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PHYSIOLOGICAL REVIEW

Epidemiology of the human circadian clock ☆

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Light

Summary Humans show large inter-individual differences in organising their behaviour within the 24-h day—this is most obvious in their preferred timing of sleep and wakefulness. Sleep and wake times show a near-Gaussian distribution in a given population, with extreme early types waking up when extreme late types fall asleep. This distribution is predominantly based on differences in an individuals' circadian clock. The relationship between the circadian system and different "chronotypes" is formally and genetically well established in experimental studies in organisms ranging from unicells to mammals. To investigate the epidemiology of the human circadian clock, we developed a simple questionnaire (Munich ChronoType Questionnaire, MCTQ) to assess chronotype. So far, more than 55,000 people have completed the MCTQ, which has been validated with respect to the Horne–Østberg morningness–eveningness questionnaire (MEQ), objective measures of activity and rest (sleep-logs and actimetry), and physiological parameters. As a result of this large survey, we established an algorithm which optimises chronotype assessment by incorporating the information on timing of sleep and wakefulness for both work and free days. The timing and duration of sleep are generally independent. However, when the two are analysed separately for work and free days, sleep duration strongly depends on chronotype. In addition, chronotype is both age- and sex-dependent.

Introduction

The days of all organisms are structured by an interaction of solar and biological cycles. Solar cycles are a consequence of the Earth's rotation, as its surface is periodically exposed to and shielded from light. Daily biological cycles are a product of an endogenous circadian clock that is present in organisms of all phyla. The alteration between light and darkness produces a host of signals (light, temperature, availability of resources, etc.) which can act as cues (zeitgebers) capable of synchronising endogenous timing systems. The introduction of technical clocks has added a third temporal

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dimension—social time—that influences the daily life of humans. Although time zones were introduced to accommodate the continuously changing solar times, they are only approximations. As a consequence, the sun rises and sets at different social times within each time zone. The most extreme example of this discrepancy exists in China where one sixth of the Earth's circumference officially lives on Peking time. The difference between solar and social time is so large in Western China that citizens do not adjust their lives according to official time.

In mammals, a clock centre (pacemaker) resides in the suprachiasmatic nucleus (SCN) located above the crossing of the optic nerves. The circadian clock controls physiology at numerous levels, from gene expression to complex behaviours (e.g., sleep and performance). When shielded from solar and social time (constant conditions), the biological clock "runs free" with an endogenous period close to 24 h. In real life, circadian clocks are usually synchronised to 24 h by zeitgebers.[1] The most important zeitgeber is light (and darkness) which is also responsible for the daily rhythmicity of all other environmental signals. Unlike many other animals, the zeitgeber light is detected exclusively by the eyes in mammals, by a combination of rods, cones, and a recently discovered additional retinal photopigment, melanopsin, which is found to be dispersed in the ganglion cell layer.[2,3] The retinal signals are transduced to the SCN via collaterals of the optic nerves where they synchronise (as glutamatergic input) the circa-daily-rhythm produced by SCN neurons to exactly 24 h.[4] Via its rhythmic outputs, the SCN coordinates all the cellular circadian clocks throughout the body's organs and tissues to adapt physiology to the Earth's rotation.

An active process called entrainment ensures that the biological clock is stably synchronised to its zeitgebers. Depending on its phase, the circadian clock responds differently to a zeitgeber stimulus[1,5]: at some phases (e.g., late night to early morning), light advances the clock, at others, e.g., afternoon and evening), light delays. In the middle of the day, the circadian clock may respond little or not at all to light.

As with other genetic traits, circadian properties depend on specific genotypes. Different variants of "clock" genes[6,7] are associated, for example, with the period length of the circadian rhythm in constant conditions. In a given population, free-running periods are distributed around a species-specific mean which has been shown in both animal experimentation[8] and human studies.[9–11] Genetic variation is also, at least in part, responsible for the individual differences of the circadian clock under entrained conditions.[12–16] Individuals adopt a specific temporal relationship to the zeitgeber (e.g., the time difference between dawn and wake-up, the core body temperature minimum, or the melatonin onset). This relationship between external and internal time is called *phase of entrainment*, and when people differ in this trait, they are referred to as different chronotypes.[17]

Daily human behaviour has mainly been assessed by questionnaires designed to associate individuals with temporal preferences called "morningness–eveningness" (ME[18]). The questions used are mostly subjective, relating sleep and activity times to a personal "feeling best rhythm"[18] to the habits of others (e.g., "I get up later than most people",[19]), or to hypothetical situations (e.g., "Approximately, what time would you get up if you were entirely free to plan your day?"[20]). The degree of "ME" is expressed as a score which correlates with the timing of individuals' temperature, melatonin, or cortisol rhythms.[21–23] The ME questionnaire (MEQ) does not explicitly assess free and workdays separately nor does it ask for actual sleep and activity times[24] or exposure to outdoor light. These points, however, are essential questions for a quantitative determination of chronotype that could be of use for genetic or epidemiological analysis. The ME-score representing preferences (the higher the score the stronger the morningness preference; range: 16–86) is not a direct measurement of phase of entrainment and is, therefore, not strictly measuring chronotype. This approach likely reflects the fact that the MEQ was developed before the first clock gene had been identified, i.e., before a genetic approach was the norm.

We have, therefore, developed a new questionnaire, the Munich ChronoType Questionnaire (MCTQ) to assess individual chronotype with great precision.[17,25–27] The MCTQ asks people simple questions about their 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? All questions are asked separately for work and for free days. It has been validated with highly significant correlations by over 600 sleep-logs, by actimetry, and by correlations to biochemical rhythms such as melatonin and cortisol (unpublished data). The MCTQ has been also validated against the widely used MEQ,[18] showing high correlations.[28] The questionnaire has now been answered by more than 55,000 people and, thus, represents an excellent database for studying the epidemiology of human chronotypes.

Distribution of human chronotypes, based on the MCTQ

To quantify chronotype, a single phase marker ideally has to be extracted from the different times queried in the MCTQ. We started by assigning mid-sleep on free days (MSF; the half-way point between sleep-onset and sleep-end) as a definition of chronotype.[28] The distribution of chronotypes within a population (judged by MSF, mainly assessed in Germany, Switzerland, the Netherlands, and Austria) is almost normal with a slight over-representation of later chronotypes (Figure 1 top). The most frequent chronotype (14.6% of the population when using half-hour-bins of MSF times) sleeps on average—without social obligations—between 9 min past midnight and 8:18 a.m. (mid-sleep at 4:14 a.m.; Figure 1 top). 35.02% of the population sleep earlier and 50.38% later. While only 1% begin their sleep on free days at around 10:00 p.m. or earlier, 8.2% fall asleep around 3:00 a.m. or later. Although human chronotypes clearly cluster around a mean phase of entrainment, the differences between extreme early and extreme late types span over three quarters of the day.

This free day sleep–wake behaviour was initially used because sleep timing changes drastically on workdays. For example, the number of individuals falling asleep at 10:00 p.m. or earlier increases to 4.4% while only 1.5% fall asleep at 3:00 a.m. or later. While the average wake-up time on free days is quarter to 9, it is 2 h earlier on workdays; 55% of the population wakes up (or rather is woken up by the alarm clock) between 6:30 and 8:00 a.m. The differences in sleep-onset are much smaller. On free days, the sleep-onset (averaged over the

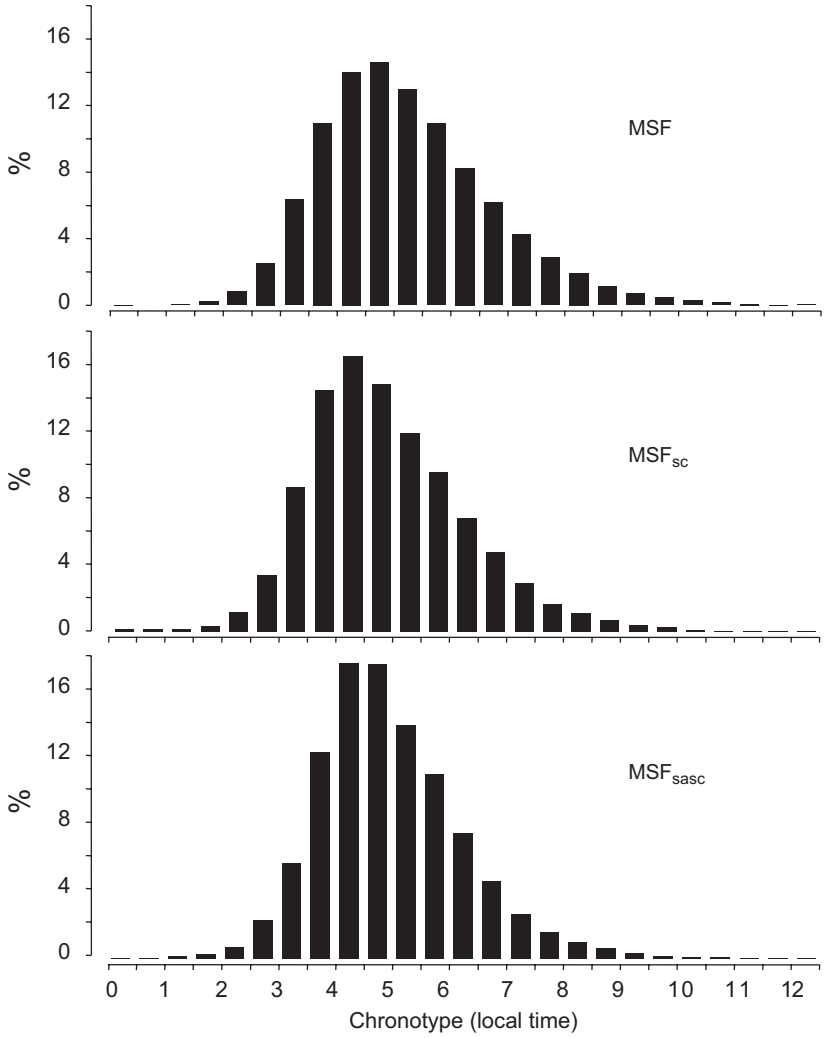

Figure 1 Distributions of chronotypes judged by different calculations of mid-sleep time: (top) mid-sleep on free days (MSF) with no adjustments; (middle) MSF corrected for the sleep-debt accumulated during the workweek (MSF$_{sc}$; see text for details); (bottom) MSF$_{sc}$ further corrected for age- and sex-dependent changes (MSF$_{sasc}$; see text for details).

entire population) is at around half-past midnight while it is only 50 min earlier on workdays.

These epidemiological results are intuitive to most of us and, therefore, appear almost banal. However, only with their quantification, can we start to ask questions about the reasons that lead to this ''normal'' behaviour and its impact on behaviour and physiology. One of the obvious consequences is that people accumulate a considerable sleep-debt over the workweek.

Chronotype and sleep duration

In addition to sleeping at different times, people also sleep for different durations. This poses a difficulty for an unambiguous chronotype determination. Sleep duration is, as suggested above, often different between work and free days (Figure 2 top and middle, respectively). Analysing the length of sleep in half-hour-bins shows that 21% of the population, sleeps between 7 and 7.5 h on workdays. Forty-one percent sleep shorter and 38% sleep longer, resulting in an almost perfect normal distribution. On free days, the largest binned group (15.5%) sleeps for 7.5–8 h, but the majority of the population (50.5%) sleeps even longer. Under the assumption that most of the individuals in our database follow a 5-day workweek (we ask people who work in shifts to fill out a different dedicated questionnaire), we have estimated the mean individual sleep duration by averaging the values of five workdays and two free days (Figure 2 bottom). This calculation allows an estimation of a person's individual sleep need. According to this

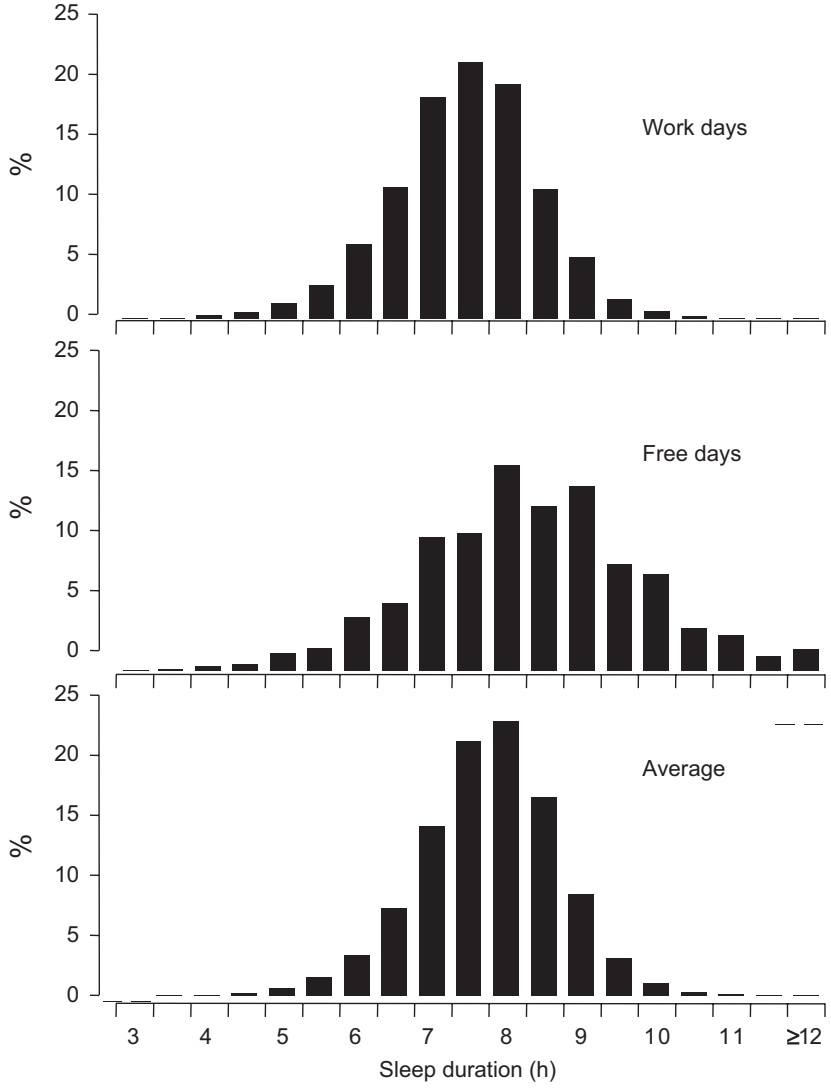

Figure 2 Distribution of sleep duration on workdays (top), free days (middle) and calculated as a weekly average (bottom, approximated by averaging 5 work and 2 free days).

theoretical estimation of a weekly average in sleep duration, the largest of the half-hour-bins (22.5%) is found in the same category as on free days without sleep correction (7.5–8 h), but in this case, 48% sleep shorter and only 29.5% longer.

MSF and average sleep duration correlate only slightly ($r = -0.03$; only significant due to the large numbers) with a slope of only 2.45 min. However, when short and long sleepers are compared ($\leqslant 6$ h vs. $\geqslant 9$ h, respectively), the chronotype (MSF) distributions of these two groups are almost identical (see also Ref. [26]). We, therefore, conclude that sleep timing and sleep duration are essentially independent traits. The two variables also clearly separate when the data is analysed by factor analysis (unpublished data).

There is, however, a significant relationship between sleep duration on work and free days and chronotype (MSF). The later the chronotype, the shorter the workday sleep and the longer the free day sleep (Figure 3).

This result suggests that both duration and timing of sleep on free days are influenced by the sleep-debt accumulated over the workweek. 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 by subtracting the difference between the sleep duration on free days and its weekly average (see distribution of MSF_{sc} in Figure 1, middle panel). Since the majority of the population is sleep deprived on workdays, MSF_{sc} lies, in most cases, slightly earlier than MSF.

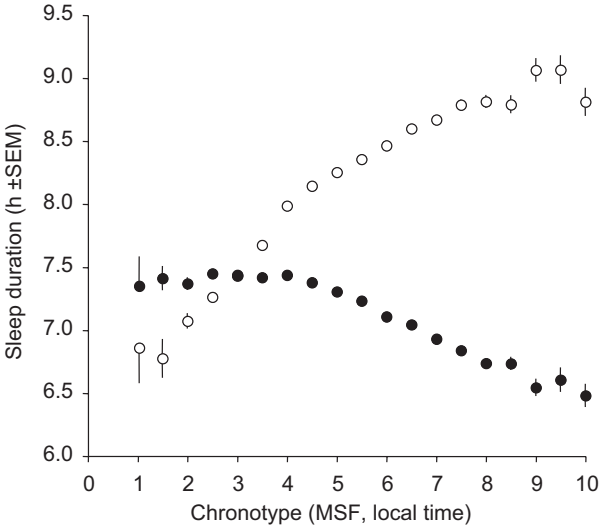

Figure 3 Relationship between chronotype (MSF) and sleep duration analysed separately for work and free days (filled and open circles). Early chronotypes are sleep deprived on free days while late chronotypes sleep less than their weekly average on workdays. People who sleep voluntarily approximately between 11:00 p.m. and 6:00 a.m. are the only chronotypes who show no difference in sleep duration between work and free days. Vertical bars represent the SEM in each category (to avoid overlap, they are, in some cases only drawn in one direction); most errors are smaller than the data points. The correlations for the raw data for both work and free days are highly significant ($r = -0.174$ and 0.266, respectively; $N = 60{,}000$; $p \ll 0.0001$ for both).

An independence of sleep duration and its timing (based on the MEQ) has been found previously[18] and has also been demonstrated in research with rodents.[29] This suggests that sleep duration and rest-time are not the same; while sleep duration appears independent of chronotype, the ratio between activity- and rest-time (α/ρ ratio) changes systematically with period length and, thereby, also with phase of entrainment.[30]

Chronotype and age

Chronotype has been shown to depend not only on genetic[14,31,32] and environmental factors[17] but also on age.[17,33–36] The large MCTQ database accumulated with our MCTQ survey allowed us to examine this age-dependency as an epidemiological phenomenon because there are many subjects for each age group.[25] Within each age group, the shape and width of the chronotype distribution (MSF_{sc}) is similar to that of the general population (Figure 1, middle panel). The respective means, however, vary systematically (Figure 4). Children are generally earlier chronotypes, progressively becoming later (delaying) during development, reaching a maximum in their "lateness" at around the age of 20, and then becoming earlier again (advancing) with increasing age.

The general phenomenon that females tend to mature earlier than males is also apparent for the ontogeny of chronotype (Figure 4). Women reach their maximum in lateness at around 19.5 years of age,[25] while men continue to delay their sleep until around the age of 21 and are, on average, later chronotypes for most of their adulthood (see also Ref. [37]). This sex (rather than gender) difference disappears at around the age of 50, which coincides with the average age at menopause.[38,39] People over 60 years of age, on average become even earlier chronotypes than they were as children.

The systematic changes of chronotype with age, together with the significant sex differences between puberty and menopause indicate that—directly or indirectly—endocrine factors are involved in the age-dependent changes of chronotype.[33,40]

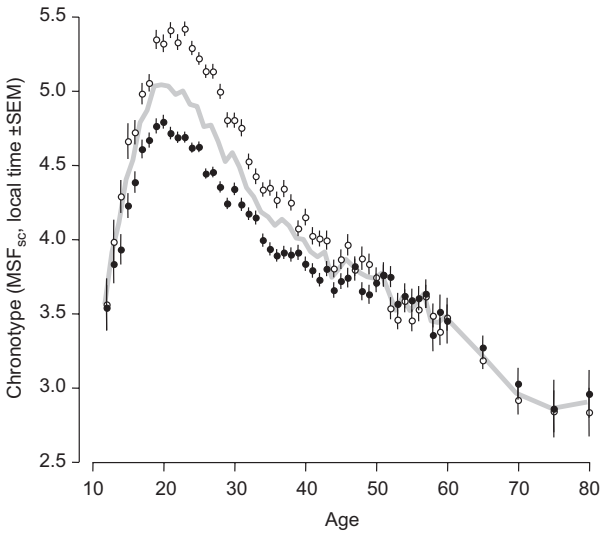

Figure 4 Chronotype depends on age. These changes are highly systematic and are different for males and females (filled circles, females; open circles, males; the grey line shows the averages for the entire population). The first data points represent the averages for subjects aged 12 or younger. Between ages 12 and 60 data were averaged for each year of age while those showing the mean chronotype for subjects above 60 years of age are averaged over groups of 5 years. Vertical lines represent SEM.

Thus, incorporation of hormonal aspects could enhance the description and understanding of the circadian phenotype. Both concentration and timing of many hormones are age-dependent.[41] In young people (16–25 years of age), the time-of-day-dependent secretion of growth hormone reaches its maximum (and cortisol its minimum) at around 1:00 a.m.—approximately 1 h later than in the elderly (>70 years). The structure of sleep changes along with these endocrine changes.[41,42]

The sharp maximum of lateness at around the age of 20 coincides with the suggested end of adolescence which has, so far, been defined based on a mixture of several biological and socio-biological variables.[43] We suggest that adolescence ends and individuals enter adulthood at the age when their chronotype stops delaying and starts advancing.

If an individual preserved his/her relative position within the distribution throughout life, Figure 4 would also result if we had undertaken a longitudinal study, in that case representing the average of somewhat parallel individual age-dependencies. However, this cross-sectional study cannot distinguish whether individuals become earlier chronotypes with age or whether chronotypes (independent of age) have become later over the last decades. To distinguish between these possibilities, the MCTQ asks for self-assessment of present, childhood, and teenage chronotype (giving a choice of seven categories, ranging from extreme early to extreme late). All self-assessments highly correlate with MSF$_{sc}$. With progressing age, subjects recall themselves to have been relatively more delayed as teenagers and less advanced as children (compared to their present chronotype), thereby mimicking the age-dependent progression of chronotype shown in Figure 4. This indicates that subjects retain their relative positions within the distribution throughout life and that the pattern of the age-dependent chronotype changes must have been similar during the last century.

The data also cannot formally rule out behavioural and environmental factors influencing the age- and sex-dependent differences in chronotype (e.g., do teenagers sleep late because they go to the disco or do they go to the disco because they cannot sleep until late?). This will be followed up in subsequent studies also, but if life-style were the driving force, a different kinetic would be predicted, namely stabilisation to a given chronotype by about 30 years. Furthermore, in a parallel study, we collected chronotype information (MCTQ) in three secluded valleys of South Tyrol ($N \approx 800$). The age-dependent progression of chronotype in these culturally distinct areas is practically identical to that shown here (data not shown), suggesting that this age dependency is not specific for a given database but is rather a characteristic of chronotype with respect to age.[25]

Given the age dependency of chronotype and the sex differences that occur between the ages of 20 and 52, an additional correction to the MSF$_{sc}$ is necessary when genetic or epidemiological questions are addressed. The resulting chronotype is standardised for the effects of age and sex (MSF$_{sasc}$). The distribution of MSF$_{sasc}$ in the population is shown in the bottom panel of Figure 1 (for the correction algorithms, see supplemental data to Ref. 27).

Comparison between the MCTQ-derived chronotype and the morningness–eveningness score

In a study at the University of Groningen, 2481 subjects have responded to both the MEQ and the MCTQ.[28] Since then, approximately 250 additional subjects have filled out both questionnaires. Table 1 shows the correlation coefficients (r) between MCTQ-derived chronotype variables and the corresponding ME-scores. The basic chronotype calculation for MSF highly correlates with the ME-score ($p \ll 0.0001$). However, when MSF is corrected

Table 1 Correlations between MCTQ-derived chronotypes and the morningness–eveningness score.[18]

MCTQ variable	*r*
Mid-sleep calculations	
MSF	−0.74
MSF_{sc}	−0.66
MSF_{sasc}	−0.59
Self-assessment	
At present	−0.80
As child	−0.47
As teenager	−0.56

2726 subjects have filled out both questionnaires. Correlations are given for mid-sleep on free days (MSF), its correction for sleep-debt accumulated during the workweek (MSF_{sc}), and for the age- and sex-corrected chronotype (MSF_{sasc}).

for sleep-debt accumulated during the workweek, the correlation decreases (the duration of the workweek is highly variable in the Netherlands, so we also considered an average 4-day workweek, which only leads to slightly smaller decreases). This result indicates that the ME-score cannot account for the confounding effect of work-related sleep deprivation. The correlation decreases further when MSF_{sc} is corrected for sex- and age-dependencies (MSF_{sasc}). This weaker correlation is not surprising because by correcting for age-specific trends, the MSF_{sasc} is a standardised chronotype measurement and does not necessarily reflect a subject's chronotype corresponding to his/her present situation. It does, however, indicate that an ME-score that is not corrected for age and sex dependencies may be a weak phenotype for evaluation of genetic or epidemiological questions about the human circadian clock. Age dependencies have also been reported for the ME-score and corrections have been applied in some studies.[44]

Interestingly, the best correlation between ME-scores and the MCTQ concerns the subjective, self-ratings ($r = -0.80$). After subjects have given their average times of sleep and wakefulness, the MCTQ asks them to give a self-assessment of their chronotype by choosing from seven categories (extreme, moderate and slightly early, normal, and slightly, moderate and extreme late). This self-assessment is introduced by the following description: *After you have answered the preceding questions, you should have a feeling for which chronotype (time-of-day-type) you belong to. If, for example, you like (and manage) to sleep quite a bit longer on free days than on workdays, or if you cannot get out of bed on Monday mornings, even without a Sunday-night-party, then you are more a late chronotype (owl). If, however, you regularly wake up before the alarm goes off, feel perky once you jump out of bed, and if you would rather go to bed early than to an evening concert then you are an early chronotype (lark). In the following questions, you should categorise yourself*. It is remarkable that an introduction combined with a single question of self-assessing one's chronotype gives almost the same results as a questionnaire consisting of 19 items. In addition to assessing oneself in the present situation, the MCTQ asks subjects to assess what chronotype they were as children and teenagers. As to be expected, both of these assessments give weaker correlations to the ME-score (see Table 1).

A recent study performed by the Groningen laboratory investigated extreme chronotypes under constant conditions in a time-isolation facility. The participants, who were selected on the basis of their actual sleep and activity behaviour and not on diurnal preference, also filled out the MEQ. Although their ME-scores correlated well with their mid-sleep phase on free days (judged by both the MCTQ and their actimetry) the ME-score variation among the extreme early types was so large that it almost was indiscriminative: only one subject fell into the category of clear morning preference, five into the category of moderately morning preference, two assessed no preference, and one subject even scored as moderately evening type.

Conclusions

Dependencies of chronotype on age and sex as well as insights into how social schedules as experienced during the workweek may confound sleep/wake behaviour have not only made this accurate estimation possible, but also allow standardisation of the chronotype of an individual so that it becomes independent of age and sex. These adjustments are an important prerequisite for genetic and epidemiological studies. Our understanding of the complex genetics that underlie chronotype (which is the only circadian variable accessible in large-scale human studies) is still at the beginning and will profit from accurate phenotyping. Further refinements of chronotyping are still necessary. For example, we are presently not confident in accurately assessing the chronotype of people actively involved in rotating shift work because we still do not have an adequate algorithm for estimating the confounding effects of sleep deprivation under these conditions (a study to prevent these limitations is under way).

We are only beginning to realise the usefulness of accurate chronotype assessment. Individual shift work scheduling, interpretation of medical results and optimal timing of medical interventions are only a few examples. The possibility that internal time (as represented by chronotype) may be more important than external time (either sun time or social time) for understanding temporal human biology is becoming increasingly evident. We have shown recently, for example, that the human circadian clock entrains predominantly to sun time, in contrast to social time,[27] showing that its entrainment is comparable to that of other animals. However, most probably due to increased shielding from high light intensities as we find them outdoors during the day (15,000 to over 100,000 lx), the distribution of chronotypes in industrial societies increases in width, leading to large phase differences in circadian timing between individuals. Under these conditions the definition of a night shift from 10:00 p.m. to 6:00 a.m., for example, may turn out to be not very useful for investigations of its consequences on health and well-being—these external night-shift times must have very different effects on an extreme early vs. an extreme late chronotype. In addition, more global studies are needed to understand the influences of different cultures, of climatic environments, or of latitude on chronotype and its change (or stability) across seasons.

Our daily timing is such an obvious aspect of our lives and may, therefore, have been neglected in its exact quantification. It is, however, also enormously influential on many other aspects in our lives that range from alertness, performance and social competence to physiological and mental health.

Practice points

1. Circadian research combines molecular biology, genetics, physiology, medicine, psychiatry and epidemiology to understand the mechanisms of the endogenous daily clock which exists in organisms of all phyla.
2. Biological clocks in temporal isolation run with their own, not exactly 24-h periodicity (hence the term circadian, about 1 day) and are synchronised to the daily rotation of the Earth by a mechanism called entrainment.
3. Circadian clocks regulate and/or modulate functions at all levels, ranging from gene expression and physiology to behaviour and cogitation.
4. Entrainment of circadian clocks relies on signals (zeitgebers) that represent the regular daily changes of the environment of which light is the most important zeitgeber signal.
5. Individual differences in how circadian clocks entrain within the day (early or late) can be measured in humans under natural conditions using a simple questionnaire (Munich ChronoType Questionnaire, MCTQ). The resulting ''chronotype'' has a genetic basis but also depends on light exposure, age and sex. Knowledge about chronotype is important for both medical diagnostics and therapy (e.g., chronopharmacology).
6. Modern society poses many challenges on circadian entrainment in humans, due to lack of zeitgeber strength (by working predominantly inside), transmeridian travel (jetlag) or working schedules in a 7/24 society (shift work).

Research agenda

Issues to be defined in the future include:

1. Measuring properties of the human circadian clock in real life, without retaining subjects in expensive and highly artificial constant routines.
2. Incorporating our knowledge about circadian rhythms and the effects of light into everyday medical praxis, thereby tailoring individual examination and treatment to optimise both diagnostics and therapy.
3. Using our knowledge about circadian rhythms and the effects of light to improve health and quality of life in shift workers and transmeridian travellers as well as those who suffer from clock-related sleep problems or any other form of dys-entrainment.
4. Understanding the circadian system as a whole. How do all the clocks in the brain and the periphery communicate, what are the optimal phase relationships between, for example, the liver clock and the central pacemaker in the brain?

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