantified by subtracting the time [ZT] of the occurrence of the entraining cue (i.e., “lights off” in a light entrainment regime) from the time of the onset, the main activity phase. This time span is slightly positive in most nocturnal mouse strains, which become active shortly after “lights off,” and negative in the few ones (29) that become active before the onset of darkness. This method does, however, not permit to reliably distinguish chronotypes between the most widely used strains of mice, and it also will not quantify the obvious differences in the temporal dynamics of the activity profiles that occur after the onset of activity. Some mice (C3H/HeN (C3H)) have a single activity peak early in the night, while others (C57Bl/6J (C57Bl/6)), extend their activity well into the early morning.

In search for a parameter that defines the chronotype of mice, it was found that cumulative plots of the activity profiles allow reproducible measurements of the chronotype by determining the “median of activity” (MoA) defined by spontaneous locomotor activity of the mice kept under a standard 12/12 LD regime (28). The MoA is the time-point on the timescale at which the mouse has completed 50% of its daily “activity work” under entrained conditions. “Early” mice will have accomplished that “task” at an earlier time (ZT) than “late” ones. Indeed, the inbred mouse strains that are routinely used in circadian research (33–38), namely the C3H, C57Bl/6 and CBA/J mice, do have significantly different chronotypes, even though they do not differ with regard to the onset of the activity phase: the C57Bl/6 are the “owls” among them, the C3H are the “larks,” and the CBA/J are of intermediate type (Figure 1).

Notably, the MoA – in its current definition (28) – can only be applied to nocturnal strains of mice (see above). The starting point of the cumulative plots that are used to determine the 50% values is currently fixed at ZT 12. If this “fixed starting point” procedure was used to chronotype the “early runner” mice (29), the results would obviously be nonsensical, as the calculus would result in a late chronotype. However, this weakness can be overcome by combining the MoA with Wisor et al.’s (29) method of determining the onset of the main activity phase in hours ZT and starting the cumulative procedure at that point in time. The MoA – like any other chronotyping method – can only be applied to animals that do entrain to external stimuli, arrhythmicity, and chronotypic behavior are mutually exclusive.

Free-Running Period Length, Molecular Clockwork, and Chronotype

Rhythmic behavior persists even in constant darkness (DD), although with a period slightly shorter or longer than that of the 24-h-day, demonstrating that the free-running oscillation is the output of an endogenous temporal program. The length of the period under DD is denoted by the Greek letter τ (tau), followed by a “DD.” The τ DD is thought to be mainly determined by the endogenous clock and clock genes, the key components of the molecular clockwork (40).

Indeed, mice carrying mutations or targeted deletions of clock genes, such as Clock, Cry, or Per (41–47), either show a dramatic change in the length of their τ DD or become arrhythmic under constant conditions [Bmal1; (48)]. In humans, a missense mutation in the clock gene hPER2 is associated with a variant in human sleep behavior, the familial advanced sleep phase syndrome (FASPS), which is characterized by a significantly shorter period length of circadian rhythms (49). However, the clock-genes are certainly *not* the sole genes that determine the τ DD. If the Per-1 gene is knocked out in C57Bl/6 mice, their τ DD is 1 h shorter than that of their wild-type littermates (47). If the very same gene – Per-1 – is deleted in C3H mice (50), the τ DD is not affected (Figure 1).

It was generally assumed that there is a strict correlation between the τ DD and the chronotype; however, recent studies have shown that this is not always the case. In finches and humans, a correlation between τ DD and chronotype has been demonstrated (49, 51–53). Also, in laboratory mice, the chronotype correlates with

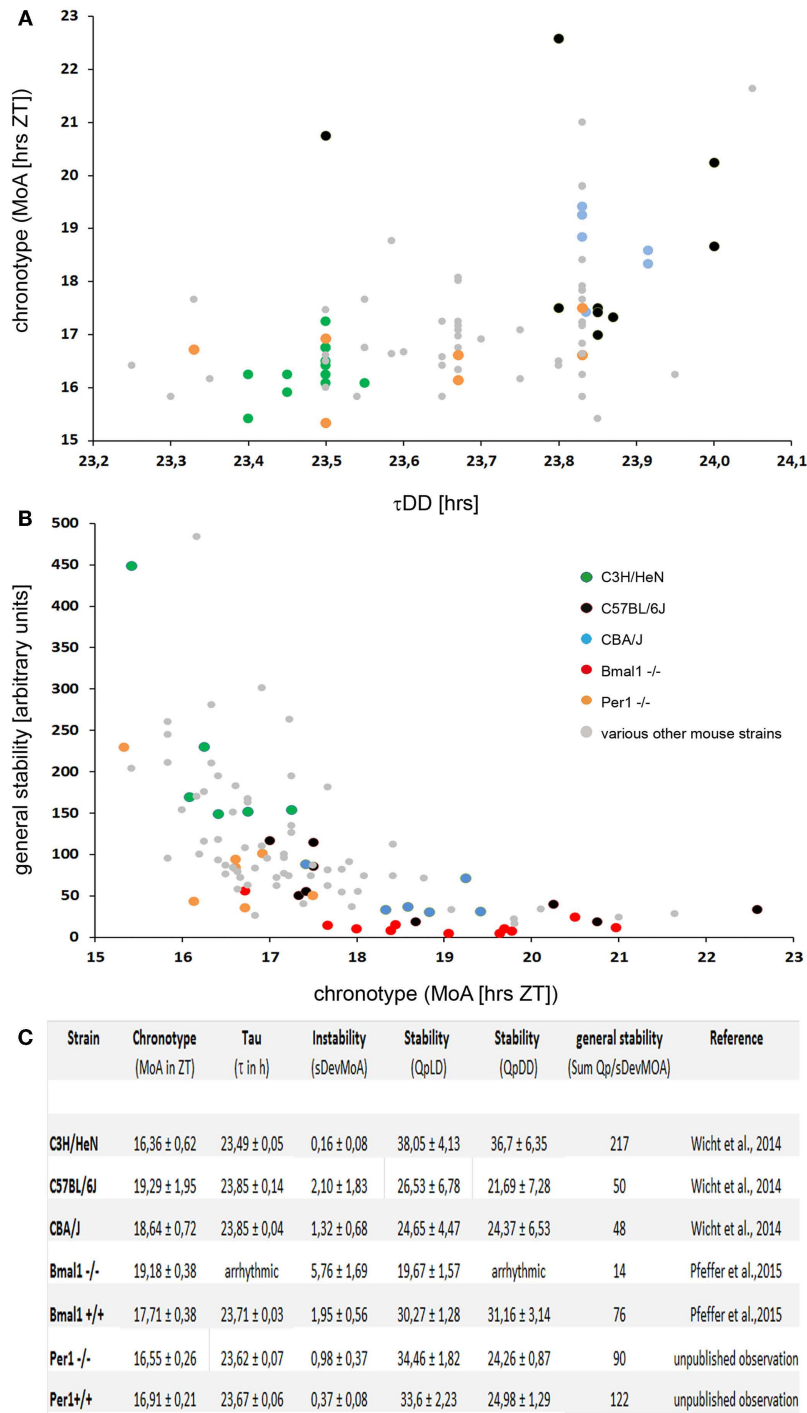


FIGURE 1 | (A) A plot of the τ DD vs. chronotype (MoA) in 89 individual mice of various strains. Some of the strains mentioned in the text are highlighted by colored dots, see insert in **(B)** for the code. The MoA was determined as described in Ref. (28). There is a moderate correlation of $\rho = 0.47$ (Spearman's rank correlation) between the two parameters. Note that the differences in the τ DD are less than an hour, while the chronotypes differ by more than 6 h. **(B)** A plot of the chronotype (MoA) vs. the general stability of the locomotor rhythms in 103 individual mice of various strains. The general stability is scaled in arbitrary units; high numbers indicate stable rhythms. The numbers were calculated as described in Ref. (28), in short; they represent a combined

measure of stability using Refinetti's (39) Qp-values and the accuracy with which the individual mice reproduce their chronotype in a day-to-day comparison. Note that the Bmal1^{-/-} mice (red dots) which do not show up in **(A)**, as they have no τ DD, do have a (late) chronotype. Yet, they are not the least stable mice; some individuals of the C57BL/6 strain have even less stable locomotor rhythms. There is a good correlation of $\rho = -0.75$ (Spearman's rank correlation) between the two parameters. **(C)** Table with the chronotype (MoA), instability (sDevMoA), stability (QpLD and QpDD), and general stability of several mouse strains measured so far. The "general stability" [in **(B)**] is calculated by dividing the sum of the Qps (QpLD and QpDD) by the SDev of the MoA.

the τ DD; C57Bl/6 mice have a longer τ DD and a later chronotype as compared to C3H mice, but the correlation between the τ DD and the chronotype is quite moderate [(28); **Figure 1**]. Notably, this correlation between the τ DD and the chronotype is absent in some cases: In Cry1 KO as well as in Cry2 KO mice, the chronotypic differences in LD (measured by the onset of activity relative to lights-off) are minimal compared to their background-matched wild-types, despite their large differences in free-running periods (54). In accordance with these observations, Wisor et al. (29) report the absence of a correlation between the τ DD and the onset of the activity period in their “early runner” mice.

The BMAL1 KO (*Bmal1*^{-/-}), a mouse with a defective molecular clockwork, is arrhythmic in constant darkness, and therefore has no τ DD (48). But under entrained conditions, these animals do display a rhythmic behavior and a chronotype. It is later than that of their wild-type littermates (C57Bl/6 background), and they display a less stable rhythm (55). It seems that the chronotype and the τ DD are not correlated in a simple and predictable manner. In fact, a τ DD is not even necessary in order to have a chronotype.

It should also be noted that – in terms of absolute duration – the τ DDs of the different mouse strains differ by about 20–40 min, while the differences between the chronotypes amount to several hours [see **Figure 1**; (24–29, 55)]. Furthermore, the τ DD cannot be causal for the establishment of the chronotype – simply because the chronotype manifests itself only under entrained conditions, while the τ DD requires free-running (non-entrained) conditions in order to be realized. Under conditions of normal nycthemeral entrainment, however, the period length of the behavioral rhythms will be 24.00 h. These data suggest that the chronotype is influenced by genetic factors, and many of them probably reside outside the classical clock genes as shown for several mouse strains (24–27, 29).

Chronotype and Rhythm Stability

Different mouse strains also differ with respect to the inter- and intra-individual variances and the stability of the behavioral rhythms. The timely precision with which mice (and other beings) reproduce their behavioral rhythms under different conditions (be they entrained or free-running) can be quantified using a combination of different methods [(28, 39); and **Figure 1**]. The standard deviation of the MoA (SDevMoA) is a measure of the rhythm instability, which quantifies the more or less pronounced daily variation of the activity pattern. In contrast, the Qp-analysis quantifies the rhythm stability in LD and in DD by estimating the stability and robustness of an animal's τ over several periods as compared to an ideal, sinusoidal wave with the same wavelength (39). Both measures correlate inversely and were used to calculate the “general stability” by simply dividing the summed Qps (LD and DD) for each mouse by the SDev of its MoA [(28); **Figure 1**].

Interestingly, a quite robust correlation exists between rhythm stability in locomotor activity and the chronotype (**Figure 1**). This could be observed in all mouse strains examined so far – mice with an early chronotype reproduce their rhythms (both free-running and entrained) more precisely than those with a late one (28, 55). The factors constituting the causal link for the correlation between chronotype and rhythm stability are currently unknown.

A similar correlation between chronotypes and rhythm stability has been found in humans (13, 20). But in humans, the rhythmic instability of the late chronotypes (“owls”) is attributed to the repeated shifts in the (social) entrainment that they suffer – the phase of entrainment on their “work days” differs (more than in “larks”) from their phase on “free days,” while the stability differences in mice were observed under conditions of constant, non-shifting entrainment.

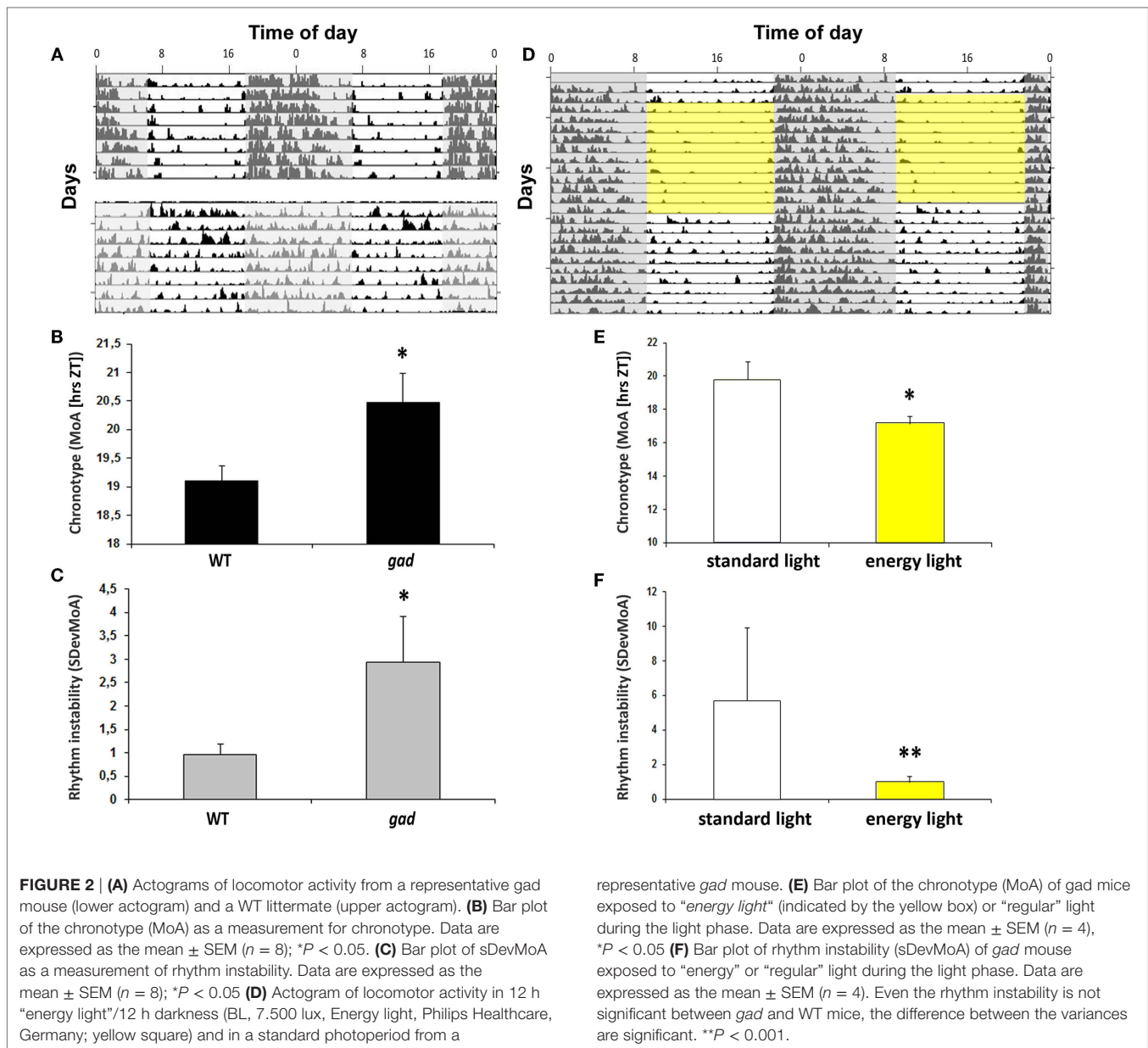
Researches into the stability (or lability) of circadian and diurnal rhythms have a long tradition [see, for example, Ref. (56)]. In spite of the presence of a plethora of data on various species under varying conditions, the biological role of rhythm stability vs. lability is still unknown.

Chronotype, Entrainment, and Disease

The photoperiod has been shown to influence the chronotype in humans – short days (in winter) will shift the average chronotype of a population to a later while long days (in summer) will shift it to an earlier one (57, 58). A similar effect can be observed in mice. Under short days and long nights, their night activity profiles will become “expanded” – i.e., “later,” if the “MoA” were applied (39). Inversely, short nights will compress the activity profiles resulting in an earlier chronotype. Notably, the different photoperiods do *not* affect the onset of activity in relation to the onset of darkness (39). However, not only the duration of light exposure affects the chronotype but also the capacity of an individual to entrain to a certain light stimulus.

Aged *mPer1/mCry2* mutant mice lose their rhythmic wheel-running behavior under LD conditions. This loss is caused by an impaired light signal transduction (59). This suggests that an impairment of circadian light perception and processing can be associated with the rhythm instability and the chronotype. Indeed, many neurodegenerative diseases such as Parkinson's or Alzheimer's disease are associated with sleep disturbances (60) presumably as a consequence of disturbances of proteostasis, resulting in a compromised circadian clock (61). However, in the gracile axonal dystrophy (*gad*) mice with a spontaneous mutation in the *Park5* gene which encodes for ubiquitin carboxy-terminal hydrolase L1 (UCHL1), the endogenous rhythm generator in the SCN stays intact. The τ DD is thus not affected (62), but the chronotype is – in comparison to the wild-type littermates – shifted to a later point in time. Along with the change in chronotype (**Figure 2B**), the mice show an increasingly unstable rhythm (**Figures 2A,C**). The *gad* mice suffer from a loss of the circadian photopigment (melanopsin) in the retina (62), which may be responsible for changes in the chronotype and the development of rhythm instability. Notably, the chronotype became earlier, and rhythm instability was less variable at higher luminance levels (**Figures 2E,F**). This is consistent with reduced daytime activity at higher irradiance levels (**Figure 2D**; 62). Thus, impaired circadian light perception as a consequence of neurodegeneration resulting in circadian disruption was rescued by light “therapy” to some extent.

In addition, age also affects the chronotype. In humans, the chronotype becomes progressively later during adolescence and then gradually shifts to an earlier chronotype again (63). Also in mice, there is evidence for a pubertal shift in the timing of daily



rhythms (64). The circadian organization changes with further aging in mice including fragmentation of the activity rhythm and a decreased precision in onset of daily activity (65). It is most likely that these changes seen in the activity rhythm are accompanied by a change in the chronotype, even though this has not been analyzed yet numerically. This indicates that the chronotype is not static and may be influenced by external and internal factors.

Conclusion

With respect to the current researches on that topic, Ehret's (11) definition of the chronotype should be expanded to “the temporal phenotype of an organism in relation to entraining cues (*zeitgebers*)”, in particular, since it has become clear that behavioral characters that are observed under (artificial) constant conditions (i.e., in the

absence of external *zeitgebers*) are of restricted predictive value with respect to entrained behavior, and also that the chronotype is malleable and can be influenced by external or internal changes. Therefore, the chronotype is the overt behavioral manifestation of the interplay of external and internal factors that makes us more or less susceptible to problems and diseases that arise from the “false timing.” Laboratory mice offer the opportunity to analyze this interplay under controlled external (entrainment) and internal (genetic) conditions. Furthermore, they provide an excellent model system to develop new therapeutical strategies to alleviate chronodisruption.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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